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- To provide a forum for organizing symposia/conferences with a view to develop close relationship among the scientists engaged and interested in plant genetic resources activities.

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CONTENTS

1. Role of Potato in Entrepreneurship Development <i>Brajesh Singh and Tanuja Buckseth</i>	189
2. Morphological Characterization and SSR Marker Assay of Mango Landraces of West Coast of Kerala, India <i>Anuradha Sane, Joseph John, C Vasugi, M R Dinesh and K Pradheep</i>	195
3. Morphological and Molecular Diversity Analysis in Forage Sorghum (<i>Sorghum bicolor</i> (L.) Moench) <i>Arvinth S, RN Patel, RA Gami, Kapil K Tiwari and AH Joshi</i>	206
4. Variability and Character Association in Different Classes of Rice (<i>Oryza sativa</i> L.) of Assam for Root and Yield Attributing Traits <i>Priyanka Bairagi, Dikshita Gogoi and Kishore Kumar Sharma</i>	215
5. Identifying Potential Donor Parents for Breeding Against Leaf Curl Virus and Anthracnose Diseases in Chilli <i>Solanki Bal, Arup Chattopadhyay and Asit Kumar Mandal</i>	222
6. Evaluation of Long-Day Onion (<i>Allium cepa</i> L.) Genotypes for Yield and Quality Traits Under Temperate Conditions of Kashmir Valley in India <i>Geetika Malik, Rafiq A Shah, Alima Shabir, Vishal Dinkar, Javid I Mir and Om C Sharma</i>	232
7. Seeds of Harmony: Conservation of Landraces by Farmers, Communities and NGOs in the Western Ghats, a Case Study <i>G M Puneeth, Ravi Gowthami, Sanjay Patil, Kailash Chandra Bhatt, Ramesh Vasudeva and Sunil Archak</i>	246
8. Molecular Characterization of Grapes (<i>Vitis</i> spp.) Using EST-derived Simple Sequence Repeat (SSR) Markers <i>Sharmistha Naik, Jagesh K Tiwari, Rasna Zinta, Narender Negi, Rajnish Sharma, Gopal Singh, and DP Sharma</i>	255
9. Restoration Reaction in the F₁ Hybrids Derived from F₃ <i>maldandi</i> Restorers on Different Cytoplasm in <i>Rabi Sorghum</i> [<i>Sorghum bicolor</i> (L.) Moench] <i>Prashant Kariyannanavar*, M. C. Wali, B. D. Biradar, V. S. Kubsad, L. K. Verma, B. N. Prashantha, N. Pavan Kumar and Revanasiddayya</i>	263
10. Ensemble Habitat Suitability Studies of <i>Vetiveria zizanioides</i> (Linn.): An Important Genetic Resources with Aromatizing, Medicinal and Land Restoration Properties <i>Manish Mathur and Preet Mathur</i>	266
11. Genetic Diversity Assessment of <i>Cordia gharaf</i> (Forsk.) Germplasm for Morpho-physiological and Fodder Traits in Hot-arid Kachchh Region of India <i>Rahul Dev, Anil Patidar, M Sureshkumar, Sushil Kumar, K Venkatesan, R Saranya, Traloki Singh and Devi Dayal</i>	283
12. Stem of Woody Pepper (<i>Piper pendulispicum</i> C. DC) as a Source of Phenolic Acids and Piperine <i>Ajit A. Waman, Pooja Bohra, Karthika Devi R., Lokesh A.N. and Shivashankara K.S.</i>	294
13. Collecting Plant Genetic Resources from Remote and Isolated Marwah-Warwan Valley of Jammu and Kashmir, India <i>Sheikh M. Sultan, Susheel Kumar Raina and Praveen Kumar Singh</i>	301
14. Characterization of Gola Ber Genotypes under Arid Conditions of Western Rajasthan for Qualitative and Quantitative Characters <i>DK Sarolia, Kamlesh Kumar, Lokesh Kumar, D Singh and MK Choudhary</i>	310
15. Comparative Assessment of Different Earth System Models for Habitat Suitability of <i>Cuminum cyminum</i> (Linn.) Crop: A Machine Learning Evaluation from Arid and Semi-Arid Hot Areas of the India <i>Manish Mathur and Preet Mathur</i>	316
Plant Germplasm Registration Notice	341
Author Guidelines	

REVIEW ARTICLE

Role of Potato in Entrepreneurship Development

Brajesh Singh and Tanuja Buckseth

Abstract

Potatoes can play various roles in entrepreneurship development, especially in sectors related to agriculture, food processing, and retail. Potatoes are a staple crop in many regions, and cultivating them can be a profitable venture for farmers. Entrepreneurial farmers can innovate in terms of cultivation techniques, such as adopting organic farming practices or implementing technology-driven precision agriculture methods. They can establish businesses related to potato farming inputs, such as supplying high-quality seeds, fertilizers, and pesticides. Additionally, there's potential for startups to provide farm machinery, irrigation systems, and other equipment tailored to potato cultivation. Potatoes are versatile ingredients used in various food products like chips, fries, mashed potatoes, and snacks. Entrepreneurs can set up food processing units to manufacture these products, focusing on quality, flavor innovation, and health-conscious options to meet consumer demands. Beyond traditional potato products, there's an opportunity for entrepreneurs to create value-added products like dehydrated potato flakes, starch, flour, and specialty potato-based foods. These ventures can cater to niche markets or capitalize on trends such as gluten-free or vegan diets. Distribution networks or retail outlets specializing in potato products can be established. This could involve setting up standalone stores, online platforms or partnering with supermarkets and grocery chains to reach a broader customer base. In regions with surplus potato production, entrepreneurs can explore export opportunities by tapping into international markets. This might involve complying with export regulations, ensuring product quality and packaging standards, and building relationships with overseas buyers. Overall, potatoes offer a multitude of opportunities for entrepreneurship across the agricultural and food industry value chain, from farming and processing to distribution and retail. Entrepreneurs who innovate, adapt to market trends, and focus on quality and sustainability can unlock the full potential of potato-based ventures.

Keywords: Seed potato, Value-added products, Startups, Export, Retail.

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Introduction

The growth of any nation depends upon the proper and optimal utilization of different resources of a nation. With globalization and economic liberalization concomitant with growing urbanization, consumer preferences are changing (Devaux et al., 2014). To address this situation, the emphasis is on enabling farmers to increase their level of competitiveness, to produce for an identified market and seek new market opportunities that offer higher levels of income. Agriculture and allied sectors are the largest enterprises in the country and an enterprise can survive only if it can grow consistently. Potato is one of the most crucial food crops in the entire world. Potato is the third most important food crop worldwide. The Indian economy is primarily based on agriculture and over 58% of the Indian population is involved in livelihood related to agriculture works (Kaur et al., 2023). Potato contributes strongly to the agricultural economy of the country. The current share of potatoes in agricultural growth domestic product (GDP) from a unit area of cultivable land is about 3.7 times (2.86% agricultural GDP from 1.32% cultivable area) higher than rice (18.25% GDP from 31.19% cultivable area) and 5.4 times higher than wheat (8.22% GDP from 20.56% cultivable area), which are the two principal food crops of the country (Singh et al. 2020a). India is the

second largest producer of potatoes in the world, but when it comes to processing, it is not even in the top ten nations. Its global contribution to processing is minuscule. There are 9 major potato-producing states in India, i.e., Punjab, Andhra Pradesh, West Bengal, Gujarat, Uttar Pradesh, Haryana and Assam, Bihar, and Madhya Pradesh. Potatoes occupy an important place in Indian cuisine and it is one of the most demanding items of Indian cuisine. Potatoes are also contributing majorly to the food security of the nation in addition to cereals.

Entrepreneurship is commonly referred to as a process of setting up one's own business and at the same time having the capabilities to identify the fruitful opportunities that come in the way. It is the process of creating something new with value by devoting the necessary time and effort, assuming the accompanying risks, and receiving the resulting rewards. Entrepreneurs are innovators or developers who recognize and seize opportunities, convert those opportunities to marketable ideas (products or services that can be sold); add value through effort and skills, assume the risks of the competitive marketplace and realize the rewards or losses. ICAR-CPRI technologies related to mini-tubers production through Aeroponics systems, Indigenous varieties, value-added products managed and transferred by the institute technology management unit (ITMU) have spread over almost different states of the country, building farmers' and enterprises' confidence and contributing to potato productivity, production and development of entrepreneurship. In the journey of successful entrepreneurship in the potato sector, the entrepreneur must have traits like decision-making ability, being resourceful, personable, adaptable, persuasive and hungry for business development. ICAR-CPRI is marching ahead with its vision of advancing professional competency for pursuing excellence in research and entrepreneurship development in relation to potato and associated sectors with ethical values to meet the regional and national needs and offering specialized services to the farmers and entrepreneurs for decent livelihood and earning. The potato sector has a bright scope of entrepreneurship development (Fig. 1).

Entrepreneurship in seed potato sector

The seed potato sector offers significant scope for entrepreneurship due to its critical role in ensuring quality and high-yielding potato crops. Entrepreneurship in producing quality seed mini-tubers using aeroponics technology presents a promising opportunity to address the challenges facing the seed potato sector while fostering innovation, economic growth, and sustainability in agriculture.

Seed potatoes: Indian scenario

The availability of virus-free planting material is a prerequisite for micropropagation and seed production of the

clonally propagated potato crop. The Government of India is committed for ensuring the timely availability of certified seeds to the farmers cultivating different crops. However, for high-volume high-seed rate crops like potatoes, the production and supply of good quality certified seeds is a challenge. Annually, India requires 5.4 million tons of potato tubers as seeds. The challenge becomes even more complex as the vegetative mode of propagation makes the seed vulnerable to several pathogens in its multiplication process and the seed multiplication ratio (SMR) of 1:6 is meager. Farmers, mainly marginal and small farmers, rarely store their harvests as seeds for the next growing season. Often, they buy seed potatoes every season, making the seed replacement rate (SRR) almost 100%. However, there is no institutional mechanism to monitor the quality of seed potatoes. Most often, degenerated produce is sold as seed, especially to small and marginal farmers who lack finances (Buckseth et al., 2022). The seed alone comprises over one-third of the total cost of production of potatoes. There is a considerable gap between the requirement and supply of certified seed potatoes in India. The Central Potato Research Institute of the Indian Council of Agricultural Research produces about 3,000 tons of nucleus and breeder seed annually and supplies 80% of it to the states and other agencies for its multiplication. If this stock were to multiply in three stages, i.e., Foundation 1, Foundation 2, and Certified grades, we could produce only about 0.5 million tons of certified seed. On the assumption of 100% SRR, it meets only 10% of the total seed requirement, leaving a deficit of about 4.9 million tons. It is virtually impossible to produce such a massive quantity of certified seeds using traditional methods. Besides its low rate of multiplication in a longer span of 7 to 8 years, the traditional seed production system suffers from several other constraints like (i) the requirement of a huge number of disease-free propagules in the initial stage, (ii) the slower process of generating 100% healthy seed stock from the infected material, (iii) progressive accumulation of degenerative viral diseases in each field exposure, and (iv) field multiplications of initial

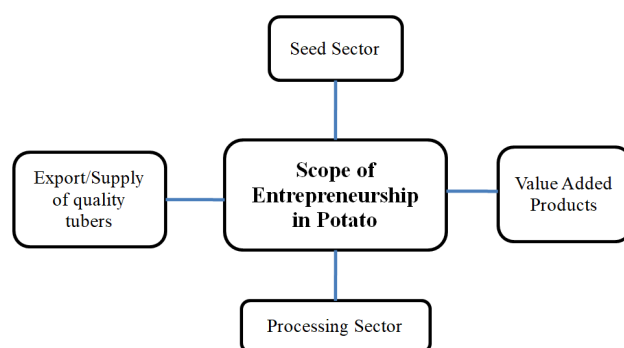


Fig. 1: Entrepreneurship scope in potato sector/farming

disease-free material. It is further complicated because the breeder Seed supplied by ICAR-CPRI is seldom multiplied in all three generations by the State Seed Certification Agencies maintaining the recommended procedures (Singh et al., 2019). About 1.2 million tons of potato seed is sold by private seed producers, especially those from Punjab, western Uttar Pradesh, West Bengal and Haryana, though there are no mechanisms and infrastructures for monitoring seed quality. It is, therefore, imperative to evolve a seed production system encompassing innovative techniques to improve the quality of seed (NAAS, 2021) and to reduce field exposure, along with a robust system of certification and quality assurance of the seed produced and supplied by private seed growers.

Role of ICAR-CPRI for the development of the seed industry

Of the key technologies developed by the institute, the tissue culture-based hi-tech seed system developed has led to the opening of > 30 labs across the country and has allowed the supply of healthy mother stock (in vitro plants) to different seed-producing organizations/agencies for seed production in the country. One in vitro tube with 2 to 3 plants, sub-cultured at least ten (2^{10}) times will generate 2048 plants. During 2010-2020, ~1296 culture tubes were supplied to 64 firms, which would have generated 2654208 healthy microplants. These plants would have covered 8 ha area under G0 crop followed by 40 ha G1, 200 ha G2, 1000 ha G3, 50000 ha G4 and 25000 ha G5 area respectively,

thereby producing 6250000 quintals of certified seed. This has resulted in covering 12.5% of the total potato area of the country, resulting in a total monetary gain of Rs. 1250 crores, assuming that seed potatoes were sold @ Rs. 20000/ton. Further, the aeroponics technology, which has revolutionized the seed system in the country, has been commercialized to 28 entrepreneurs associated with mini-tubers production and 6 entrepreneurs involved with the fabrication of aeroponics units in 10 different states of the country viz, Haryana, Punjab, Madhya Pradesh, Bihar, Uttar Pradesh, Mizoram, Gujarat, New Delhi, West Bengal and Maharashtra. There is a huge scope for entrepreneurship to produce quality seed mini-tubers in other states of the country. The combination of seed production technology advancement and the availability of varieties in the Indian market has resulted in tremendous development in the seed industry recently. There is an excellent transformation in the overall potato portfolio across the sectors in the country and farmers need to rationalize their production as per the requirement of various categories of potatoes. Many of the entrepreneurs like M/s Sekhon Biotech, Rupnagar and M/s Bhatti Agritech, Jalandhar are, directly established and benefited from the ICAR-CPRI technologies and started quality planting material production after licensing CPRI aeroponic technology. Another successful entrepreneur in potato mini-tuber production for fulfilling the seed requirement is M/s Raghuvansh Agro Farm, Kanpur (Fig. 2).

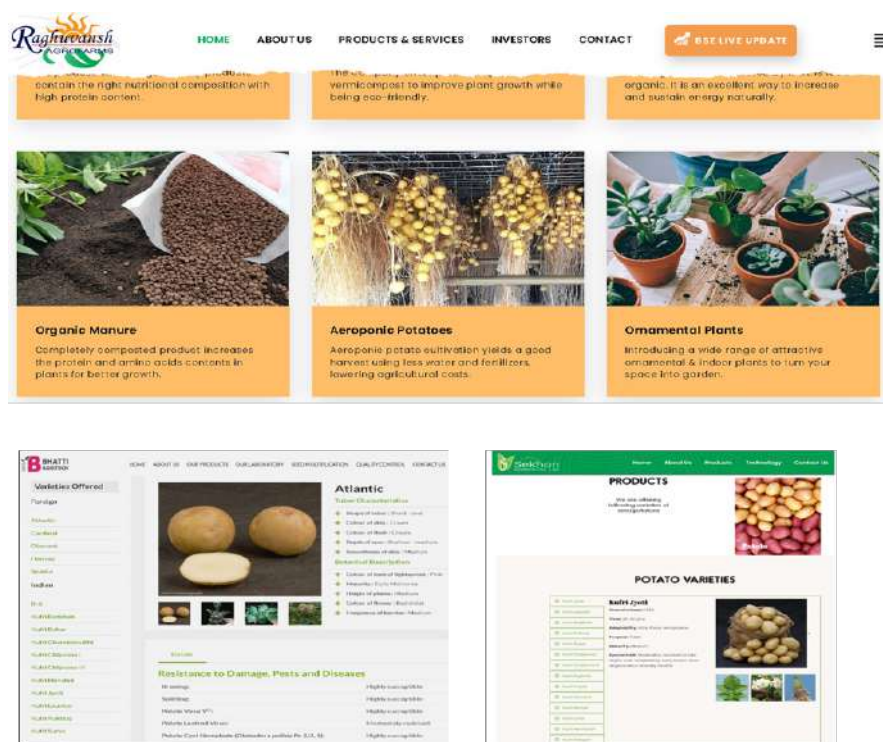


Fig. 2: Aeroponics entrepreneurs involved in the seed sector M/s Raghuvansh Agrofarm, Kanpur; M/s Bhatti Agritech, Jalandhar; M/s Sekhon Biotech Pvt. Ltd. Rupnagar Punjab

Entrepreneurship in Potato Processing Sector

Entrepreneurship in the potato processing sector offers a multitude of opportunities for value addition, innovation, and market expansion. The rate of population growth and urbanization is increasing as is the level of potato consumption, especially in the form of chips and crisps. The global potato market has been segregated into frozen products, potato chips and snack pellets, dehydrated and other products. Potato chips remain the most popular snack food across the globe, valued at USD 31.2 billion in 2020 and is expected to grow at a compound annual growth rate (CAGR) of 6.4% during 2020–2026 (IMARC 2020). The frozen potato market is growing fast due to the rise in the market for French fries supplied in restaurants and fast food outlets (Rana and Singh, 2017). The worldwide frozen potato market is dominated by French fries, capturing a share of about 40% (market share of USD 21.8 billion in 2020) (IMARC 2021). The market for dehydrated potato flakes is also witnessing substantial growth due to their longer shelf life, ease of handling and all-season availability. These instant potatoes mimic the original flavor of fresh potatoes and provide excellent thickening properties. Globally, the dehydrated potato market is dominated by flakes (capturing 36.5% market share), followed by potato powder, dice, value-added products and shreds (Fig 3). The potato processing sector contributes immensely towards global food security.

Growing urbanization and consumers' shift to convenient ready-to-eat foods have led to tremendous growth in the Indian potato processing sector, particularly with French fries being one of the largest-selling food items. India is the major producer of potatoes in the world, but only 7% of total produce is processed (compared to industrialized countries, this percentage is over 80). Recent trends in

changing food consumption habits worldwide indicate that the Indian potato processing industry has potential scope for further expansion. With a global production of 371 million tonnes, India is producing over 53 million tons of potatoes which is second next to China. Also, India has the prospects for growth of processed potato products where the economic boom is apt to create a sturdy demand for convenient ready-to-eat snack foods. In India, most of the crop is cultivated for table and seed purposes while processing is still in its infancy stage. Hence, there is ample scope to invest in potato processing industries in India. The Indian frozen French fry industry and dehydrated potato flake industry has undergone enormous growth in the past one and half decades. In the year 2010, the potato processing level was only 3 to 4%, but now, due to the setting up of more potato processing units, the consumption of potatoes for processing has almost doubled up to 7–8%. Potato training activity is carried out as one of the solutions to increase entrepreneurial interest and courage to start a business among housewives from the smallest social sphere, namely the household. This can encourage women's independence and empower the potential of housewives who still have a lot of free time. The training and exposure of this material is expected to increase the understanding and spirit of entrepreneurship for housewives and the practice of making potato chips that is done can increase the skills of housewives. There are many startups with the products developed by ICAR-CPRI Shimla. Investing in research and development is essential for staying ahead of market trends and consumer preferences. Entrepreneurs can conduct product testing, flavor innovation, and sensory evaluations to continuously improve existing products and develop new offerings that meet evolving consumer demands.



Potato muffins (Gluten free)



Potato Jalebi (Gluten free)



Potato Porridge and Semolina



Gluten-free Potato-Sorghum cookies

Fig. 3: Value-added and processing products of potato

Entrepreneurship in the potato processing sector offers vast potential for growth, innovation, and profitability. By leveraging technological advancements, market insights, and strategic partnerships, entrepreneurs can establish successful ventures that contribute to the development and sustainability of the potato industry.

Export scope in Potato and its products

Exports surged beginning in 2017 as India established the capacity to produce international-grade French fries. There was a surplus of domestically created products available, resulting in a decline in import quantities. The production capacity of French fries increased by 0.1 million tonnes over the last decade, which is directly related to the expansion of fast food chains (McDonald's) and the creation and growth of food merchants (The Economic Times 2013). India managed to export around 10,000 tonnes of dehydrated potato flakes to countries like Malaysia, Indonesia, Thailand, Philippines and Vietnam in 2019 (FAOSTAT 2019). India also witnessed remarkable growth in export of fresh potatoes during the last decade. India entered the market in 1987 and now, it is Asia's third largest exporter of fresh potatoes (table and processing) after Iran, China and Pakistan. Export of fresh potatoes from India increased from 0.1 million tonnes in 2010 to 0.42 million tonnes in the year 2019 (FAOSTAT 2019), with 14% growth per annum, valued at 76.6 million USD (Gondalia et al., 2017).

Way Forward

The article highlights the importance of promoting entrepreneurial behavior among potato growers and underscores the potential of potato processing to contribute to India's self-sustainability in food production and nutrition. Here are some key points:

Enhancing entrepreneurial behavior

There is a need to enhance the entrepreneurial behavior of potato growers by providing them with specific knowledge and encouraging the adoption of value-added practices. This could involve training programs, workshops, and educational initiatives aimed at fostering entrepreneurship skills and mindset among farmers.

Understanding human behavior

Analyzing entrepreneurship and entrepreneurial behavior among potato farmers can aid in understanding and predicting human behavior in agricultural contexts. By gaining insights into the factors influencing entrepreneurial decision-making and actions, policymakers and stakeholders can design targeted interventions to support entrepreneurial growth.

Promoting Entrepreneurial Performance

The analysis of entrepreneurship among potato farmers can help in enhancing motivation and entrepreneurial

performance within the agricultural sector. By identifying barriers to entrepreneurship and addressing them through policy measures and support programs, the overall performance and productivity of potato growers can be improved.

Future Growth Prospects

The growth of the potato-based industry in India is expected to be lucrative, offering opportunities for high-profile growth and development. Sustainable growth in the potato processing sector can benefit both producers and processors, creating employment opportunities, generating income, and supporting rural livelihoods.

Contribution to Food Security

Potato processing can contribute to achieving self-sufficiency in food security by diversifying food sources, reducing postharvest losses, and ensuring year-round availability of potato-based products. This can strengthen India's position in the global food market and enhance its resilience to food supply disruptions.

In conclusion, promoting entrepreneurship among potato growers and leveraging potato processing for value addition can contribute significantly to India's agricultural development, economic growth, and food security objectives. By investing in entrepreneurship development and supporting the growth of the potato processing industry, India can harness the full potential of its agricultural resources to meet the nutritional needs of its population and achieve self-sustainability in food production.

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RESEARCH ARTICLE

Morphological Characterization and SSR Marker Assay of Mango Landraces of West Coast of Kerala, India

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Abstract

Mango is an indigenous crop that originated in North East India-Myanmar region. Local landraces of mangoes found on the South West coastal regions and North East India were found to have many useful traits, including the tendency of polyembryony. To estimate the diversity available in the landraces maintained at ICAR-NBPGR, Regional Station, Thrissur, morphological characterization was carried out using twelve qualitative and ten quantitative traits. To complement the morphological data, the molecular assay was performed using SSR markers. Qualitative leaf traits accounted for less variation, although large variability was observed in all quantitatively measured traits, indicating high genetic variability among local types. The molecular genetic distance derived from the similarity coefficient ranged between 0.12 to 0.96. Twenty genotypes were grouped into two, having a significant degree of polymorphism. This study supports the availability of high variability between landraces, which is an important factor in the effective utilization and *in situ* conservation of genetic resources.

Keywords: *Mangifera indica*, Morphological characters, Molecular characterization, Variability, Yield.

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Introduction

Mango is an important commercial fruit crop of India which is the leading producer in the world (69,110 hectares having yield potential of 5.5 lakh tons) (NMD, 2020). Though monoembryonic types are commercially cultivated to a larger extent, mangoes from tropical regions of Southeast Asia, namely Myanmar, Cambodia, Laos, Malaysia, Singapore, Thailand, Vietnam, coastal parts of Kerala, Karnataka and Goa, are stated to be polyembryonic. These are in high demand as these largely retain their true-to-type nature due to nucellar origin. Polyembryony is common in landraces found in Kerala (Radha and Manjula, 2000). The traditional varieties of Kerala viz., Chappikudiyan, ChenkaVarikka, Chandra Karan, Koonan, Kolambu, Kalkandamanga, Karaka manga, Vattan (=Bappakkai, Komanga), Kotturkonam, Kilichundan Tholikaippan, and Muvandan are prized for special preparations. Although commercial mango cultivation is limited to a few hundred hectares in the village of Muthalamada in Palakkad, local mangoes are ubiquitous on farms in Kerala. On the other hand, the mango growing area has decreased considerably due to the change of social and economic status, including a shift in cultivation pattern, which has led to genetic erosion of the state's traditional mango landraces. Therefore, there is a need to document and preserve the traditional landraces that are on the brink of extinction (Simi *et al.*, 2013).

Information on the extent of genetic variation for fruit characteristics is crucial in identifying valuable traits that the use of morphological/ molecular markers could achieve. Though morphological markers are easily detectable, inherited, reliable and scored visually, the precise estimate of variability could not be determined due to several environmental factors, including different stages of plant growth and environmental conditions. Thus, the use of molecular markers plays an important role in cultivar identification although expensive, are independent of these external factors (Sennhenn *et al.*, 2014). SSR-based DNA fingerprints, besides their use in selection and mapping (Mc Couch *et al.*, 1997) are reliable markers because of their presence extensively in the genomes, high degree of polymorphism and codominant inheritance.

To protect the precious genetic resources of Kerala from various anthropogenic and environmental threats, ICAR-NBPGR Regional Station, Thrissur, has collected 102 accessions (comprising of about 50 landraces) from various regions of Kerala and adjoining areas over the past 24 years and conserved them in the field gene bank (FGB). The present investigation aimed to characterize Kerala-specific mango types using morphological descriptors and molecular markers that will identify elite trees with high

yield, desirable fruit quality and other important economic traits.

Materials and Methods

Experimental Site

Mango germplasm consisted of 20 landraces (Table 1) originally collected from six districts of Kerala along with the allotted indigenous collection (IC) numbers, maintained in the Field Gene Bank of the ICAR-NBPGR, RS, Thrissur, Kerala, India (10.54 N, 76.27 E, 46 m) formed the source of study. Soils of this area are lateritic and a little acidic. Three seedlings for each accession were planted in 1997 at 6 x 6 m spacing and grown under natural conditions without following a package of practices.

Morphological Data

Observations on 12 qualitative and ten quantitative traits were recorded according to the Bioversity international descriptor (IPGRI, 2008) from 2011-2014 and 2019. The qualitative leaf traits and states viz., the shape of leaf blade, the shape of leaf apex, the shape of leaf base, margin type, leaf pubescence, leaf texture, leaf attitude in relation to branch, the color of fully developed leaf, leaf fragrance, leaf blade twisting, and thickness of pulvinus were recorded by

Table 1: Indigenous collection number, landrace name and locations of collection from different regions of Kerala*

S No.	Indigenous collections	Local name	Place of collection	District
1	IC202177-A	Nadashala/ Panchasara manga	Padoor	Palakkad
2	IC202202	Vattan	Cheruthuruthy	Thrissur
3	IC202209	Kolambu	Athani	Thrissur
4	IC212569	Chunayan	Kumbala	Kasaragod
5	IC470613	Muvandan-1	Kumbla	Kasaragod
6	IC470614	Vellarimanga	Nadathara	Thrissur
7	IC470615	Ullitholiyan	Chenganoor	Alappuzha
8	IC470616-A	Mylapoovan (Variant)	Chenganoor	Alappuzha
9	IC470616-B	Kilimukan	Chirayinkeezhu	Thiruvananthapuram
10	IC470621	ValiyaKilichundan	Chirayinkeezhu	Thiruvananthapuram
11	IC470622	Muvandan-2	Vellanikkara	Thrissur
12	IC470627-B	Kilichundan	Vellanikkara	Thrissur
13	IC470656	Nattu maavu-1	Mattathur	Thrissur
14	IC470667	Mallusseri/ Njettikuzhiyan	Karumassery	Ernakulam
15	IC470677	Pulimanga	Karumassery	Ernakulam
16	IC470679	Nattu maavu-2	Mattathur	Thrissur
17	IC470682	Nattu manga	Kuzhikkattuserry	Thrissur
18	IC470692	Mailgoa	Cherpu	Thrissur
19	IC470697	Apple manga	Palissery, Annamanada	Thrissur
20	IC548486	Kalletty	Karumasserry	Ernakulam

*Landraces are conserved in the field gene bank at ICAR-NBPGR, Regional station, Thrissur

comparing the specimen with the figures and descriptor states given in the descriptors. The qualitative fruit traits and states, namely fruit shape, the skin color of ripe fruit, fruit pulp color, the quantity of fiber in pulp, fruit apex, fruit skin surface, fruit stalk insertion, fruit beak type, pulp texture of ripe fruit, pulp aroma and slope of fruit ventral shoulder were recorded by comparing the specimen with the figures and descriptor states given in the descriptors. The quantitatively measured leaf traits were leaf blade width (cm), leaf blade length (cm) and petiole length (cm). The fruit characteristics recorded include the weight of the individual fruit (g), fruit length (cm), fruit width (cm), stone length (cm), stone width (cm), stone weight (g), TSS (°B) and yield/tree. The total yield per tree was recorded in kilograms and rated as high (> 500 kg/tree), medium (100–500 kg) and poor (< 100 kg). Quality of the fruit was measured with sensory evaluation considering the sweetness, flavor, acidity, aroma and astringency of ripe fruits. Based on the sensory evaluation, the taste was rated as excellent (4), good (3), fair (2) and poor (1). About 25 seeds (stone) extracted from mature ripe fruits of each landrace were sown in moist sand beds. Days taken for initiation of germination and number of sprouts per stone were recorded and those accessions with more than one sprout from a stone were considered to be polyembryonic.

DNA Isolation and SSR Marker Analysis

The young, green and just mature leaves were used for DNA isolation using CTAB protocol (Doyle and Doyle, 1990) involving few modifications. DNA quality was estimated on 0.8% agarose gel and the quantity was calculated based on 260/280 spectrophotometer readings. The DNA working solution was prepared to 10 ng/μL and preserved at -20°C. About 50 SSR oligo-nucleotide sequences were taken from the published data by Ravishankar *et al.* (2011) from which 10 were selected based on reproducibility and polymorphism. The 10 polymorphic SSR primers were used for the PCR analysis aimed at generating DNA profiles (Table 4). PCR reactions were performed in 10 μL including 60 ng genomic DNA, buffer (10X with MgCl₂), Forward and Reverse primer at 1μM each, 2.0 mM dNTP mix (10 mM) and 0.2 U Taq DNA polymerase (3 U/μL) (Bangalore Genei). PCR assay was carried out in master cycler (Eppendorf) set at 94°C (4 min) initial denaturation, 94°C (1-minute) denaturation, 55°C (45 seconds) annealing and 72°C (1-minute) extension for 30 cycles with a final extension at 72°C (8 minutes). The PCR products were separated on 3% standard agarose (Sanbiomed) gel in TBE (10 mM Tris–borate, 1 mM EDTA) running buffer solution. Amplified products were run on agarose gels and documented after visual examination under UV light. Explicit and reproducible alleles amplified by each of the 20 SSRs were compared with 100 bp DNA ladder for calculating the molecular weights of the amplified products.

Data Analysis

Quantitative traits were analyzed statistically and summary statistics were performed with XLSTAT 2012. Scoring was done using UV tech and allele sizes were obtained as base pairs in integers. These base pairs were converted to binary code for data analysis and interpretation using PAST software. Since accessions exhibited different ploidy levels, SSR integer data were converted into a binary matrix. If the allele is present then one (1), and if absent, it is zero (0). Allelic differences of 1–5 bp difference were considered identical. To compare the effectiveness of SSR markers in diversity analysis parameters *viz.*, number of polymorphic bands, average number of polymorphic bands per unit assay and number of loci per assay unit were considered. Polymorphic information content (PIC) for each SSR primer was calculated using the formula provided by Smith *et al.* (1997). The scored binary data were used to derive similarity coefficients using PAST software (Hammer *et al.* 2001)

Results and Discussion

Morphological Characterization

Morphological characterization as per Bioversity International descriptors revealed that moderate variations were observed for the qualitative traits. Leaf texture was leathery (coriaceous) for nine accessions and papery (chartaceous) for the remaining 11 accessions (Table 2). The leaf margin was wavy (9), entire (9), or intermediate (2). However, leaf pubescence was not observed in any of the collections. The color of the leaves ranged from pale green (7) to dark green (2) and green (11). Leaf fragrance (smell emanating from crushing) was mild in 10 accessions and strong in 9 accessions, with one accession, IC202177, showing no leaf scent. The uniqueness of the leaf fragrance is an important feature of Kerala Landraces. Out of 20 accessions, 7 showed twisted leaves to varying degrees and 13 accessions showed no twisting. Variation was observed in the shape of the leaf blade, which was highly variable and ranged from elliptic (6), obovate (8), lanceolate (3), and one accession each in obtuse, oblanceolate and ovate. Leaf apex shape varies from acute (10) to acuminate (10). Leaf base shape was mostly acute in most of the accessions. Leaf attitude in relation to the branch was semi-erect in 14 accessions and, horizontal in 4 and semi drooping in 2 accessions. Leaf pulvinus was thick in 10 accessions, thin in 8 accessions and thin to thick in IC470616-A and IC470622. Earlier findings also revealed large variability for nine leaf traits and nineteen fruit characters among Kuttiaoor mango accessions of Kerala (Dinesh *et al.*, 2015). Joshi *et al.* (2013) examined leaf apex variation among nine mango cultivars. Krishna Pillai and Wijeratnam (2016) were of the opinion that young leaf color could be one of the important traits for classification. With respect to leaf quantitative traits

Table 2: Variability in the mango landraces for qualitative leaf traits with descriptor states

Indigenous collections	Leaf blade shape	Leaf apex shape	Leaf base shape	Leaf attitude in relation to branch	Thickness of petiole	Leaf blade twisting	Leaf texture	Leaf margin	Leaf pubescence	Color of fully developed leaf	Leaf fragrance
IC202177A	Elliptic	Acute	Acute	Semi erect	Thin	Absent	Chartaceous	Entire	Absent	Green	Absent
IC202202	Obovate	Acute	Obtuse to acute	Semi erect	Thin	Absent	Chartaceous	Entire to wavy	Absent	Pale green	Mild
IC202209	Elliptic	Acuminate	Acute	Semi erect	Thick	Absent	Coriaceous	Wavy	Absent	Dark green	Strong
IC212569	Lanceolate	Acute	Acute	Semi erect	Thick	Absent	Chartaceous	Entire	Absent	Green	Strong
IC470613	Lanceolate	Acuminate	Acute	Semi erect	Thick	Absent	Chartaceous	Entire	Absent	Pale green	Strong
IC470614	Obtuse	Acuminate	Obtuse	Semi erect	thin	Absent	Chartaceous	Wavy	Absent	Green	Strong
IC470615	Obovate	Acute	Acute	Semi erect	Thin	Present	Coriaceous	Wavy	Absent	Pale green	Strong
IC470616A	Elliptic	Acute	Acute	Semi erect	Thin to thick	Absent	Coriaceous	Entire	Absent	Green	Mild
IC470616B	Obovate	Acute	Obtuse	Horizontal	Thin	Present	Coriaceous	Wavy	Absent	Green	Strong
IC470621	Ovate	Acute	Obtuse	Semi erect	Thin	Present	Coriaceous	Entire	Absent	Green	Mild
IC470622	Elliptic	Acute	Acute	Horizontal	Thin to thick	Absent	Coriaceous	Wavy	Absent	Green	Mild
IC470627B	Elliptic	Acuminate	Acute	Semi drooping	Thin	Absent	Chartaceous	Entire	Absent	pale green	Strong
IC470656	Obovate	Acuminate	Acute	Semi erect	Thick	Absent	Coriaceous	Entire	Absent	Green	Strong
IC470667	Elliptic	Acute	Acute	Semi erect	Thick	Absent	Chartaceous	Entire	Absent	Green	Strong
IC470677	Obovate	Acute	Acute to Obtuse	Semi drooping	Thick	Present	Coriaceous	Entire to wavy	Absent	Green	Mild
IC470679	Obovate	Acuminate	Acute	Semi erect	Thick	Present	Coriaceous	Wavy	Absent	Pale green	Mild
IC470682	Obovate	Acuminate	Acute	Horizontal	Thin	Present	Chartaceous	Wavy	Absent	Dark green	Mild
IC470692	Obovate	Acute	Acute	Horizontal	Thick	Absent	Chartaceous	Entire	Absent	Pale green	Mild
IC470697	Lanceolate	Acuminate	Acute	Semi erect	Thick	Absent	Chartaceous	Wavy	Absent	Pale green	Mild
IC548486	Ob lanceolate	Acute	Acute	Semi erect	Thick	Present	Chartaceous	Wavy	Absent	Green	Mild

Table 3: Variability in the quantitative traits studied in the mango landraces

Indigenous collections	Leaf blade length (cm)	Leaf blade width (cm)	Leaf blade length-width ratio	Petiole length (cm)	Fruit length (cm)	Fruit width (cm)	Fruit weight (g)	Stone length (cm)	Stone width (cm)	Stone weight (g)	Pulp content (g)	Pulp recovery (%)	TSS (°Brix)
IC202177-A	16.04	3.83	4.21	1.97	7.10	5.82	126.28	5.92	3.48	34.80	91.48	72.44	21.40
IC202202	15.73	5.23	3.03	3.27	7.16	6.72	141.33	5.57	3.97	26.04	115.29	81.58	11.33
IC202209	23.13	5.87	3.93	3.17	11.60	5.97	135.50	8.12	4.02	24.42	111.08	81.98	17.33
IC212569	21.97	5.07	4.33	4.73	4.92	4.08	41.10	3.86	2.73	10.26	30.84	75.04	14.64
IC470613	18.13	4.63	3.90	2.27	7.02	5.90	131.75	5.17	5.09	19.33	112.42	85.33	13.50
IC470614	25.37	6.93	3.67	4.13	16.92	8.08	428.64	14.06	3.48	37.50	391.14	91.25	24.00
IC470615	13.37	3.97	3.37	2.77	5.92	4.31	67.75	4.90	2.77	14.66	53.09	78.36	23.40
IC470616-A	16.97	4.53	3.76	2.37	8.30	6.29	132.49	6.25	3.60	20.50	111.99	84.53	19.00
IC470616-B	20.77	5.67	3.68	4.17	7.58	7.40	167.15	6.40	3.56	24.60	142.55	85.28	13.80
IC470621	11.46	5.57	2.04	1.92	11.54	7.92	323.60	8.53	4.27	45.76	277.84	85.86	12.50
IC470622	20.83	6.63	3.16	2.47	7.28	6.54	157.53	5.33	3.70	18.67	138.86	88.15	14.00
IC470627-B	17.53	4.68	3.73	2.71	9.42	6.10	166.87	7.96	3.37	25.33	141.54	84.82	13.50
IC470656	27.47	7.87	3.56	5.27	6.38	4.70	80.38	5.28	3.15	12.50	67.88	84.45	15.33
IC470667	26.57	6.07	4.38	6.27	11.55	6.89	183.42	10.38	4.43	35.18	148.24	80.82	13.00
IC470677	24.64	6.12	4.09	2.13	5.80	4.15	57.08	4.08	2.53	10.67	46.41	81.31	18.67
IC470679	28.07	7.21	4.03	2.96	6.54	5.36	95.47	5.12	3.43	20.31	75.16	78.73	16.00
IC470682	29.14	7.83	3.72	6.03	7.18	4.91	99.16	5.91	3.56	23.87	75.29	75.93	16.00
IC470692	23.21	4.23	5.48	3.78	7.13	6.35	147.25	5.63	3.68	23.81	123.44	83.83	19.00
IC470697	25.57	5.43	4.74	2.63	9.07	7.80	263.88	6.33	3.93	27.66	236.22	89.52	16.90
IC548486	18.77	4.33	4.41	2.27	12.63	9.68	658.37	8.66	4.97	78.12	580.25	88.13	16.20
Mean	21.24	5.59	3.86	3.36	8.55	6.25	180.25	6.67	3.69	26.70	153.55	82.87	16.48
Minimum	11.46	3.83	2.04	1.92	4.92	4.08	41.10	3.86	2.53	10.26	30.84	72.44	11.33
Maximum	29.14	7.87	5.48	6.27	16.92	9.68	658.37	14.06	5.09	78.12	580.25	91.25	24.00
Standard deviation	5.10	1.23	0.70	1.33	2.94	1.46	145.42	2.40	0.67	15.15	131.59	4.95	3.54
Standard Error	1.14	0.28	0.16	0.30	0.66	0.33	32.52	0.54	0.15	3.39	29.42	1.11	0.79
CV (%)	24.03	22.03	18.16	39.60	34.38	23.40	80.68	35.97	18.07	56.75	85.70	5.97	21.48

Table 4: Variability in Qualitative parameters of fruits

Indigenous collections	Fruit shape	Skin color of ripe fruit	Fruit pulp color	Quantity of fibre in pulp	Fruit apex	Fruit skin surface	Fruit stalk insertion	Fruit beak type	Pulp texture of ripe fruit	Pulp aroma	Slope of fruit ventral shoulder
IC202202	Obovoid	Green	Orange	Intermediate	Round	Smooth	Oblique	Perceptible	Soft	Strong	Sloping
IC212569	Ovoid	Greenish yellow	Light yellow	High	Acute	Smooth	Oblique	Pointed	Soft	Mild	Sloping
IC470613	Obovoid	Yellow	Yellow	Intermediate	Acute	Smooth	Oblique	Perceptible	Intermediate	Intermediate	Sloping
IC470614	Oblong	Yellow	Orange	Absent	Acute	Smooth	Oblique	Mammiform	Soft	Intermediate	Sloping
IC470615	Oblong	Greenish yellow	Yellow	High	Acute	Smooth	Oblique	Pointed	Soft	Strong	Ending in a long curve
IC470616-A	Obovoid	Yellow	Dark orange	Intermediate	Round	Smooth	Oblique	Pointed	Firm	Mild	Sloping
IC470616-B	Ovoid	Yellow	Light orange	High	Acute	Smooth	Oblique	Pointed	Firm	Mild	Ending in a long curve
IC470621	Oblong	Greenish yellow	Orange	High	Acute	Smooth	Oblique	Perceptible	Soft	Strong	Sloping
IC470622	Obovoid	Greenish yellow	Yellow	Low	Acute	Smooth	Oblique	Perceptible	Firm	Mild	Sloping
IC470656	Oblong	Greenish yellow	Orange	Intermediate	Acute	Smooth	Oblique	Perceptible	Firm	Mild	Sloping
IC470667	Obovoid	Yellow	Orange	Low	Acute	Rough	Vertical	Perceptible	Soft	Mild	Ending in a long curve
IC470677	Oblong	Greenish yellow	Orange	High	Acute	Smooth	Oblique	Perceptible	Soft	Intermediate	Sloping
IC470679	Obovoid	Green	Light orange	Intermediate	Obtuse	Smooth	Oblique	Perceptible	Soft	Intermediate	Ending in a long curve
IC470682	Oblong	Greenish yellow	Light orange	High	Obtuse	Smooth	Oblique	Perceptible	Firm	Mild	Sloping
IC470692	Obovoid	Greenish yellow	Yellow	Intermediate	Round	Smooth	Oblique	Mammiform	Soft	Intermediate	Ending in a intermediate curve
IC470697	Obovoid	Yellow with red blush	Golden yellow	Low	Acute	Smooth	Oblique	Pointed	Firm	Intermediate	Ending in a intermediate curve
IC202177-A	Oblong	Greenish yellow	Yellow	Intermediate	Obtuse	Smooth	Oblique	Prominent	Soft	Intermediate	Sloping abruptly
IC202209	Oblong	Greenish yellow	Golden yellow	Intermediate	Obtuse	Smooth	Oblique	Pointed	Soft	Intermediate	Ending in a long curve
IC470627-B	Oblong	Greenish yellow	Yellow	Intermediate	Obtuse	Smooth	Oblique	Perceptible	Intermediate	Intermediate	Ending in a long curve
IC548486	Oblong	Greenish yellow	Yellow	Intermediate	Acute	Smooth	Oblique	Perceptible	Intermediate	Intermediate	Rising and then rounded

(Table 3) wide variations were observed viz., length (11.46 cm in IC470621 to 29.14 cm in IC470682), width (3.83 cm in IC202177A to 7.87 cm in IC470656) and petiole length (1.92 cm in IC470621 to 6.27 cm in IC470667). The ratio of blade length and blade width was maximum (5.48) in IC470692 and minimum (2.04) in IC470621.

The fruit characters viz., length (4.92 cm in IC212569 to 16.92 cm in IC470614), diameter (4.08 cm in IC212569 to 9.68 cm in IC548486) and weight (41.10 g in IC212569 to 658.37 g in IC548486) also recorded wide variability among the accessions. The pulp recovery and TSS are the important parameters that decide consumer preference. High variation was recorded for pulp recovery percent (72.44 % in IC202177A to 91.25% in IC470614) and TSS (11.33°B in IC202202 to 24°B in IC470614) among the accessions. The accession IC470614 registered high pulp recovery (91.25 %) coupled with high TSS (24°B). Stone parameters are important in selecting the genotypes with good pulp content. Consumers prefer fruits with the lowest values for stone parameters. Stone weight (10.26 g in IC212569 to 78.12 g in IC548486) and length (3.86 in IC212569 to 14.06 cm in IC470614) registered lot of variations. This huge variation in local mango cultivars (Fig. 1) is likely the result of a long period of cultivation, thus providing ample scope for active hybridization and selection mechanisms. According to Rajan *et al.* (2013) north Indian accessions were found to be superior for yield as well as quality attributes compared to accessions of south and western regions. Morphological traits such as leaf length, bearing habit, shape of inflorescence, flowering pattern, the shape of fruit, resistance to diseases, and color of fruit skin serve as morphological markers that can be easily differentiated visually (Khan *et al.*, 2015).

Morphological characterization revealed variations for the fruit qualitative traits (Table 4, Fig. 1). Fruit shape ranged from ovoid (2), obovoid (8) and oblong (10) (Table 4). Fruit apex was acute (12), obtuse (5) or round (3). Skin color was greenish yellow (12) in the majority of the accessions (12), yellow in 6 accessions and green in 2 accessions. Only one accession was yellow with red blush (IC470697). The fruit

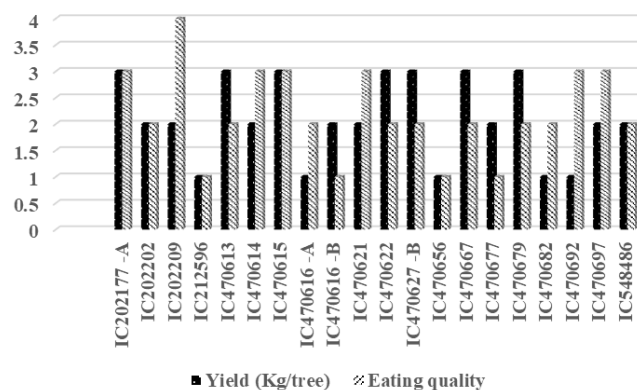


Figure 1: Variations in fruit shape, size and pulp color of mango landraces used in the study

skin surface was smooth in all accessions except IC470667, where it was rough. Fruit stalk insertion was oblique in all accessions except IC470667 where it was vertical. Fruit beak type was perceptible in most of the accessions (11), pointed in 6 accessions, prominent in IC202177-A and mammiform in IC470692 and IC470614. The slope of the fruit ventral shoulder was slopping abruptly in 10 accessions, ending in a long curve in 8 accessions and rising and then rounding in 2 accessions. A wide spectrum of colors was observed in fruit pulp color. Fruit pulp color was orange (7), yellow (6), light orange (3), golden yellow (2), light yellow (1) and dark orange (1). The quantity of fiber in the pulp was absent in IC470614, low in 3 accessions, intermediate in 10 accessions and high in 6 accessions. The pulp texture of ripe fruit was soft (11), intermediate (3) or firm (6). Pulp aroma varied from mild (7), intermediate (10), to strong (3). Morphological variability may be due to probable genotypic variation among the different cultivars (Mitra, 2016)

Eating quality is important which determines the market demand. The accessions IC 202177 A, IC202209, IC470614, IC470615, and IC548486 had good eating quality with good taste (Fig. 2). As yield is a quantitatively inherited trait and its expression is based on cultivar, climatic conditions, crop age, pest and disease incidence and others, the study resulted in the identification of accessions IC202177-A, IC470613, IC470615, IC470622, IC470627B, IC470667, IC470679 with high yield (>500 kg/plant) (Fig. 2). Yield variations among different mango cultivars have been reported earlier (Archit Singh and Sanjay Pathak, 2018).

Since mango exhibits nucellar embryony, the seedlings tend to be true to type. These seedlings serve as good rootstocks. The days taken to germination ranged from 6 (IC470613) to 31 days (IC470679 and IC470677) (Fig. 3). Polyembryony was confirmed if the stone produced multiple seedlings/stone. Based on the number of seedlings per stone, seven accessions were found to be polyembryonic. According to Dinesh *et al.* (2016), polyembryony is a



Yield: High- >500 kg/plant, Medium-100–500 kg, Poor <100kg/plant
Eating quality: Excellent Good, medium and poor

Figure 2: Variability in yield and eating quality of mango landraces

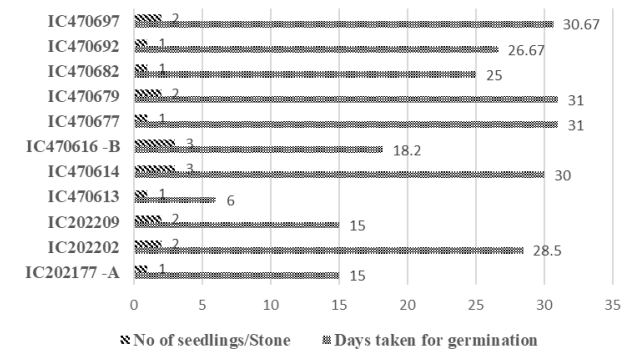


Figure 3: Days taken for germination and number of seedlings/ stones in mango landraces

common trait in mango cultivars of Southeast Asian origin, particularly Kuttiattoor varieties of Kerala, which are regular, heavy-bearing compared to monoembryonic varieties. A similar opinion was expressed by Radha and Manjula (2000) that Kerala, being hot and humid, has both mono and polyembryonic types and collections showed great variation with respect to vegetative and floral characters and the number of seedlings produced per stone (1-6). Unlike monoembryonic types, variability in the polyembryonic cultivars is much less and there is a need to widen the genetic base in polyembryonic varieties. There is a need to create variability by producing new recombinants, which, while retaining the polyembryonic nature, should have other desirable characteristics, either for use as rootstocks or as commercial types. (Sane *et al.*, 2015; Dinesh *et al.*, 2016).

Molecular Profiling using SSR Markers

Out of fifty SSR primers used in the study, ten primers were consistent in producing clear and reproducible bands (Table 6), which were utilized for generating DNA profiles of 20 accessions (Fig. 4). The allele size ranged from 91 bp (MillHR 18) to 347 bp (MillHR 24) with a total of 83 polymorphic bands. The alleles per SSR locus were 4 (MillHR 17) to 11 (MillHR 24) (Table 6), with an average of 8.3. All the primers have shown 100% polymorphism. Polymorphic information content (PIC) values varied from 0.37 (MillHR 19) to 0.98 (MillHR17), with an average value of 0.66. Most of the SSR primers used in the study detected multiple loci, which is in conformity with results reported by and Kumar *et al.* (2013) that were reported to be due to the allopolyploid nature (Mukherjee, 1950). The polymorphism detected varies based on factors like the number of varieties used and the presence of variability among them. By using just three SSR markers, Viruel *et al.* (2005) identified 28 mango genotypes. Dinesh *et al.* (2016) developed DNA barcodes for 10 Kuttiattoor mango accessions for registration with the PPV&FRA.

Cluster Analysis

In our study, the coefficient of similarity based on Jaccard's ranged between 0.12 to 0.96 and grouped 20 individual accessions into two major clusters (Fig. 5). IC470697 and

Table 5: Mango landraces with unique traits

Unique traits	Indigenous collections
Good fruit quality suitable for fresh fruits	IC470621, IC470627, IC202177A
Late bearing	IC202177A, IC202209
Regular bearing	IC470613, IC470622
Polyembryony	IC470621, IC470692 IC470622, IC470613, IC470677
Firm flesh	IC470616A
Thin peel, juicy type with good quality fruits	IC470615
Tender mango pickle	IC470656
Culinary preparations (mainly fish curry)	IC470677
Fruits with attractive red skin color	IC470697

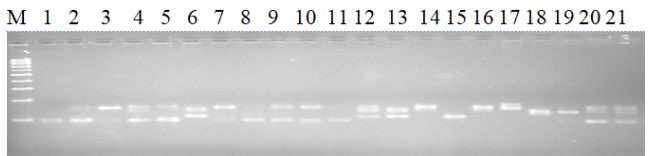


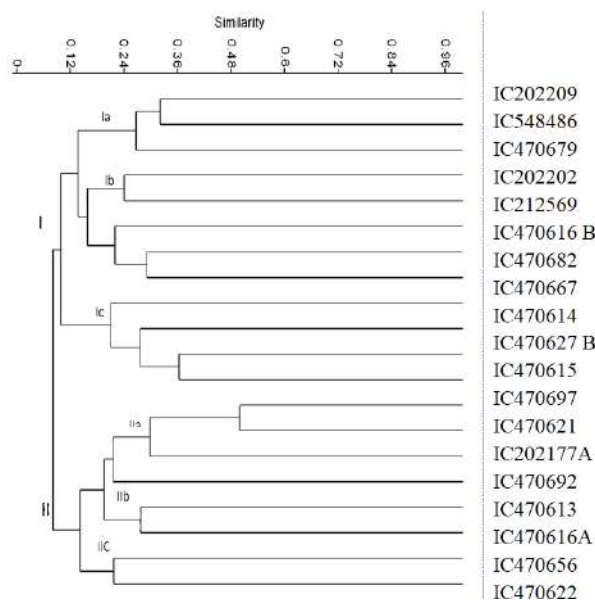
Figure 4: DNA profile of 20 landraces with MillHR18. M- 100 bp ladder, 1- IC470622, 2- IC IC470616A, 3- IC470677, 4- IC470656, 5- IC470621, 6- IC 870393*, 7- IC470613, 8- IC470692, 9- IC202177A, 10- IC202209, 11- IC470614, 12- IC548486, 13- IC202202, 14- IC470679, 15- IC212569, 16- IC470616 B, 17- IC670682, 18- IC470667, 19- IC470627, 20- IC470615, 21- IC470697

*- Not included as phenotypic data is not available

IC470621 exhibit maximum genetic similarity (0.48). According to Simi *et al.* (2013), similarity coefficient values varied between 0.217 and 0.83, estimated by RAPD in 30 mango varieties. Each major cluster was split into sub-clusters primarily based on a high similarity coefficient. The first major cluster contained 12 accessions and was divided into three sub-clusters. The first sub-cluster consisted of 3 genotypes, IC202209, IC548486 and IC470679 (Kolambu, Kalletty and Nattumaavu-2, respectively). IC202209 (Kolambu) was late bearing, and produces good quality medium size fruits. IC548486 (Kalletty) fruits were very big and were originally collected from saline areas. Nattumavu (IC470679) is used for pickle-making and curry purposes. The IC470679 (Nattumaavu/Nattumanga) were highly heterogeneous, where tree-to-tree variation for fruit quality traits was very high. In this group, the majority of them are pickling types, bunch-bearing, containing high latex, and soft, juicy types with high variation for sweetness and flavor. The second sub-cluster consists of 5 accessions viz., IC202202, IC212569, IC470616-B, IC 470682 and IC470677. Accessions IC202202 and IC212569 (Vattan and Chunayan, respectively) produced round fruits and were very prolific

Table 6: Properties of primers, primer sequences and amplification products used in the study

Primer ID	Sequence (5'-3')	Repeat motif	Expected range (bp)	Allele range (bp)	No. of alleles per locus	No. of polymorphic alleles	Polymorphic information content (PIC)
MillHR 12	F: GCCCCATCAATACGATTGTC R: ATTTCCACCATTGTCGTTG	(GA) ₁₁	178	175-219	6	6	0.67
MillHR17	F: GCTTGCTTCCAAGTACGAGACC R: GCAAAATGCTCGGAGAAGAC	(GT) ₁₃ GAGT(GA) ₁₀	244	166-193	4	4	0.98
MillHR18	F: TCTGACGTCACCTCCTTTCA R: ATACTCGTCCTCGTCCTGT	(GT) ₁₂	168	91-123	9	9	0.48
MillHR 19	F: TGATATTTTCAGGGCCCAAG R: AAATGGCACAAGTGGGAAAG	(AC) ₁₁	187	96-289	10	10	0.37
MillHR 23	F: TCTGACCAACAAAGAACCA R: TCCTCCTCGTCCTCATCATC	(GA) ₁₇ GG (GA) ₆	144	142-312	10	10	0.71
MillHR 24	F: GCTCAACGAACCAACTGAT R: TCCAGCATTCATGAAGAAGTT	(CA) ₉ TACC(CATA) ₆	246	209-347	11	11	0.71
MillHR 26	F: GCGAAAGAGGAGAGTGCAAG R: TCTATAAGTGCCCCCTCACG	(GA) ₁₄ GGA (GAA) ₂	166	106-180	10	10	0.57
MillHR 31	F: TTCTGTTAGTGCGGTGTTG R: CACCTCCTCCTCCTCTTT	(GAC) ₆	229	191-295	7	7	0.88
MillHR 34	F: CTGAGTTTGGAAGGGAGAG R: TTGATCCTTCACCACCATCA	(GGT) ₉ (GAT) ₅	237	123-172	8	8	0.65
MillHR 36	F: TCTATAAGTGCCCCCTCACG R: ACTGCCACCGTGAAAGTAG	(TC) ₁₇	246	212-273	8	8	0.57
Average number of alleles					8.3	83	

**Figure 5:** Dendrogram depicting genetic relationships 20 mango landraces analyzed with 10 SSR loci

bearers. Kilimukan (IC470616-B) produces fruits with more fiber content without fruit fly incidence. Here, the control measures were not adopted for controlling the fruit fly population and fruit fly incidence was not observed under natural epiphytotic conditions. IC470682 (Nattu manga)

exhibited a bunch-type bearing habit; IC470677 (Puli manga) was acidic and used in culinary preparations. The common factor in this sub-cluster was that all of them were used for culinary purposes.

The third sub-cluster consists of four accessions, namely, IC470614, IC470627, IC470615 and IC470697 (Vellari manga, Kilichundan, Ullitholiyan, and Apple manga, respectively). IC470614 (Vellari manga) fruits were long and cucumber type; IC470627 (Kilichundan) fruit quality was good and used for fresh fruit purposes. IC470615 (Ullitholiyan) fruits had a thin peel, juicy with good quality fruits. IC470697 (Apple manga) was a variant of Muvandan with good bearing and the fruit peel was red in color. Here all of them produced good quality fruits with good eating quality.

The second major cluster II had eight genotypes and was subdivided into three sub-clusters. The first sub-cluster had IC470621, IC202177A, IC470692 and IC470613 (Valiya Kilichundan, Nada Shala/Panchasaramanga, Mailgoa and Muvandan, respectively). The fruit quality of IC470621 (Valiya Kilichundan) was good and used for table purposes; IC202177A (Nada Shala/Panchasaramanga) fruits were late bearing, sweet, firm flesh type and used for table purposes. IC470692 (Mailgoa) was originally collected from Goa; IC470613 (Muvandan) is a regular bearing type. In this sub-cluster, all three are polyembryonic types and also suitable for fresh fruit with good eating quality. The

second sub-cluster included IC470616A and IC470677 (Mylapooan-variant, Puli manga, respectively). IC470616A (Mylapooan) was firm flesh type, and IC470677 (Puli manga) was polyembryonic. The third sub-cluster had IC470656 and IC470622 (Nattu manga, Muvandan, respectively). IC470656 (Nattu manga), appemidi type, was used for tender mango pickle; IC470622 (Muvandan) was a typical regular bearing variety. Two Muvandan types, viz., IC470613 and IC470622 collected from two different places, have broadly grouped in the same cluster but in different sub-clusters.

In this study, we observed that there was no clear separation of landraces belonging to different regions of Kerala. Ward *et al.* (2005) also reported that there was no partition of genotypes based on their geographical vicinity because the populations studied originally were seedlings and not vegetative propagated ones. He attributed it to high variability, which was the result of the allogamous breeding. Simi *et al.* (2013) also got a similar result that the grouping of varieties did not follow any regular pattern with respect to geographical location in 30 local varieties of mango collected from the four districts of southern Kerala. The genotypes of the same cluster have a high similarity coefficient based on genetic distance, but morphologically, they are different with respect to fruit traits.

According to Karihaloo *et al.* (2003), in mangoes, due to heterozygous nature and outcrossing nature, high diversity is prevalent in regions in India among naturally produced seedlings. The commercial cultivars were selected from the existing seedling population based on consumer preferences. Kerala does not consider mango to be a commercial crop, although mango trees are a necessary part of every homestead there. Kerala's production of mangoes is distinguished for its earliness. The first mango fruits of the season arrive from Kerala in Indian markets. Due to early bearing habit (the harvesting in March to April), they fetch the highest price due to the great demand for fruits in the major marketplaces in other parts of the country (Radha and Nair, 2000)

In the current study, we looked at the quantitative and qualitative characteristics of mango and ascertained that there was a noticeable difference between the 20 mango accessions that were taken from various eco-geographical regions of Kerala. With regard to leaf and fruit characteristics, the majority of mango landraces are unique morphologically. Furthermore, there is a lot of genetic variation in mangoes, both within and across landraces and well-known cultivars, providing room for development. All of the native landraces that were gathered from six districts in Kerala were found to be morphologically unique from the commercial cultivars, indicating their potential for use either directly or through breeding programs.

Also noteworthy is the presence of significant cultivar sources for enhancement based on regular bearing habits, pickle and curry type mangoes, besides juicy and good

quality fruits for fresh consumption in this germplasm collection (Table 5). Thus, the morphological variability data, along with molecular data and genotype grouping, should aid breeders in choosing genotypes for crosses to create new cultivars appropriate to the current tropical high, humid climate. The polyembryonic accessions with specific traits would help in enhancing the genetic base of polyembryonic types and those with desirable traits could be utilized as parents in crop improvement programs.

Authors' Contribution

Conceptualization of research (AS&MRD); Designing of the experiments (AS&MRD); Contribution of experimental materials (JJ & KP); Execution of field/lab experiments and data collection (AS&CV); Analysis of data and interpretation (AS, JJ & KP); Preparation of the manuscript (AS&CV).

Declaration

The authors have declared that they do not have any conflict of interest. The submissions are original and unpublished and are not under consideration for publication elsewhere. Authors also declare that the manuscript is based on their own original work and the manuscript is checked for plagiarism.

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RESEARCH ARTICLE

Morphological and Molecular Diversity Analysis in Forage Sorghum (*Sorghum bicolor* (L.) Moench)

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Abstract

The present investigation was carried out to find the diverse genotypes by using D² statistics and simple sequence repeat (SSR) markers. The genetic divergence assessed by Mahalanobis D²-statistics grouped 22 genotypes into eight clusters. The maximum inter-cluster distance was observed between clusters V and VIII (645.77). Thus, genotypes included in these clusters may be utilized under an inter-varietal hybridization program for forage yield improvement. The molecular diversity analysis shows that the number of alleles for SSR primers ranged from two to four, with an average number of alleles per locus was 2.79. The similarity coefficients among all the 22 genotypes ranged from 0.185 to 0.875. The clustering pattern of the dendrogram generated by pooled molecular data of seventeen SSR loci generated two clusters, viz., A and B, at a similarity coefficient of 0.33. The combined results for morphological and molecular diversity estimates showed that genotype "SSG-59-3" was found to be distinct from other genotypes.

Keywords: Cluster, Diversity, D²-statistics, Sorghum, SSR.

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Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) ranks first among the cereal fodder crops because of its growing ability in low fertile soil, faster growth habit, higher fodder yield, palatability and nutritious quality. It gives almost uniform green fodder yield throughout the year. It is mostly grown in the semi-arid tropics of Asia and Africa, where water availability is limited due to its drought-tolerant nature (Kumar *et al.*, 2011).

To create forage sorghum as an enterprising and remunerative crop, there's a necessity to develop varieties or hybrids having faster growth, good vigor and high forage yield, as well as high protein content and low HCN content at the flowering stage. The collection, maintenance and evaluation of germplasm are the most important and primary steps of any crop improvement program. The best way to perceive the potential of the available germplasm is by analyzing its genetic diversity. Consideration of geographical diversity as a reasonable index of genetic diversity might result in inaccurate conclusions. Mahalanobis' D² statistics of multivariate analysis is recognized as a powerful tool in quantifying the degree of genetic divergence among the breeding material.

The information obtained from phenotypic characterization does not reflect the real genetic variation because of genotype × environment interaction. The use of molecular markers has numerous advantages in genetic diversity analysis as their expression is not affected by the environment (Gepts, 1993).

Table 1: Experimental material used in the study

S. No.	Genotypes	Origin	Types
1	SRF-316	Surat, Gujarat	Single-cut forage type
2	MALWAN	Local landraces Gujarat	Dual purpose; Forage & Grain type
3	GFS-5	Surat, Gujarat	Single cut forage type
4	CSV-21	Surat, Gujarat	Single cut forage type
5	SRF-286	Surat, Gujarat	Single cut forage type
6	DS-1053	Deesa, Gujarat	Dual purpose; Forage & Grain type
7	DS-1168	Deesa, Gujarat	Dual purpose; Forage & Grain type
8	DS-1111	Deesa, Gujarat	Dual purpose; Forage & Grain type
9	DS-1146	Deesa, Gujarat	Dual purpose; Forage & Grain type
10	DS-1187	Deesa, Gujarat	Dual purpose; Forage & Grain type
11	UTML-529-10(8)	IIMR, Hyderabad	Forage type
12	PDJP-1612/621513	IIMR, Hyderabad	Forage type
13	PDJP-1614/621515	IIMR, Hyderabad	Forage type
14	DSF-168	Deesa, Gujarat	Single cut forage type
15	DSF-117	Deesa, Gujarat	Single cut forage type
16	GAFS-12	Anand, Gujarat	Single cut forage type
17	GFS-4	Surat, Gujarat	Single cut forage type
18	DSF-172	Deesa, Gujarat	Single cut forage type
19	SH1813	Hissar, Haryana	Multicult Forage type
20	S-652	Hissar, Haryana	Multicult Forage type
21	SSG-59-3	Hissar, Haryana	Multicult Forage type
22	SH-1488	Hissar, Haryana	Multicult Forage type

Several DNA-based markers have been successfully used, in which SSR is found to be the marker of choice for diversity analysis in sorghum because of their ability to produce informative multi-allelic loci and greater genotypic differentiation. SSRs are highly polymorphic (Anas and Yoshida, 2004) and possess wider genome coverage than restriction fragment length polymorphism (RFLP), random-amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) markers. Their co-dominant nature, simple and quick operation, good stability and simplicity make SSR markers a versatile choice for genetic differentiation studies. The present investigation aimed to characterize forage sorghum genotypes concerning their morphological traits and molecular markers.

Materials and Methods

Morphological diversity analysis

The experimental material comprising 22 forage sorghum genotypes was given in Table 1, along with their place of origin and single-cut/multi-cut type. These genotypes were evaluated in randomized block design with three replications at Sorghum Research Station, Sardarkrushinagar Dantiwada Agricultural University, Deesa during *Kharif* – 2020.

Each genotype was sown in one row of three-meter length with an optimum inter-row spacing of 30 cm. The observations were recorded both as visual assessment for days to 50% flowering and measurement on five randomly selected plants for plant height (cm), number of leaves per plant (no), stem girth (mm), length of leaf blade (cm), width of leaf blade (cm), leaf: stem ratio, dry fodder yield per plant (g), brix content (%), hydrocyanic acid content (ppm), crude protein content (%) and green fodder yield per plant (g). The replication-wise mean values were used for statistical analysis. The morphological divergence analysis was carried out by D^2 statistics as proposed by Mahalanobis (1936) and described by Rao (1952). Grouping of genotypes into different clusters was done by *tocher's* method. The intra-cluster and inter-cluster distances were calculated by the formula given by Singh and Chaudhary (1977) using TNAUSTAT software.

Molecular diversity analysis

The molecular analysis was carried out at the Department of Genetics and Plant Breeding, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar. Fresh, healthy leaves were collected from all the genotypes at the time of flowering for DNA extraction. High molecular weight genomic DNA was isolated from 22 sorghum genotypes by

Table 2: Analysis of variance (ANOVA) showing mean sum of squares for twelve wtraits in forage sorghum

S. No	Characters	Mean sum of squares		
		Treatment	Replication	Error
	Degree of freedom (df)	21	2	42
1.	Days to 50% flowering	68.02**	11.56	10.53
2.	Plant height (cm)	1356.29**	856.49	323.00
3.	Number of leaves per plant (no)	1.79**	2.00	0.607
4.	Stem girth (mm)	9.69**	1.35	1.35
5.	Leaf length of blade (cm)	166.46**	22.03	46.06
6.	Leaf width of blade (cm)	2.76**	0.02	0.49
7.	Leaf: Stem ratio	0.01**	0.00007	0.001
8.	Dry fodder yield per plant (g)	1421.81**	14.98	66.91
9.	Brix (%)	4.51**	0.12	0.51
10.	Hydrocyanic acid content (ppm)	155.58**	2.97	1.95
11.	Crude protein (%)	3.99**	0.06	0.21
12.	Green fodder yield per plant (g)	9433.12**	109.50	393.47

*Significance at 5% probability level **Significance at 1% probability level

following the cetyl trimethyl ammonium bromide (CTAB) extraction method as described by Doyle and Doyle (1987) with some modifications. The list of primers selected for the study is shown in Supplementary Table 2. About 17 SSR primers after their optimization and screening were selected and used.

Scoring and statistical analysis of data

The amplified products of each primer were scored separately based on the presence or absence of a band across 22 sorghum genotypes, *i.e.*, the use of binary codes 1 and 0 for the presence or absence of a band, respectively. The data was entered into a binary matrix and subsequently analyzed using the NTSYSpc version 2.20 software package (Rohlf, 2000).

The similarity coefficient among the 22 sorghum genotypes was calculated by using Jaccard's similarity coefficient by SimQual function. The dendrogram, including all the 22 genotypes, was constructed by using the un-weighted pair group method with arithmetic mean (UPGMA) method by SAHN clustering function of NTSYSpc (version 2.20). The diversity parameters, such as the number of alleles per locus, heterozygosity and polymorphism information content, were calculated using power marker software. The software program Alpha Ease FC version 4.0.0 (Alpha Innotech Corporation, USA) was used for determining the molecular weight (MW) of bands separated on the gel. The polymorphism information content (PIC) values measure the informativeness of a given DNA marker for the 22 sorghum genotypes. Heterozygosity (H) is a parameter indicating the average frequency of a heterozygous individual's occurrence.

Results and Discussion

Morphological diversity analysis

The analysis of variance for all the twelve traits is shown in Table 2. The mean sum of squares due to treatments showed highly significant differences among the genotypes for all the traits, which indicates the wide spectrum of variation among them. The mean performance of 22 genotypes studied for 12 traits in three replications is presented in Supplementary Table 1. A wide range of variation among the genotypes was found for all the traits *viz.*, days to 50% flowering (57.33–78.67), plant height (157.33–243.00 cm), number of leaves per plant (6.6–10.2), stem girth (5.37–14.00 mm), leaf length of blade (45.73–81.64 cm), leaf width of blade (4.35–8.75 cm), leaf: stem ratio (0.18–0.46), dry fodder yield per plant (42.51–135.49 g), brix content (7.56–12.23%), hydrocyanic acid content (11.82–37.06 ppm), crude protein (6.65– 11.03%) and green fodder yield per plant (92.39–351.27 g). The wide range of variations in forage yield and components were also reported earlier by Jadhav *et al.* (2011) and Rana *et al.* (2016).

For improving complex traits like forage yield, the selection of parents having wide divergence for various characters is of prime importance which can be assessed by D² statistics developed by Mahalanobis (1936). The greater distance between two clusters indicates greater divergence and vice versa. The genotypes falling in the same cluster indicate that they are more closely related than those belonging to other clusters. In view of this, Mahalanobis D² statistics was used to assess the genetic divergence in different forage sorghum genotypes to identify superior

Table 3: Distribution of 22 genotypes of forage sorghum in different clusters on the basis of D^2 statistics

Cluster	Number of genotypes	Name of genotype
I	5	DS-1187, UTML-529-10(8), PDJP-1612/621513, GAFS-12 and GFS-4
II	8	MALWAN, CSV-21, SRF-286, DS-1053, DS-1111, DSF-168, S-652 and SH-1488
III	3	SRF-316, GFS-5 and DSF-117
IV	2	DS-1168 and DS-1146
V	1	PDJP-1614/621515
VI	1	SSG-59-3
VII	1	SH-1813
VIII	1	DSF-172

Table 4: Average Intra-cluster (Diagonal) and Inter-cluster (off-diagonal) distance of 8 clusters of forage sorghum

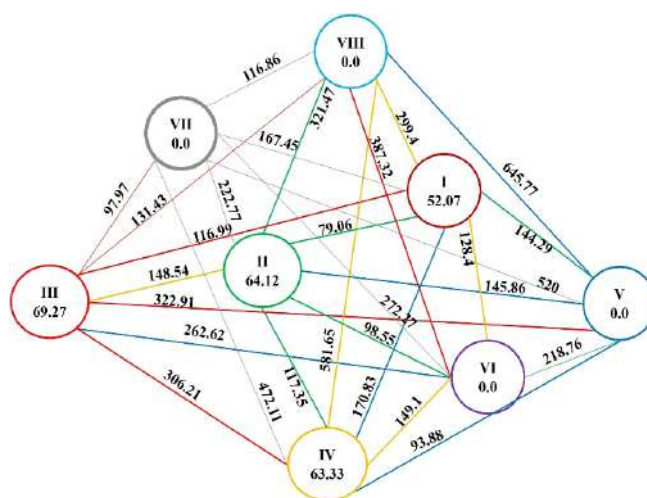
Cluster	I	II	III	IV	V	VI	VII	VIII
I	52.07	79.06	116.99	170.83	144.29	128.40	167.45	299.40
II		64.12	148.54	117.35	145.86	98.55	222.77	321.47
III			69.27	306.21	322.91	262.62	97.97	131.43
IV				63.33	93.88	149.10	472.11	581.65
V					0	218.76	520.00	645.77
VI						0	272.27	387.32
VII							0	116.86
VIII								0

genotypes which can be utilized for future breeding programmes.

In the present study, the clustering was done based on the relative magnitude of D^2 values by the tocher's method. With the help of D^2 values, eight clusters were formed from 22 genotypes with 12 traits of forage sorghum. The composition of clusters is given in (Table 3).

The results indicated that a maximum number of eight genotypes appeared in cluster II followed by five genotypes in cluster I, three genotypes in cluster III, two genotypes in cluster IV and each genotype in clusters V, VI, VII and VIII. This indicated that there is a presence of diversity among the 22 genotypes studied. Each of the following genotypes PDJP-1614/621515, SSG-59-3, SH-1813 and DSF-172 occupied their position individually in separate clusters, which shows there is a presence of distinguished traits from the rest of the genotypes studied. The average intra and inter-cluster distance between all possible pairs of eight clusters are presented in Table 4 and Fig. 1.

A study on data revealed that the inter-cluster distance D^2 values ranged from 79.06 to 645.77. The maximum inter-cluster distance was observed between clusters V and VIII (645.77) followed by clusters IV and VIII (581.65), cluster V and VII (520.00), cluster IV and VII (472.11), cluster VI and VIII (387.32), cluster III and V (322.91), cluster II and VIII (321.47) and cluster III and IV (306.21). The least inter-cluster distance was observed between clusters I and II (79.06) followed by clusters IV and V (93.88), clusters III and VII (97.97), clusters

**Fig. 1:** Cluster diagram showing interrelationship among eight clusters using D^2 analysis

II and VI (98.55), clusters VII and VIII (116.86), cluster I and III (116.99), cluster II and IV (117.35), cluster I and VI (128.40), cluster III and VIII (131.43), cluster I and V (144.29), cluster II and V (145.86), cluster II and III (148.54), cluster IV and VI (149.10), cluster I and VII (167.45), cluster I and IV (170.83), cluster V and VI (218.76), cluster II and VII (222.77), cluster III and VI (262.62), cluster VI and VII (272.27) and cluster I and VIII (299.40).

The intra-cluster distance was ranged from 0.0 to 69.27. The maximum intra-cluster distance of 69.27, followed by

Table 5: Percent contribution of different characters towards total genetic divergence of forage sorghum

S. No	Character	Number of times character ranked first	Percent contribution
1	Days to 50% flowering	4	1.73
2	Plant height (cm)	2	0.87
3	Number of leaves per plant (no)	0	0
4	Stem girth (mm)	3	1.30
5	Length of leaf blade (cm)	1	0.43
6	Width of leaf blade (cm)	0	0
7	Leaf:Stem ratio	8	3.46
8	Dry fodder yield per plant (g)	16	6.93
9	Brix (%)	9	3.90
10	Hydrocyanic acid content (ppm)	133	57.58
11	Crude protein (%)	30	12.99
12	Green fodder yield per plant (g)	25	10.82

64.12, 63.3 and 52.07, was observed in clusters III, II, IV and I, respectively. In contrast, the least intra-cluster distance (*i.e.*, 0.0) was observed in clusters V, VI, VII and VIII.

In the present investigation, the inter-cluster distance was higher than the intra-cluster distance which indicated the wide genetic diversity among the different accessions of different clusters than those of the same cluster. Similar results were reported by Elangovan *et al.* (2014), Meena *et al.* (2016) and Ahalawat *et al.* (2018). The maximum inter-cluster distance observed between clusters V and VIII indicates that the genotypes included in these clusters are more diverse and may generate high heterotic responses in a breeding program. Similar results were reported by Mohanraj *et al.* (2006).

The contribution of different traits towards divergence is shown in Table 5. HCN content (57.58%) was the main contributor to the total genetic divergence, followed by crude protein content (12.99%) and green fodder yield per plant (10.82%). The traits *viz.*, the number of leaves per plant and width of leaf blade showed no contribution toward total genetic divergence. HCN content contributed more towards divergence and a similar result was reported by Damor *et al.* (2017). Meena *et al.* (2016) also showed that the number of leaves per plant had no contribution towards divergence.

Molecular Diversity Analysis

SSR marker analysis of 17 SSR primer pairs *viz.*, msbCIR238, msbCIR240, msbCIR276, msbCIR300, msbCIR329, Xcup14, Xcup53, Xtxp12, Xtxp15, Xtxp57, Xtxp67, Xtxp136, Xtxp141, Xtxp265, Xtxp289, Xtxp317 and Xtxp358 generated a total

of 42 alleles among 22 forage sorghum genotypes. The results were depicted in Table 6 and the SSR amplification profile of primer msbCIR240 is given in Fig. 2. The number of alleles ranged from two to four, with an average number of 2.79 alleles per locus. This result was closely related to the findings of Agrama and Tuinstra (2003) and Akansha *et al.* (2020). The maximum number of four alleles was recorded in Xtxp289 and msbCIR240, followed by three alleles in msbCIR238, Xcup53, Xtxp12, Xtxp15, Xtxp67, Xtxp141, and Xtxp265, and a minimum number of two alleles in msbCIR300, msbCIR329, Xcup14, Xtxp57, and Xtxp317. The primers msbCIR276, Xtxp136 and Xtxp358 were found to be monomorphic. Kondombo *et al.* (2010) also reported the primer Xtxp136 was monomorphic.

The genotypes were subjected to Jaccard's coefficient analysis to find the similarity between all possible pairs of genotypes. The results indicated that the similarity coefficients among all the 22 genotypes ranged from 0.185 to 0.875. The genotypes SRF-316 and DS-1168 were found to be most dissimilar, with a similarity coefficient of 0.185. The study of results from pair wise combinations indicated that the genotypes GFS4 and SH-1813 were highly related to each other as the value of similarity coefficient 0.875 was higher as compared to other genotypic combinations.

The molecular size of the amplified PCR products ranges from 91bp (msbCIR238) to 348bp (Xtxp289). The PIC value ranged from 0.15 (msbCIR329) to 0.64 (Xtxp289), with an average of 0.42.

To study the phylogenetic/evolutionary relationship among different genotypes, the dendrogram was constructed by using 17 SSR markers through NTSYS-pc software (Fig. 3). Based on the dendrogram, two main clusters were formed from 22 parental genotypes, *i.e.*, cluster A and cluster B, which were formed at a similarity coefficient of 0.33. Cluster A is subdivided into sub-clusters A₁, A₂ and A₃. The sub-cluster A₁ includes seven genotypes *viz.*, SRF-316, DSF-168, CSV-21, UTML-529-10(8), DSF-117, DSF-172



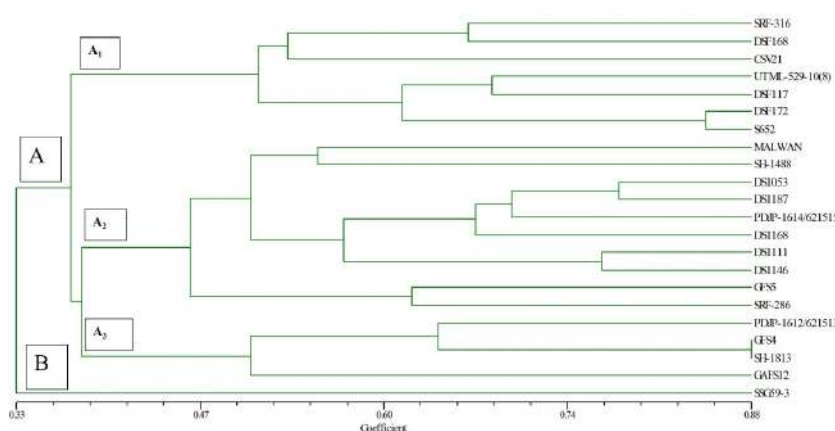
Where,

M=Ladder	6.DS-1053	12.PDJP-1612/621513	18.DSF-172
1.SRF-316	7.DS-1168	13.PDJP-1614/621515	19.SH1813
2.MALWAN	8.DS-1111	14.DSF-168	20.S-652
3.GFS-5	9.DS-1146	15.DSF-117	21.SSG-59-3
4.CSV-21	10.DS-1187	16.GAFS-12	22.SH1488
5.SRF-286	11.UTML-529-10(8)	17.GFS-4	

Fig. 2: SSR amplification profile of primer msbCIR240 in 22 forage sorghum genotypes

Table 6: Results of SSR analysis in twenty-two genotypes of forage sorghum

S. No.	Primer	Molecular band size (bp)	No. of alleles	PIC	Heterozygosity
1.	msbCIR238	91-112	3	0.58	0.00
2.	msbCIR240	108-171	4	0.60	0.00
3.	msbCIR276	235	1	0.00	0.00
4.	msbCIR300	106-114	2	0.35	0.00
5.	msbCIR329	114-135	2	0.15	0.00
6.	Xcup14	202-217	2	0.26	0.00
7.	Xcup53	180-204	3	0.38	0.00
8.	Xtxp12	163-200	3	0.48	0.00
9.	Xtxp15	196-228	3	0.25	0.10
10.	Xtxp57	230-264	2	0.38	0.00
11.	Xtxp67	163-194	3	0.44	0.06
12.	Xtxp136	250	1	0.00	0.00
13.	Xtxp141	132-170	3	0.56	0.09
14.	Xtxp265	179-205	3	0.37	0.00
15.	Xtxp289	261-348	4	0.64	0.05
16.	Xtxp317	153-168	2	0.38	0.00
17.	Xtxp358	210	1	0.00	0.00
		Average	2.79	0.42	0.02

**Fig. 3:** Dendrogram showing clustering of 22 forage sorghum genotypes constructed using UPGMA based on Jaccard's similarity coefficient obtained from SSR-based PCR analysis

and S652. The sub-cluster A_2 includes ten genotypes viz., MALWAN, SH-1488, DS-1053, DS-1187, PDJP-1614/62151, DS-1168, DS-1111, DS-1146, GFS-5 and SRF-286. The sub-cluster A_3 includes four genotypes viz., PDJP-1612/62151, GFS-4, SH-1813 and GAUS-12. The sub-cluster B includes single genotype SSG-59-3. Based on molecular data of the present investigation, it was observed that genotype SSG-59-3 is the most diverse genotype, as it was found placed in a separate cluster from the rest of the genotypes.

Conclusion

In the present investigation, the diversity of twenty-two genotypes of forage sorghum was estimated through

morphological and molecular data (*i.e.*, SSR marker). A total of eight distinct clusters were formed through D^2 analysis whereas two main clusters (A & B) with three subclusters, *i.e.*, A_1 , A_2 , A_3 in the main cluster A, were formed through NTSYSpc version 2.20 program, which revealed that grouping of genotypes through Mahalanobis D^2 analysis was not utterly similar to a grouping of genotypes based on the molecular data through NTSYSpc version 2.20 program. It may be due to the gene concerned with morphological traits is stage-specific, while molecular analysis of the genome represents evolutionary variation that may be functional or non-functional.

Based on combined results for morphological and SSR

genetic diversity estimates, genotype “SSG-59-3” was found to be distinct from other genotypes and can be exploited to harness their unique features in breeding programs.

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Supplementary Table 1: Mean performance of twenty-two genotypes for different characters in forage sorghum

S No.	Genotypes	Days to 50 per cent flowering (days)	Plant height (cm)	Number of leaves per plant (no)	Stem girth (mm)	Leaf length of blade (cm)	Leaf width of blade (cm)
1	SRF-316	76.33	157.3	8.53	11.55	69.53	7.27
2	MALWAN	66.00	214.7	8.20	10.17	61.00	6.21
3	GFS-5	76.67	162.1	8.67	9.34	52.42	6.36
4	CSV-21	70.00	198.0	8.20	9.76	61.40	6.49
5	SRF-286	78.67	214.7	9.07	11.22	61.47	6.01
6	DS-1053	69.67	243.0	9.13	9.10	57.40	6.67
7	DS-1168	75.67	236.0	10.20	8.28	54.13	6.05
8	DS-1111	67.00	224.7	8.07	8.56	59.80	6.55
9	DS-1146	71.67	215.3	9.07	9.01	63.80	6.49
10	DS-1187	70.67	199.7	7.87	8.37	61.60	6.37
11	UTML-529-10(8)	69.67	185.3	7.33	8.15	65.47	5.76
12	PDJP-1612/621513	57.33	180.6	6.60	5.37	45.73	4.67
13	PDJP-1614/621515	72.67	200.0	7.73	8.36	66.93	6.21
14	DSF-168	75.67	190.0	8.70	9.06	63.22	5.62
15	DSF-117	69.33	211.7	8.20	9.57	54.73	6.26
16	GAFS-12	68.67	212.0	7.33	8.47	68.13	4.51
17	GFS-4	66.00	205.8	7.93	5.79	53.27	4.35
18	DSF-172	76.67	204.4	9.34	14.00	81.64	8.75
19	SH-1813	69.33	199.0	8.13	9.76	58.40	6.07

20	S-652	70.67	196.0	8.40	10.70	67.20	6.46
21	SSG-59-3	69.00	233.0	8.20	9.61	62.80	5.00
22	SH-1488	73.33	200.7	8.07	10.00	67.47	6.85
	Mean	70.94	203.81	8.32	9.28	61.71	6.14
	Maximum	78.67	243	10.2	14	81.64	8.75
	Minimum	57.33	157.33	6.6	5.37	45.73	4.35
	S.Em. ($\pm$)	2.64	14.67	0.84	0.94	5.54	0.57
	CD at 5%	7.53	41.86	2.40	2.68	15.81	1.63
	CV (%)	4.57	8.82	12.35	12.53	10.99	11.4

Supplementary Table 1: Continued

S No.	Genotypes	Leaf: stem ratio	Dry fodder yield per plant (g)	Brix content (%)	Hydrocyanic acid content (ppm)	Crude protein content (%)	Green fodder yield per plant (g)
1	SRF-316	0.35	109.86	11.07	31.4	8.93	255.84
2	MALWAN	0.24	103.90	11.56	24.7	8.40	219.59
3	GFS-5	0.27	77.96	9.87	26.0	10.68	214.01
4	CSV-21	0.23	62.61	12.23	18.4	8.58	155.85
5	SRF-286	0.37	75.25	9.02	19.9	9.45	196.83
6	DS-1053	0.27	90.96	10.35	24.9	9.45	208.19
7	DS-1168	0.33	82.08	9.59	11.8	10.68	176.87
8	DS-1111	0.24	66.14	9.71	14.1	9.10	146.52
9	DS-1146	0.46	85.69	10.67	14.5	8.75	169.29
10	DS-1187	0.29	72.53	10.26	21.9	8.05	147.62
11	UTML-529-10(8)	0.25	61.72	8.43	26.7	9.10	136.37
12	PDJP-1612/621513	0.25	53.21	7.56	22.2	7.53	104.26
13	PDJP-1614/621515	0.33	42.51	7.69	13.4	10.15	92.39
14	DSF-168	0.23	57.06	10.23	15.8	9.10	146.75
15	DSF-117	0.21	99.83	9.51	33.8	10.85	208.26
16	GAFS-12	0.25	62.85	8.98	28.7	9.45	161.86
17	GFS-4	0.18	75.58	9.84	21.0	9.45	162.46
18	DSF-172	0.23	135.49	9.46	37.1	10.33	351.27
19	SH-1813	0.22	95.25	11.10	35.0	7.53	220.97
20	S-652	0.23	101.75	11.24	21.2	11.03	221.91
21	SSG-59-3	0.24	86.92	8.53	17.7	6.65	199.89
22	SH-1488	0.26	97.14	11.01	19.9	9.28	243.75
	Mean	0.27	81.65	9.91	22.73	9.22	188.23
	Maximum	0.46	135.49	12.23	37.06	11.03	351.27
	Minimum	0.18	42.51	7.56	11.82	6.65	92.39
	S.Em. ($\pm$)	0.029	6.68	0.58	1.14	0.37	16.19
	CD at 5%	0.08	19.06	1.66	3.25	1.06	46.20
	CV (%)	13.35	10.02	7.17	6.12	4.98	10.54

Supplementary Table 2: List of SSR primers used in the study

S No.	Name	Seq. (5'-3')	T _m value	% GC content	Bases
1	msbCIR238F	AGAAGAAAAGGGTAAGAGC	55.25	45.00	20
2	msbCIR238R	CGAGAAACAATTACATGAACC	53.97	38.10	21
3	msbCIR240F	GTTCTTGGCCCTACTGAAT	54.51	47.37	19
4	msbCIR240R	TCACCTGTAACCCGTCTTC	57.30	50.00	20
5	msbCIR276F	CCCCAATCTAATTATTTGGT	53.20	40.00	20
6	msbCIR276R	GAGGCTGAGATGCTCTGT	55.97	55.56	18
7	msbCIR300F	TTGAGAGCGGCGAGGTAA	55.97	55.56	18
8	msbCIR300R	AAAAGCCCAAGTCTCAGTGCTA	58.39	45.45	22
9	msbCIR329F	GCAGAACATCACTCAAAGAA	53.20	40.00	20
10	msbCIR329R	TACCTAAGGCAGGGATTG	53.69	50.00	18
11	Xcup14F	TACATCACAGCAGGGACAGG	59.35	55.00	20
12	Xcup14R	CTGGAAAGCCGAGCAGTATG	59.35	55.00	20
13	Xcup53F	GCAGGAGTATAGGCAGAGGC	61.40	60.00	20
14	Xcup53R	CGACATGACAAGCTCAAACG	57.30	50.00	20
15	Xtxp12F	AGATCTGGCGGCAACG	54.30	62.50	16
16	Xtxp12R	AGTCACCCATCGATCATC	53.69	50.00	18
17	Xtxp15F	CACAAACACTAGTGCCTTATC	55.92	42.86	21
18	Xtxp15R	CATAGACACCTAGGCCATC	56.67	52.63	19
19	Xtxp57F	GGAACCTTTGACGGGTAGTGC	59.82	52.38	21
20	Xtxp57R	CGATCGTGATGCCCAATC	56.67	52.63	19
21	Xtxp67F	CCTGACGCTCGTGGCTACC	63.14	68.42	19
22	Xtxp67R	TCCACACAAGATTCAGGCTCC	59.82	52.38	21
23	Xtxp136F	GCGAATAGCATCTTACAACA	53.20	40.00	20
24	Xtxp136R	ACTGATCATTGGCAGGAC	53.69	50.00	18
25	Xtxp141F	TGTATGGCCTAGCTTATCT	52.35	42.11	19
26	Xtxp141R	CAACAAGCCAACCTAAA	47.95	41.18	17
27	Xtxp265F	GTCTACAGGCGTGCAAATAAAA	56.53	40.91	22
28	Xtxp265R	TTACCATGCTACCCCTAAAAGTGG	61.01	45.83	24
29	Xtxp289F	AAGTGGGTGAAGAGATA	51.41	44.44	18
30	Xtxp289R	CTGCCTTTCCGACTC	50.57	60.00	15
31	Xtxp317F	CCTCCTTTCTCCTCCTCCC	63.73	61.90	21
32	Xtxp317R	TCAGAATCCTAGCCACCGTTG	59.82	52.38	21
33	Xtxp358F	CAAGGACAAGATTCATTTAAGGG	57.59	37.50	24
34	Xtxp358R	TCACACCTCACAAAATAAAGTGC	57.59	37.50	24

RESEARCH ARTICLE

Variability and Character Association in Different Classes of Rice (*Oryza sativa* L.) of Assam for Root and Yield Attributing Traits

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Abstract

Root and yield-attributing traits for plant selection in rice have been major breeding objectives for the development of varieties with higher yield and stress tolerance. Therefore, the present investigation was conducted for a comparative study of root and yield attributing traits in different classes of rice in Assam. The study revealed the presence of genetic variability among the genotypes for all the traits. Bao varieties showed maximum root development throughout its growth period up to maturity. The highest phenotypic and genotypic coefficient of variation was observed for the root length, root volume and root: shoot ratio. Similarly, in yield attributing traits number of spikelets per panicle showed maximum PCV and GCV followed by total tillers per plant and Days to 50% flowering. The correlation coefficient revealed a positive association of fresh root weight, root: shoot ratio, total tillers per plant, and effective tillers per plant with grain yield per plant, respectively at the genotypic level. A higher genotypic correlation coefficient indicates less influence of environmental effects on traits.

Keywords: Root traits, Assam rice, Yield attributing traits, Moisture stress.

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Introduction

Rice is one of the most widely consumed cereals and is the primary food source for about two-thirds of the global population (Pirdashti *et al.*, 2009). For approximately 100 million Indian families, rice serves as their main source of carbohydrates and minerals. The most significant food crop in Northeast India is rice, including Assam, which is widely grown on 72% of the total cultivated land in upland, lowland, and deepwater conditions (Singh *et al.*, 2006). About 5.2 million tonnes of rice are produced on 2.5 million hectares of paddy land in Assam. Although the crop is mainly cultivated in rainfed conditions, the production of rice is impacted by the climatic fluctuation of the region.

In rainfed conditions, water scarcity is the major limiting factor for the growth and productivity of rice crops. In addition to that climatic variation due to global warming contributed more towards moisture stress in rice production. According to Babu *et al.* (2003), moisture stress is the primary obstacle to rice production and yield stability in rainfed ecosystems, resulting in a yield that is less than one-third of the global average. It is imperative to identify or develop rice cultivars that can withstand such threats during this critical period to address the increasing global food crisis.

Due to its extreme difficulty studies on root traits in the field condition is very limited. In Assam, a few attempts have been undertaken to study root traits in rice besides yield attributing

traits, which are confined to the shoot portion only. Roots are the principal organ for the uptake of nutrients and water from soil. Besides the uptake of water and nutrients, it provides mechanical anchorage to the plant. The root system serves as vital in situations of stress since it is the first organ to encounter different abiotic stimuli. The current study aims to find genotypes that possess desirable root and yield-attributing traits that could impact rice productivity and production.

Materials and Methods

The experimental materials consisted 23 genotypes that belong to three diverse traditional rice classes of Assam, namely *Bao*, *Ahu*, *Sali* and two high-yielding varieties were also included (Table 1). These genotypes were collected in pure form from Assam Agricultural University Zonal Agricultural Research Station, Lakhimpur, Assam. The experiment was conducted during *Sali* season (June–November) of 2020 at Instructional cum Research Farm, Department of Plant Breeding and Genetics, Assam Agricultural University, Jorhat. The material was planted in a randomized block design with three replications in PVC pipes with dimensions of 11 cm diameter and 1 m length placed in 30 cm depth and filled with a mixture of soil

and farm yard manure in the ratio of 10:1. The spacing for the tubes was 50 x 50 cm. Altogether 15 parameters were recorded. Different root traits considered were root and shoot weight (g), maximum root length (cm), root volume (cc) and root: shoot ratio. Root sampling was done at 21 and 55 days and at maturity in the month of November. At the time of sowing, five seeds per pipe were sown, but at 21 days of sowing, one seedling from each pipe was uprooted to record the root weight of the seedlings. Similarly, root sampling was done at 55 days for recording root weight, allowing only one plant per pipe to grow from then onwards. A suitable growing condition was maintained so that less possible extraneous effect is shown on the phenotypic expression of the characters. The PVC pipes were removed carefully and soaked in water to loosen the soil. Roots were cleaned thoroughly and carefully under running water. The intact root system of each plant was collected and stored. Root fresh weights were recorded after sampling, root dry weights were recorded by drying in room temperature (25°C) for one week and dry weights (g) were recorded. Maximum root length (cm) was measured from the base of the plant (collar region) to the tip of the longest root. Root volume (cubic centimeter; cc) was measured based on the water displacement method. A graduated measuring cylinder was filled with water and the initial volume was recorded. Then, the root system was immersed in the measuring cylinder and the final volume was noted down after the proportionate water displacement occurred. The difference between the two readings gave root volume (cc). Root: shoot ratio was computed by dividing the root dry weight of a sample plant by the respective shoot dry weight. Data were collected and subjected to analysis of variance (ANOVA) for all the traits following the standard protocols. Genetic parameters of variation, heritability and genetic advance were estimated following Burton and Devane (1953). Genotypic and phenotypic correlation coefficients were determined by following Singh and Choudhury (1985).

Table 1: Details of rice genotypes used in the experiment

S No.	Genotypes	Class	Type of cultivar
1	Panindra	Bao	Improved
2	Sunmoti 2	Bao	Traditional
3	Negheri	Bao	Traditional
4	Amona	Bao	Traditional
5	Pachanan	Bao	Improved
6	Kekuwa	Bao	Traditional
7	Ahina	Bao	Traditional
8	Betguti 1	Ahu	Traditional
9	Lahi Ahu	Ahu	Traditional
10	Kola Ahu	Ahu	Traditional
11	Bengungutia Ahu	Ahu	Traditional
12	Kopougutia Ahu	Ahu	Traditional
13	Disang	Ahu	HYV
14	Luit	Ahu	HYV
15	Arize Bold	Sali	Hybrid
16	Arize Diamond	Sali	Hybrid
17	Kola Pakhi	Sali	Traditional
18	Ranjit	Sali	HYV
19	Gitesh	Sali	HYV
20	Bor Aijung	Sali	Traditional
21	Solpona	Sali	Traditional
22	Bordubi 3	Sali	Traditional
23	Chakua	Sali	Traditional

Result and Discussion

The analysis of variance revealed significant variation among the genotypes for root and yield attributing traits, which

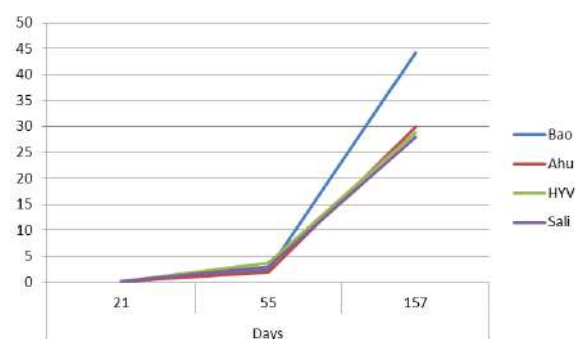


Fig 1: Rate of change of root development (root weight) of different classes of rice

Table 2 (a): Analysis of Variance for root traits of rice genotypes

Source of variation	df	Mean square						
		Root Fresh Weight (g)	Root Dry Weight (g)	Shoot Fresh Weight (g)	Shoot Dry Weight (g)	Root Length (cm)	Root Volume (cc)	Root: Shoot Ratio
Replication	2	110.88**	15.84	13.5	33.46	15.79	37.96	0.02
Genotype	22	310.74**	291.18**	402.17**	330.97**	503.47**	900.92**	0.20**
Error	44	12.02	10.56	25.19	28.16	5.92	18.75	0.01
CD (%)		5.71	5.88	8.27	8.76	4.02	7.15	0.18
CV		7.38	10.61	5.95	9.77	6.30	9.05	16.71

Table 2 (b): Analysis of Variance for yield attributing traits of rice genotypes

Source of variation	df	Mean square							
		Days to 50% Flowering	Plant Height(cm)	Total Tillers per Plant	Effective Tillers per Plant	Panicle Length(cm)	Number of Spikelet per Panicle	1000-Grain Weight(g)	Grain yield per plant (g)
Replication	2	127.14	0.23	0.91	8.74**	4.52	376.41	4.15	30.13*
Genotype	22	879.32**	1016.61**	42.03**	6.30**	32.55**	4577.49**	26.43**	34.80**
Error	44	40.27	16.54	4.08	1.41	1.73	219.25	2.22	7.91
CD (%)		10.48	6.72	3.34	3.34	2.17	24.45	2.46	4.65
CV		6.78	3.61	11.33	14.82	5.59	9.98	8.03	12.21

*Significant at 5%; **significant at 1%

might be due to diverse sources (*Ahu*, *Sali*, *Bao*, Modern cultivars) amongst the experimental material (Table 2(a) & (b)). Similar findings were reported by many workers in the past, which revealed the presence of wide genetic variability among root traits and for different quantitative traits (Anbumalaramathi *et al.*, (2008); Courtois *et al.*, (2013)). The rate of change of root weight was computed graphically (Fig.1) based on the observations made at 21, 55 and 157 (maturity) days after sowing (DAS) for *Bao*, *Ahu*, *Sali* and the hybrid group. The mean root weight of all the classes of rice exhibited a similar trend up to 55 DAS. The mean root weight increased at a faster rate in the case of *Bao* varieties up to 40 g at maturity, while in all other classes, the rate of increase was similar (30 g). *Bao* or deepwater rice exhibited the highest root growth as compared to other rice classes. Since *Bao* or deepwater rice encounters drought in the early vegetative stage and prolonged submergence in the later part of the life cycle, it is likely to show higher root growth than the others. Varietal differences were obtained from genetic parameters calculated for all the characters presented in Table 3(a) & (b). Genotypic Coefficient of Variation (GCV) and Phenotypic Coefficient of Variation (PCV) were recorded as highest for root: shoot ratio followed by root volume root length. Similarly, for yield attributing traits number of spikelets per panicle recorded maximum PCV and GCV followed by total tillers per plant and Days to 50% flowering. Minimum differences between GCV and PCV were recorded, which depicts the minimum role of the environment in the expression of these characters. These

findings concurred with Roy *et al.*, (2009) and Hijam *et al.*, (2012).

The presence of high magnitude of variation is not sufficient for the selection to become effective unless a heritable fraction of variation is not known (Burton, 1952). Hence it becomes imperative to estimate the heritable variation. In addition, it is also important to find out genetic gain under selection along with heritability to reach a more reliable conclusion. In the present investigation, heritability estimates were observed to be highest for root length, followed by root volume, root fresh weight and root dry weight. Genetic advance as a percentage of the mean was observed to be highest for root shoot ratio, followed by root volume, root length, root fresh weight, and root dry weight. Similarly, in yield attributing traits maximum heritability was observed for plant height followed by days to 50% flowering and panicle length. Genetic advance as a percent of mean was found to be the maximum for the number of spikelets per panicle, followed by total tillers per plant and days to 50% flowering. Similar findings were reported by Hijam *et al.*, (2012); Phung *et al.*, (2016). High heritability, along with high genetic gain, indicated the presence of additive gene action. Both high heritability coupled with high genetic advance together serve as a better indication for the efficiency of selection than considering alone (Johnson *et al.*, 1955). Based on the above observations, root traits such as root volume, root fresh weight, and root dry weight can be considered for further investigation in the present germplasm under study.

The mean value of root fresh weight was 47 g, with a

Table 3 (a): Estimate of range, general mean, phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability in broad sense, genetic advance as percentage of mean for root traits:

Traits	Range	MEAN $\pm$ Sem	PCV (%)	GCV (%)	Heritability (bs) (%)	GA as % of mean
Root Fresh Weight(g)	28–62.33	46.9 9 $\pm$ 2.00	22.48	21.23	89.22	41.32
Root Dry Weight (g)	19–50.67	33.64 $\pm$ 2.06	30.54	28.64	87.93	55.32
Shoot Fresh Weight (g)	60.67–104	84.40 $\pm$ 2.90	14.55	13.28	83.30	24.97
Shoot Dry Weight (g)	38.67–79.83	54.35 $\pm$ 3.06	20.90	18.48	78.18	33.67
Root Length (cm)	22.5–73.67	38.62 $\pm$ 1.405	33.94	33.35	96.55	67.50
Root Volume (cc)	9.17–80.33	47.87 $\pm$ 2.50	36.94	35.82	94.00	71.54
Root: Shoot Ratio	0.31–1.33	0.65 $\pm$ 0.06	2.52	39.10	84.56	74.07

Table 3 (b): Estimates of range, general mean, phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability in broad sense, genetic advance as percentage of mean for yield attributing traits

Traits	Range	MEAN $\pm$ Sem	PCV (%)	GCV (%)	Heritability (%)	GA as % of mean
Days to 50% Flowering	70–124	93.55 $\pm$ 3.66	19.12	17.87	87.41	34.43
Plant Height(cm)	86.33–161	112.54 $\pm$ 2.35	16.62	16.22	95.27	32.62
Total Tillers per Plant	9.67–23	17.83 $\pm$ 1.17	22.94	19.95	75.61	35.73
Effective Tillers per Plant	5.33–10	8.00 $\pm$ 0.69	21.78	15.97	53.72	24.11
Panicle Length(cm)	17.73–29.33	23.53 $\pm$ 0.76	14.72	13.62	85.57	25.95
Number of Spikelet per Plant	92–216	148.42 $\pm$ 8.55	27.55	25.68	86.88	49.31
1000-Grain Weight(g)	10.83–22.4	18.56 $\pm$ 0.86	17.28	15.30	78.42	27.91
Grain Yield per Plant(g)	14.17–27.46	23.03 $\pm$ 1.62	17.83	12.99	53.10	19.51

range of 28 g in *Lahi Ahu* (Fig. 2: Plate no. 5) to 62.33 g in *Pachanan* (Fig. 2: Plate no. 1) (Tables 3 (a and b) & 4). Similarly, dry root weight varied from 19 g in *Lahi Ahu* to 50.67 g in *Sunmoti 2* (Fig 2; Plate no. 2) with a mean value of 55.33 g. The wide range of variation for root weight revealed the scope for the selection of desirable genotypes having high root weight. Genetic variation for root biomass was also reported by Hijam *et al.*, (2012). Considerable genetic variations in root biomass were also reported by various authors (Ingram *et al.*, 1994 and Kato *et al.*, 2009). Shoot fresh weight varied from 60.67 in *Bordubi 3* to 104 g in *Kekua*, with a mean value of 84 g. Similarly, the mean value for the shoot dry weight was recorded to be 54 g, with a range of 38.67 g in *Solpona* to 79.83 g in *Kola Pakhi*. Root length significantly varied from 22.5 cm in *Bor Aijung* (Fig 2: Plate no.1) to 73.67 cm in *Chakua* (Fig 2: Plate no. 5) with a mean value of 39 cm. The mean value for root volume was 48 cc with a range of 9.17 cc in *Betguti 1* (Fig 2; Plate no. 2) to 80.33 cc in *Negheri* (Fig2: Plate no.5) Similarly, root: shoot ratio varied from 0.31 in *Lahi Ahu* to 1.33 in *Sunmoti 2* with mean value of 1. *Bao* varieties are significantly superior to all other genotypes for root fresh weight, root dry weight, shoot fresh weight, root volume, and root: shoot ratio, which indicates scope for selection from this class of rice. Wang *et al.*, (2006) have also reported that the characters, viz., root volume, root: shoot ratio, root distribution pattern, have various functional significance.

In the present investigation, apart from estimating the correlation coefficient for yield and yield-attributing traits,

the effect of root traits on grain yield was also estimated (Table 5), which could serve as a reliable criterion for improving the adaptation of rice crops under water-limiting conditions.

A significant positive association was recorded for grain yield with root fresh weight, root: shoot ratio, and effective tillers per plant at the genotypic level. At the phenotypic level, the traits showed a non-significant association with grain yield. These findings were in agreement with Yogameenkashi & Vivekananda (2010); and Pushpam *et al.* (2017). Root fresh weight was found to have a significant positive correlation at phenotypic and genotypic levels with root dry weight, shoot fresh weight, root: shoot ratio and days to 50% flowering. These findings were in conformity with Hijam *et al.*, (2012); Nassir *et al.*, (2017). This strong inter-correlation among the traits indicates the scope for simultaneous selection and improvement of these traits along with grain yield

Similarly, root length showed a significant positive association with panicle length at phenotypic and genotypic levels. This was in agreement with the findings of Armenta-Soto *et al.*, (1983); Toorchi *et al.*, (2003). Therefore, selection for root length may simultaneously improve the panicle length, which is an important yield-attributing trait.

Total tillers per plant and 1000-grain weight recorded a positive association with grain yield at both phenotypic and genotypic levels. Also, total tiller per plant recorded a positive correlation with root volume, indicating the scope

Table 4: Mean performance of the genotypes for root traits

Genotypes	Mean $\pm$ Standard Deviation						
	Root Fresh Weight(g)	Root Dry Weight(g)	Shoot Fresh Weight(g)	Shoot Dry Weight(g)	Root Length(cm)	Root Volume(cc)	Root: Shoot Ratio
Panindra	60 $\pm$ 2.71	48.00 $\pm$ 2.99	93.33 $\pm$ 1.86	61.17 $\pm$ 1.42	32.33 $\pm$ 1.31	40.67 $\pm$ 1.50	0.79 $\pm$ 0.03
Sunmoti 2	61.33 $\pm$ 2.99	50.67 $\pm$ 3.55	77.33 $\pm$ 1.47	38.17 $\pm$ 3.37	28.33 $\pm$ 2.14	40.50 $\pm$ 1.54	1.33 $\pm$ 0.14
Negheri	60 $\pm$ 2.71	44.33 $\pm$ 2.23	76.33 $\pm$ 1.68	42.50 $\pm$ 2.47	42.00 $\pm$ 0.71	80.33 $\pm$ 6.77	1.05 $\pm$ 0.08
Amona	45.66 $\pm$ 0.27	32.00 $\pm$ 0.34	95.67 $\pm$ 2.35	62.50 $\pm$ 1.70	31.33 $\pm$ 1.52	35.33 $\pm$ 2.61	0.51 $\pm$ 0.03
Pachanan	62.33 $\pm$ 3.20	44.67 $\pm$ 2.30	91.67 $\pm$ 1.52	55.50 $\pm$ 0.24	30.00 $\pm$ 1.80	78.67 $\pm$ 6.42	0.81 $\pm$ 0.03
Kekuwa	61 $\pm$ 2.92	46.67 $\pm$ 2.72	104.00 $\pm$ 4.09	62.33 $\pm$ 1.66	62.33 $\pm$ 4.95	44.00 $\pm$ 0.81	0.75 $\pm$ 0.02
Ahina	52.33 $\pm$ 1.12	43.33 $\pm$ 2.02	85.00 $\pm$ 0.13	51.50 $\pm$ 0.59	59.83 $\pm$ 4.42	41.00 $\pm$ 1.43	0.85 $\pm$ 0.04
Betguti 1	38 $\pm$ 1.87	27.67 $\pm$ 1.24	67.67 $\pm$ 3.49	52.00 $\pm$ 0.49	33.33 $\pm$ 1.10	9.17 $\pm$ 8.07	0.53 $\pm$ 0.03
Lahi Ahu	28 $\pm$ 3.96	19.00 $\pm$ 3.05	84.67 $\pm$ 0.06	62.00 $\pm$ 1.60	42.67 $\pm$ 0.84	64.00 $\pm$ 3.36	0.31 $\pm$ 0.07
Kola Ahu	38.33 $\pm$ 1.80	24.33 $\pm$ 1.94	75.33 $\pm$ 1.89	53.67 $\pm$ 0.14	51.67 $\pm$ 2.72	45.00 $\pm$ 0.60	0.45 $\pm$ 0.04
Bengungutia Ahu	47.33 $\pm$ 0.07	31.67 $\pm$ 0.41	84.67 $\pm$ 0.06	42.33 $\pm$ 2.51	40.33 $\pm$ 0.36	14.67 $\pm$ 6.92	0.75 $\pm$ 0.02
Kopougutia Ahu	55.66 $\pm$ 1.81	45.00 $\pm$ 2.37	79.67 $\pm$ 0.99	41.67 $\pm$ 2.64	41.00 $\pm$ 0.50	56.67 $\pm$ 1.83	1.08 $\pm$ 0.09
Disang	52.33 $\pm$ 1.12	39.67 $\pm$ 1.26	83.17 $\pm$ 0.26	50.67 $\pm$ 0.77	30.00 $\pm$ 1.80	45.33 $\pm$ 0.53	0.80 $\pm$ 0.03
Luit	37.66 $\pm$ 1.94	22.67 $\pm$ 2.29	61.33 $\pm$ 4.81	49.87 $\pm$ 0.93	26.33 $\pm$ 2.56	66.67 $\pm$ 3.92	0.46 $\pm$ 0.04
Arize Bold	55.33 $\pm$ 1.74	32.00 $\pm$ 0.34	98.00 $\pm$ 2.84	63.67 $\pm$ 1.94	33.00 $\pm$ 1.17	65.67 $\pm$ 3.71	0.51 $\pm$ 0.03
Arize Diamond	41 $\pm$ 1.25	25.67 $\pm$ 1.66	83.67 $\pm$ 0.15	67.33 $\pm$ 2.71	45.00 $\pm$ 1.33	48.33 $\pm$ 0.10	0.38 $\pm$ 0.06
Kola Pakhi	44 $\pm$ 0.62	34.33 $\pm$ 0.14	102.67 $\pm$ 3.81	79.83 $\pm$ 5.31	44.33 $\pm$ 1.19	58.67 $\pm$ 2.25	0.43 $\pm$ 0.05
Ranjit	36.33 $\pm$ 2.22	24.33 $\pm$ 1.94	87.67 $\pm$ 0.68	56.17 $\pm$ 0.38	30.33 $\pm$ 1.73	45.00 $\pm$ 0.60	0.43 $\pm$ 0.05
Gitesh	35 $\pm$ 2.50	20.67 $\pm$ 2.70	96.33 $\pm$ 2.49	64.50 $\pm$ 2.12	30.83 $\pm$ 1.62	37.33 $\pm$ 2.20	0.33 $\pm$ 0.07
Bor Aijung	44.33 $\pm$ 0.55	30.33 $\pm$ 0.69	89.00 $\pm$ 0.96	57.00 $\pm$ 0.55	22.50 $\pm$ 3.36	30.00 $\pm$ 3.73	0.54 $\pm$ 0.02
Solpona	33.66 $\pm$ 2.78	20.33 $\pm$ 2.77	81.33 $\pm$ 0.64	38.67 $\pm$ 3.27	31.67 $\pm$ 1.45	50.67 $\pm$ 0.58	0.53 $\pm$ 0.03
Bordubi 3	44 $\pm$ 0.62	33.67 $\pm$ 0.01	60.67 $\pm$ 4.95	41.33 $\pm$ 2.71	25.33 $\pm$ 2.77	46.00 $\pm$ 0.39	0.86 $\pm$ 0.04
Chakua	47 $\pm$ 0.003	32.67 $\pm$ 0.20	82.00 $\pm$ 0.50	55.67 $\pm$ 0.27	73.67 $\pm$ 7.31	57.33 $\pm$ 1.97	0.59 $\pm$ 0.01

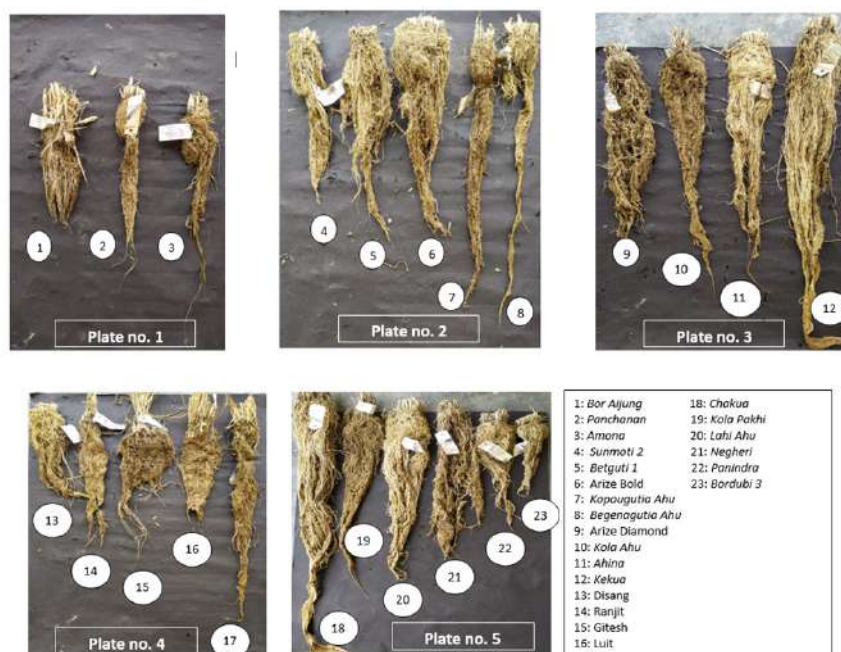
**Fig 2:** Root traits of different rice genotypes

Table 5: Correlation coefficient at genotypic level (upper diagonal) and phenotypic level (lower diagonal)

	RFW	RDW	SFW	SDW	RL	RV	RSR	DFF	PH	TTP	ETP	PL	NSP	TGW	GVP
RFW															
RDW	0.973**														
SFW	0.907**	0.265*													
SDW	0.238*	0.179	-0.176												
RL	-0.139	0.185	-0.212	0.721**											
RV	0.111	-0.192	0.721**	0.609**	0.121										
RSR	0.203	0.132	0.070	0.178	0.154	0.228									
DFF	0.729**	0.843**	-0.647**	0.030	0.099	0.058	0.811**								
PH	0.273*	0.307*	0.030	0.030	-0.024	-0.073	0.863**	0.293*							
TTP	-0.012	0.041	-0.036	0.199	0.267*	-0.155	0.233**	0.358**	-0.011						
ETP	0.145	0.112	0.199	0.066	0.186	0.250*	0.066	0.370**	0.044	0.113					
PL	0.179	0.128	0.066	0.184	0.188	0.410**	0.064	0.036	0.190	0.106	0.257*				
NSP	-0.351**	-0.354**	0.184	-0.300*	0.243*	-0.050	-0.343**	0.036	0.037	0.096	0.102	-0.425**			
TGW	0.133	0.147	-0.031	-0.053	-0.031	0.089	0.240*	0.431**	0.200	0.266*	-0.029	0.045	0.167		
GVP	0.157	0.109	0.048	-0.053	-0.170	-0.147	0.119	-0.090	-0.112	0.189	0.222	0.171	-0.368**	0.200	0.310**
	0.201	0.165	0.103	-0.173	-0.199	-0.127	0.198	0.075	-0.010	0.260*	0.119	0.245*	-0.034	0.622**	0.796**

*Significant at 5%; **significant at 1%

for selection of root volume, which may improve total tiller per plant and eventually improve the grain yield due to a strong positive correlation between grain yield and total tiller plant.

However, a negative significant association of panicle length with total tillers per plant later having a positive association with grain yield might affect the grain yield negatively. Therefore, to break this negative association, recombination breeding can be adopted.

From the above discussion, it can be inferred that direct selection for root fresh weight, root: shoot ratio, and effective tillers per plant can be advocated to improve grain yield. Drought adaptive traits such as root length, root volume, and root: shoot ratio did not show any significant association with grain yield; hence, direct selection for these traits will not be effective. However, their inter-correlation with other yield-attributing traits indicated the scope for simultaneous improvement of these traits. The breeding approach would be combining good root traits with good yield attributing traits through recombination breeding.

Conclusion

From the present investigation, we can conclude that root traits such as root volume, root: shoot ratio, root fresh weight and yield attributing traits such as total tillers per plant, days to 50% flowering, number of spikelets per panicle can be used as a reliable criterion for improving grain yield. Simultaneous improvement of root traits along with yield attributing traits might contribute to the grain yield along with adaptation of rice genotypes in moisture stress growing conditions. The findings from the above investigation can be followed by studying genetic divergence, combining ability and hybridization among the genotypes to obtain cultivars with desirable traits adapted to moisture stress growing conditions. Although in the present investigation, drought stress, submergence, and other abiotic stress were not studied, further study with the inclusion of stress tolerance may be useful.

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RESEARCH ARTICLE

Identifying Potential Donor Parents for Breeding Against Leaf Curl Virus and Anthracnose Diseases in Chilli

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Abstract

Development of multiple disease-tolerant chilli (*Capsicum annuum* L.) varieties, which can be grown successfully throughout the year, is a goal of plant breeders. The study was conducted to identify chilli genotypes that could be utilized in hybridization to retrieve potential genotypes having tolerance to both chilli leaf curl virus (ChLCuV) and chilli anthracnose diseases in addition to higher yield. About 26 chilli genotypes were screened for tolerance to ChLCuV and chilli anthracnose diseases under field and laboratory conditions, and for other quantitative traits. A wide range of genetic variation was observed for all traits under study. Genotypes were grouped into 6 clusters that do not represent the same place of origin, indicated genotypes in a cluster were geographically diverse and genotypes obtained from the same region were genetically different. The principal components, percent disease index (PDI) of ChLCuV, PDI of chilli anthracnose, and plant height, had eigenvalue >1 and together accounted for almost 100% of the variation. Based on multivariate analysis, fruit yield and tolerance to both ChLCuV and chilli anthracnose diseases, genotypes 'Bidhan Chilli 4', 'Pant C 1', and 'Chinese Bona' were identified as tolerance sources against both diseases. Highly tolerant genotypes against both ChLCuV and anthracnose diseases were identified which were less frequent in *C. annuum* than other domesticated species and inter-specific derivatives.

Keywords: *Capsicum annuum*, ChLCuV, Chilli anthracnose, Variability, Divergence.

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Introduction

An understanding of genetic diversity forms the basis of the selection of germplasm to be utilized in the hybridization program. Chilli is considered an often or facultative cross-pollinated crop and the degree of outcrossing at the field level has been reported to range from 7 to 90% (Tanksley, 1984; Singh *et al.*, 1994). India is considered to be the secondary center of diversity of chilli, especially of *Capsicum annuum* (Dhaliwal *et al.*, 2014), therefore, an ample amount of variability in *C. annuum* exists all over the country (Kumar *et al.*, 2006). Despite such variability, the crop fails to attain optimum productivity owing to the use of local unimproved cultivars and susceptibility to several biotic stresses.

In India, anthracnose, a seed-borne disease caused by *Colletotrichum capsici*, was first reported by Sydow (1928) from Coimbatore of Madras Presidency, is an emerging threat in chilli production that spreads under humid conditions, leaving very limited chances for growers to protect the crop (Srideepthi *et al.*, 2017). The disease results in dark spots, sunken necrotic tissue with concentric rings of acervuli, including die back in the stem, seedling blight, or damping off (Azad *et al.*, 1991) and has reportedly caused marketable yield loss ranging from 50 to 80% in different parts of the world (Sariah, 1994). The accessions of *C. annuum* species are known to be highly susceptible to anthracnose (Yoon *et al.*, 2004) and commonly utilized resistant genotypes are non-*C. annuum*, especially *C. baccatum* and *C. chinense* (Bal *et al.*, 2024; Lee *et al.*,

2010). A continuous search for new sources of resistance among *C. annuum* types and utilization of these genetic resources in breeding for the development of better varieties/hybrids with higher levels of tolerance against ChiLCuV and anthracnose is necessary.

Genetic divergence is an important criterion to identify potential donors in a hybridization program and proper choice of parents among indigenous materials based on multivariate analysis is essential for the development of recombinant(s) tolerant to both ChiLCuV and chilli anthracnose diseases. An investigation was carried out to determine the breeding potential of chilli genotypes against ChiLCuV and anthracnose diseases through multivariate analysis.

Materials and Methods

Breeding materials and experimental location

Twenty-six advanced breeding lines/varieties/accessions of chilli, collected across India, constituted the plant materials for this study and were screened in the research field of All India Coordinated Research Project on Vegetable Crops, Bidhan Chandra Krishi Viswavidyalaya, Kalyani, Nadia, West Bengal, India located at 23.5° N latitude and 89° E longitude at an elevation of 9.75 m above the mean sea level.

Cultural practices

The experimental soils were non-saline (EC 0.285 dS/m), sandy-loam in texture, almost neutral in action (pH 6.8), and low in organic carbon (4.2 g/kg), having good drainage facilities. Seedbeds were prepared in sandy loam soil and were 20 cm high and 1.0 m wide. Weathered cow dung @ 4 kg/m² was mixed into the beds. Thereafter, beds were drenched with chlorothalonil (2 g) + Carbendazim (1g) to keep away from damping off disease. Seeds, after treatment with Thiram (3 g/kg of seed), were sown on 28 September 2019 at a shallow depth of 5 cm apart and covered thereby with finely sieved well, rotten leaf mold (leaves of which were left to decompose for two years) which acts simultaneously as a soil improver and in preventing soil from drying out. After sowing, beds were covered with straw until germination which normally takes 7 to 10 days and hand watered regularly up to 24 October 2019. Nursery beds were then covered with 200 µm ultraviolet (UV)-stabilized polythene film supported by bamboo poles with open sides to protect seedlings from rain and direct sunlight. Seedlings were hardened by withholding water 4 days before transplanting. Thirty-day-old seedlings were transplanted to the main field on 28 October 2019. Management practices as scheduled for cultivation were followed as per Chattopadhyay *et al.* (2007).

Observations recorded

Observations were recorded from 10 randomly selected plants out of 25 plants in each of three replications for

plant height (cm), plant spread (cm), specific leaf weight (g), number of branches per plant, days to 50% flowering, number of fruits per plant, fruit retention (%), days to ripe fruit maturity from anthesis, fruit yield per plant (g), and 15 randomly selected fruits from plants of each replication for fruit length (cm), fruit diameter (cm), average fruit weight (g), number of seeds per fruit, 1000 seed weight (g).

Data on the percent disease index (PDI) of ChiLCuV was determined at 3 stages at an interval of 30 days, starting from 30 DAT and continuing up to 90 days after transplanting. PDI of ChiLCuV was obtained from all 25 plants in replication based on a disease scoring scale (0-9) as per Reddy *et al.* (2001) with slight modifications.

The maximum prevalence of anthracnose disease occurs during the *Kharif* season (July–October) under open field conditions. However, the present research work was conducted during the autumn-winter season when the prevalence of anthracnose disease under open field conditions was very low to assess the severity of anthracnose disease. The severity of anthracnose disease was judged under laboratory conditions. Artificial inoculation of fruits was done to get accurate results. Chilli fruit showing the typical symptoms of fruit rot was collected from different places in West Bengal state. The pathogen was isolated in a potato dextrose agar (PDA) medium using the collected samples. About 15 days old culture of *Colletotrichum capsici* was used for artificial inoculation. Twenty-five red ripe fruits from randomly selected plants of each genotype in each replication were considered for inoculation. The fruit surface was sterilized with 0.1% HgCl₂, and then washed in two changes of sterile water. Thereafter, the fruits were pricked with sterile pin bundles. The pinpricked fruits were then dipped in spore suspension (5×10⁵ spores/mL) of anthracnose fungus for 5 minutes and then kept for incubation on trays under a humid chamber. The humid chamber was prepared by keeping water in a tray that was placed below the tray with inoculated fruits. The wetted cotton pieces were placed on the trays and were covered with polythene to maintain the relative humidity and incubated at 27 ± 1°C thereafter for a week. After the 7th day of inoculation, infected samples were observed for anthracnose lesions. A disease rating scale following Singh *et al.* (1993) was used for the identification of tolerant/susceptible genotypes in chilli.

Statistical Analysis

The analysis of variance was done as per the standardized method described by Panse and Sukhatme (1967). Genotypic and phenotypic coefficients of variation were worked out using the formulae of Burton (1952). Heritability in a broad sense, was calculated as per the formula given by Lush (1949). The genetic advance was estimated following Johnson *et al.* (1955). Phenotypic and genotypic correlation coefficients were computed as per Johnson *et al.* (1956). Direct and

indirect effects of yield on different components and disease severity traits were calculated through path co-efficient analysis as per Dewey and Lu (1959). The D^2 statistic was used to assess genetic divergence between populations (Mahalanobis, 1936). The grouping of populations was done by using Tocher's method as described by Rao (1952). Hierarchical cluster analysis was performed to observe the degree of association according to characteristics expressed in a dendrogram (Ward, 1963). Principal component analysis (PCA) was used to identify the factor dimension of the data and to summarize varietal information in a reduced number of factors for the selection of the best-performing genotype(s). Statistical analyses were with Windostat (ver. 8.0, Indostat Services, Hyderabad, India) and SAS (ver. 9.3, SAS Inc., Cary, NC).

Results and Discussion

PDI values of genotypes for ChilCuV at different days after transplanting (DAT) varied. Most of the genotypes had comparatively lower PDI values at 30 DAT (Fig. 1). PDI values gradually increased to 60 DAT, and values varied from 3.51 to 66.11%. At 90 DAT, genotypes 'Bidhan Chilli 4' (5.18%), 'BCC 1' (7.55%), 'Chinese Bona' (7.96%) and 'Pant C 1' (10.00%) recorded the lowest disease severity and were considered as 'resistant' to ChilCuV. In addition, the genotypes 'Dhani Lanka', 'Akashi', 'PBC-824', 'PBC-613', and 'Chilli IR-8' were considered to be 'moderately resistant' according to the categorization scheme laid down by Reddy *et al.* (2001). High PDI values were observed in accessions, 'IC-255916', 'IC-383072', 'EC-390029', 'EC-382175' and 'IC-255944' at 90 DAT (Fig. 1). Resistant and moderately resistant genotypes against ChilCuV may be used under integrated production systems and in developing new resistant genotypes.

Genotypes were screened under artificial conditions to judge their potentiality to combat chilli anthracnose diseases and genotype-dependent responses were observed (Fig. 2). Chilli fruits were inoculated with *Colletotrichum capsici* under laboratory conditions and genotypes 'Bidhan Chilli 4' (1.73%), 'Pant C 1' (2.22%), 'Chinese Bona' (4.53%), and 'Chilli 38-Ragi' (5.60%) exhibited resistant disease reaction as per the categorization scheme laid down by Singh *et al.* (1993). Moderately resistant disease reaction was observed in the genotypes 'Srinagar', 'IC-570408', and 'BCC 1'. In addition, genotypes 'IC-383072', 'EC390029', 'G-4 Ziya Chibli', 'PBC-613', 'G-4', 'EC-382175', 'Akashi Lanka', 'Phule Jyoti Garima', 'IC-255944', 'BCC-25' and 'BCC-30' were considered as susceptible where disease reaction ranged from 25-50%. Disease reaction was more than 50% in the rest of the genotypes as per the categorization laid out by Singh *et al.* (1993).

Analysis of variance indicated genotype was important for genetic variability (Table 1). Very high variance (mean sum of squares for genotypes) was recorded in plant height,

plant spread, fruit retention (%), fruit yield per plant, PDI of ChilCVD (%), and PDI of chilli anthracnose disease (%), indicating a very wide range of diversity concerning these characters (Table 1).

The coefficient of phenotypic and genotypic variation (PCV and GCV, respectively), heritability in a broad sense (h^2_{bs}), and genetic advance as a percent of the mean for the characters varied (Table 2). Genotypic Coefficient of Variation (GCV) and Phenotypic Coefficient of Variation (PCV) revealed close resemblance for all the characters except the number of fruits per plant and fruit yield per plant, which indicated the fact that the contribution of these characters towards final phenotypic expression was mainly due to genetic makeup of the genotypes rather than environmental influences and selection alone could be effective based on phenotypic characters. The PCV values were found to be higher than the corresponding GCV values, indicating that there was a significant influence on the growing environment. High GCV and PCV values (>20%) occurred in plant height, plant spread, number of branches per plant, days to 50% flowering, number of fruits per plant, fruit retention (%), fruit length, fruit diameter, average fruit weight, number of seeds per fruit, 1000 seed weight, fruit yield per plant, PDI of ChilCVD (%) and PDI of chilli anthracnose disease (%) except days to ripe fruit maturity from anthesis and specific leaf weight. The high magnitude of GCV and PCV indicates ample scope for improvement through normal selection. The proportion of GCV to PCV was high (> 90%) for specific leaf weight, days to 50% flowering, fruit retention (%), fruit length, fruit diameter, average fruit weight, number of seeds/fruit, 1000 seed weight, days to ripe fruit maturity from anthesis, fruit yield per plant, PDI of ChilCuV and chilli anthracnose diseases. Traits with such high proportions are desirable in the selection process as it depicts that the traits are under genetic control rather than the environmental effect. Traits whose expressions are environmentally dependent may not be reliable descriptors for morphological characterization (Pandey *et al.*, 2008; Samaee *et al.*, 2003). The proportion of genetic contribution to the overall phenotypic expression of most traits was very high. Thus, their use as an important discriminatory variable for chilli classification studies seems relatively reliable.

The genotypic coefficient of variation is the measure to estimate the variability of characters, but GCV alone cannot determine the amount of variation that is heritable. The $GCV \times$ selection differential helps in estimating the maximum effectiveness of selection and heritability indicates how closely the goal can be achieved (Singh *et al.*, 1968; Singh and Singh, 1985).

Heritability is of interest to plant breeders primarily as a measure of the value of selection for a particular character in various types of progenies and as an index of transmissibility of characters from parent to offspring (Hayes *et al.*, 1955). The

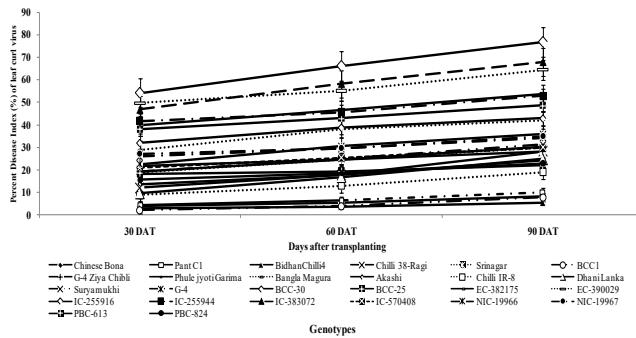


Fig. 1: Disease severity of chilli leaf curl virus (ChLCuV) at periodic intervals among chilli genotypes

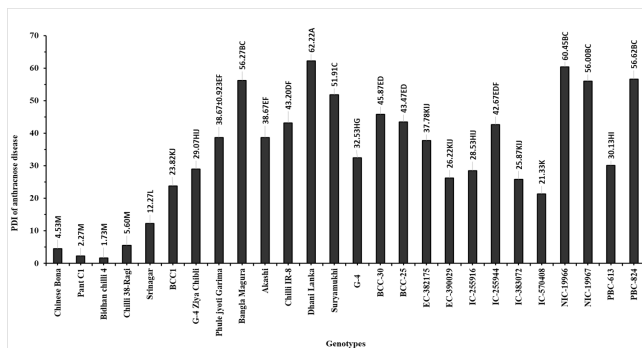


Fig. 2: Disease severity of chilli anthracnose among chilli genotypes under the artificial condition

concept of heritability is important to evaluate the relative magnitude of the effects of genes and environments on total phenotypic variability. For this reason, Burton (1952) stated that genetic variability, along with heritability, should be considered to assess the maximum and accurate effect of selection. High broad sense heritability (60% and above) occurred for all characters under study (Table 2). High heritability for fruit length, fruit diameter, and fruit weight supports the results of Chattopadhyay *et al.* (2011), and the other observations corroborate the findings of previous workers (Vaishnavi *et al.*, 2018; Bhutia *et al.*, 2015; Hasanuzzaman *et al.*, 2012; Rosmania *et al.*, 2016) who utilized other genotypes under different growing environments. High heritability indicates that the environmental influence is minimal on characters; the characters can be used for selection.

Genetic advance (GA) is an improvement in the performance of selected lines over the original population. It is not necessarily true that high heritability would always exhibit high genetic advances. For this reason, Johnson *et al.* (1955) stated that heritability in combination with genetic advance would be more reliable for predicting the effects of selection because genetic advance depends on the amount of genetic variability, the magnitude of the masking effect of genetic expression (environmental influence), and intensity of selection. In the present study, GA was very high

Table 1: Analysis of variance (ANOVA) for sixteen quantitative traits in chilli

Source of variation	Mean sum of square		
	Replication	Treatments	Error
Degrees of freedom	2	25	50
Plant height	0.7642	1249.7160**	110.3770
Plant spread	0.6132	1199.1614**	126.8037
Specific leaf weight	0.0004	2.5350**	0.0001
Number of branches per plant	0.0512	9.428205**	0.9712
Days to 50% flowering	0.6282	691.5261**	5.2015
Number of fruits per plant	9.2692	584.9994**	48.6425
Fruit retention	2.5512	8526.1153**	14.9246
Fruit length	0.0857	6.5694**	0.1129
Fruit diameter	0.0004	1.6731**	0.0124
Average fruit weight	0.0002	1.0448**	0.0029
Number of seeds per fruit	9.2692	584.9994**	48.6425
1000 seed weight	0.0008	2.6784**	0.0034
Days to ripe fruit maturity from anthesis	0.3589	157.5533**	2.7856
Fruit yield per plant	6.0823	6036.3140**	207.2829
PDI of ChLCuV	0.2648	1029.0531**	7.6296
PDI of chilli anthracnose	1.4221	1009.4875**	2.9737

** Significant at 0.05% level of probability

Table 2: Mean, range and estimates of genetic parameters of chilli genotypes

Characters	Range	GCV ^a (%)	PCV ^b (%)	GCV:PCV ^c	h^2 (%) in b.s.	Genetic advance as (%) of mean
Plant height	47.83-117.13	26.59	30.21	88.02	77.50	61.79
Plant spread	27.57-92.17	31.06	36.16	85.91	73.80	70.46
Specific leaf weight	3.20-6.59	19.32	19.32	99.98	99.00	51.00
Number of branches per plant	4.67-11.33	23.17	26.87	86.24	74.40	52.77
Days to 50% flowering	21.33-74.33	30.56	30.91	98.88	97.80	79.78
Number of fruits per plant	26.67-77.00	28.85	32.53	88.66	78.60	67.53
Fruit retention	28.00-252.33	56.28	56.43	99.74	99.50	148.21
Fruit length	3.70-9.47	21.28	21.83	97.48	95.00	54.77
Fruit diameter	0.45-2.75	47.80	48.34	98.89	97.80	124.81
Average fruit weight	0.64-2.88	37.85	38.02	99.58	99.20	99.53
Number of seeds per fruit	28.33-86.67	27.31	27.52	99.25	98.50	71.58
1000 seed weight	0.84-4.86	25.31	25.36	99.80	99.60	66.69
Days to ripe fruit maturity from anthesis	33.33-57.33	16.26	16.69	97.4	94.90	41.81
Fruit yield per plant	30.50-222.15	59.69	62.79	95.05	90.40	149.81
PDI ^e of ChiLCuV ^f	5.18-76.78	54.97	55.58	98.89	97.80	143.52
PDI of chilli anthracnose	1.73-62.22	54.26	54.50	99.56	99.10	142.62

^aGCV= Genotypic Coefficient of variation.^bPCV= Phenotypic Coefficient of Variation^cGCV:PCV (%)= ratio of the genotypic coefficient of variation and phenotypic coefficient of variation.^d h^2 in b.s. = Heritability estimate in broad sense^ePDI = Percent Disease Index^fChiLCuV = Chilli Leaf Curl Virus

(>100 %) for the fruit retention percentage, fruit diameter, fruit yield per plant, PDI of ChiLCuV, and PDI of chilli anthracnose diseases (Table 2). High GA (>20%) was also recorded for the characters plant height, plant spread, specific leaf weight, number of branches per plant, days to 50% flowering, number of fruits per plant, fruit length, average fruit weight, number of seeds per fruit, 1000 seed weight and days to ripe fruit maturity from anthesis.

High genetic advances for these traits have been previously reported (Kranthirekha *et al.*, 2016; Elahi *et al.*, 2017; Yogeshkumar *et al.*, 2018; Lakshmidheevamma *et al.*, 2021) using dissimilar genotypes and in different environments. Heritability in combination with a substantial amount of GA would be more reliable than heritability alone for predicting the effect of selection in segregating generations (Johnson *et al.*, 1955). These two genetic parameters can together substantiate the amount of genetic progress possible through normal selection. High heritability coupled with high estimates of GCV and GA for most traits under study offers opportunities for selection and indicates the presence of additive gene action, which would make the selection very effective (Panse, 1957).

Genotypic and phenotypic correlations among quantitative characters varied (Table 3). Most correlation

coefficients at the genotypic level were greater than the corresponding phenotypic ones. The higher values of genotypic than phenotypic correlation indicated that the genotypic effects were more important than environmental factors. In the presence of high environmental influence on the expression of characters, there is the possibility of overestimation of the genotypic correlation coefficient. Most characters exhibited significantly positive genotypic and phenotypic correlations with fruit yield per plant. PDI of ChiLCuV and chilli anthracnose disease were negatively correlated with fruit yield per plant. This indicated that a lower incidence of diseases helped to improve fruit yield per plant. Direct and indirect effects at the phenotypic level on fruit yield per plant varied (Table 3). Average fruit weight and number of fruits per plant had a positive, direct effect on fruit yield per plant, likely due to the positive association with fruit yield per plant. The direct effects of other characters were negligible. Direct selection could be beneficial for yield improvement since the number of fruits per plant and fruit weight exhibited significant, positive correlations with fruit yield per plant. High positive, direct effects of the number of fruits per plant and fruit weight on fruit yield per plant were obtained with other genotypes and environmental conditions by others (Naik *et al.*, 2010;

Table 3: Phenotypic and genotypic correlations among different characters of chilli and their direct effects on fruit yield per plant

Character	Genotypic correlation with fruit yield per plant	Phenotypic correlation with fruit yield per plant	Direct effects on fruit yield/ plant at phenotypic level ^a
Plant height	0.719**	0.472**	0.102
Plant spread	0.618**	0.647**	0.037
Specific leaf weight	0.474**	0.454**	-0.128
Number of branches per plant	0.554**	0.571**	0.030
Days to 50% flowering	-0.727**	-0.723**	-0.108
Number of fruits per plant	-0.329**	-0.360**	0.010
Fruit retention	0.742**	0.686**	0.108
Fruit length	0.450**	0.473**	-0.120
Fruit diameter	0.466**	0.474**	0.011
Average fruit weight	0.715**	0.704**	-0.019
Number of seeds per fruit	0.291**	0.263*	-0.058
1000 seed weight	0.709**	0.735**	0.574
Days to ripe fruit maturity from anthesis	0.795**	0.779**	0.674
PDI ^b of ChiLCuV ^c	-0.778**	-0.700**	0.073
PDI of chilli anthracnose	-0.557**	-0.547**	0.021

*** Significant at $p \leq 0.05$ or $p \leq 0.01$, respectively

^aResidual effect-0.1243

^bPDI = Percent disease index, ^cChiLCuV = Chilli leaf curl virus

Table 4: Cluster classification of chilli genotypes

Cluster	Name of the genotype with source of collection
Cluster I (4) ^a	Bangla Magura (W.B. ^b), NIC-19966 (NBPGR ^f), G-4 Ziya Chibli (W.B.), EC-382175 (NBPGR)
Cluster II (4)	Phule Jyoti Garima (MPKV ^g), PBC-613 (NBPGR), PBC-824 (NBPGR), IC-570408 (NBPGR)
Cluster III (3)	Dhani Lanka ((W.B.), Suryamukhi (W.B.), NIC-19967 (NBPGR)
Cluster IV (4)	EC390029 (NBPGR), IC-255944 (NBPGR), IC-255916 (NBPGR), IC-383072 (NBPGR)
Cluster V (3)	BCC-30 (BCKV ^h), BCC-25 (W.B.), G-4 (A.P. ^c)
Cluster VI (8)	Chilli 38-Ragi (W.B.), Srinagar (J & K ^d), Pant C 1 (U.K. ^e), Chinese Bona (W.B.), Akashi Lanka (W.B.), Chilli IR-8 (W.B.), Bidhan Chilli 4 (BCKV), BCC1 (BCKV)

^aValue in the parantheses after cluster number indicate number of genotypes.

^bW.B. = West Bengal

^cA.P. = Andhra Pradesh

^dJ & K = Jammu and Kashmir

^eU.K. = Uttarakhand

^fNBPGR = National Bureau of Plant Genetic Resources

^gMPKV = Mahatma Phule Krishi Vidyapeeth

^hBCKV = Bidhan Chandra Krishi Viswavidyalaya

Kumari *et al.*, 2010; Thakur *et al.*, 2019). The residual effect was very low indicating inclusion of maximum fruit yield per plant influenced characters in the analysis. Based on the degree of divergence (D^2 values), genotypes could be meaningfully grouped into 6 clusters (Table 4). Cluster VI had a maximum of 8 genotypes, followed by clusters I, II, and IV with 4 genotypes each; clusters III and V had 3 genotypes each. The grouping pattern of genotypes was random, indicating geographical diversity and genetic

divergence were unrelated. Genotypes grouped from different heterogeneous geographic regions in one cluster could result from a free exchange of breeding material from place to place either by farmers or breeders of different regions. The absence of a relationship between genetic diversity and geographical distance depicts that forces other than geographical origin, such as genetic drift, exchange of genetic stock, spontaneous mutation, and natural and artificial selection, are responsible for genetic diversity. The

selection of genotypes for the hybridization program should be based on genetic divergence rather than geographic divergence. Environmental influence on the composition of clusters occurs in chilli (Krishnamurthy *et al.*, 2013; Yatung *et al.*, 2014; Bhutia *et al.*, 2017).

The clustering pattern indicated that inter-cluster distance was higher than intra-cluster distance indicating high genetic diversity among genotypes taken under study (Table 5). Lower intra-cluster divergence indicated homogeneity among genotypes that were clustered together. The intra- and inter-cluster distance among the genotypes indicated cluster VI had the highest intra-cluster distance indicating genotypes in this cluster are diverse. At the inter-cluster level, the maximum inter-cluster value was in between cluster II and VI, followed by between cluster I, and V, indicating genotypes in these clusters had maximum divergence. Intermating between genotypes included in clusters VI, V, II and I would be expected to give transgressive segregates in advanced generations (Kalloo *et al.*, 1980).

The top characters which contributed maximum towards divergence were PDI of ChiLCuV followed by PDI of chilli anthracnose disease and plant height (Table 6). Such characters may be used in selecting genetically diverse parents for hybridization to exploit maximum heterosis or to accomplish selection coherently in segregating generations.

Cluster means of genotypes (Table 6) indicated mean values of clusters varied in magnitude for all 16 characters. Cluster VI showed the highest number of branches per plant, fruit retention percentage, fruit length, fruit diameter, 1000 seed weight, average fruit weight, and fruit yield per plant, followed by cluster V for maximum plant height, plant spread, specific leaf weight, number of seeds per

fruit, number of fruits per plant. Besides, cluster means for PDI of ChiLCuV and chilli anthracnose disease, days to 50% flowering and days to ripe fruit maturity from anthesis were found lowest in cluster VI. Genotypes belonging to cluster VI could be useful sources of genes for imparting tolerance against viral and fungal diseases by improving productivity with early plant types that can fit in any cropping system. Intercrossing among genotypes having outstanding mean performances can be used for improvement in chilli (Yogeshkumar *et al.*, 2018; Lakshmiddevamma *et al.*, 2021). High genetic diversity among genotypes exists along with strong relationships among them as indicated in the dendrogram (Fig. 3).

Differences in allele frequency (y) of parents and dominance effect (D) at various loci is the dependent factors for the expression of heterosis over mid-parents (H), i.e., $H = Dy^2$ (Falconer, 1981). A certain level of genetic diversity and degree of dominance are important factors for the expression of heterosis. A hybridization program involving highly divergent parents may produce transgressive segregates. There lies an optimal level of diversity beyond which heterosis may either decrease or might not increase due to unfavorable interaction of co-adopted gene complexes or physiological incompatibility (Dhillon *et al.*, 2004).

The PCA components with eigenvalues exceeding 1.0 explained 100% of the total variance (Table 7). The characters viz., PDI of ChiLCuV, PDI of chilli anthracnose disease and, plant height explained almost 100% contribution towards divergence and variable loadings for components PC₁ (PDI of ChiLCuV), PC₂ (PDI of chilli anthracnose disease), PC₃ (plant height) were shown in Table 8. The first component

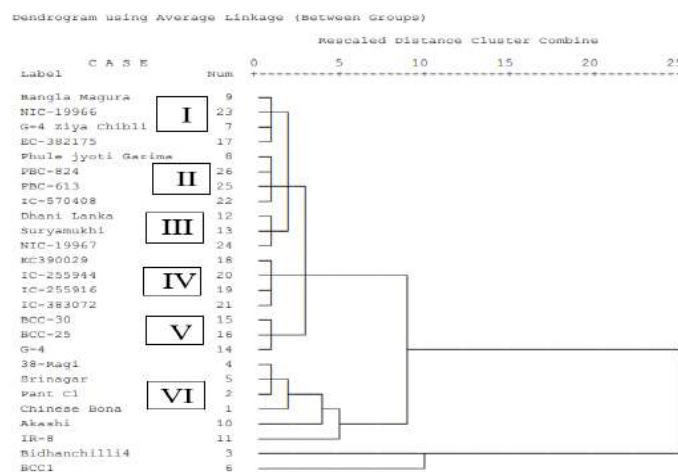


Fig. 3: Dendrogram of genotypes of chilli following Ward's method. Genotypes are in the leftmost column. The horizontal axis of the dendrogram represents the distance or dissimilarity between clusters. The vertical axis represents the objects and clusters. Each joining (fusion) of two clusters is represented on the graph by the splitting of a horizontal line into two. The horizontal position of the split, shown by the short vertical bar, gives the distance (dissimilarity) between the two clusters. Clusters are identified by Roman numerals. Genotypes placed in clusters based on 11 quantitative traits (Plant height, plant spread, specific leaf weight, number of branches per plant, days to 50% flowering, number of fruits per plant, fruit retention, fruit length, fruit diameter, average fruit weight, number of seeds per fruit, 1000 seed weight, days to ripe fruit maturity from anthesis, fruit yield per plant, PDI of ChiLCuV, PDI of Chilli anthracnose) all taken together.

Table 5: Inter and intra-cluster distances of chilli genotypes

Clusters	I	II	III	IV	V	VI
I	10730.050	38274.680	77275.630	85834.030	343362.600	232599.700
II		10968.067	161242.600	132961.900	324543.600	470168.000
III			13178.980	35155.390	134511.100	81509.670
IV				15284.670	121794.800	55772.160
V					15676.100	27353.170
VI						23246.430

^aBold diagonal values indicate intra-cluster distance; the remainder of values indicate the inter-cluster distances

Table 6: Cluster means of chilli genotypes

Characters	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	%contribution towards divergence
Plant height	51.45	66.91	78.74	66.98	95.66	94.74	10.65
Plant Spread	47.74	49.68	63.48	59.60	92.16	77.13	0.75
Specific leaf weight	4.16	3.58	4.82	5.04	6.59	5.91	0.45
Number of branches per plant	6.91	6.66	7.26	7.33	7.33	8.06	2.50
Days to 50% flowering	55.75	56.55	46.86	51.80	55.66	35.06	0.79
Number of fruits per plant	40.08	42.00	43.06	43.53	73.00	57.33	4.75
Fruit retention	64.66	58.05	95.46	84.06	84.33	174.26	0.50
Fruit length	5.33	6.58	7.47	6.70	7.30	8.03	2.29
Fruit diameter	1.57	1.24	1.49	1.38	1.93	2.07	1.36
Average fruit weight	1.05	1.25	2.22	1.24	1.16	2.04	0.21
Number of seeds per fruit	36.75	54.33	59.26	51.06	76.33	73.33	0.50
1000 seed weight	3.76	3.78	3.32	3.17	4.16	4.51	0.20
Days to ripe fruit maturity from anthesis	44.41	46.22	44.46	46.86	43.66	38.60	0.35
Fruit yield per plant	41.19	49.41	102.09	54.281	85.03	118.34	3.38
PDI ^a of ChiLCuV ^b	55.99	36.20	24.51	42.162	24.26	14.76	50.50
PDI of chilli anthracnose	34.02	44.62	29.11	40.711	38.66	17.21	20.82

^aPDI = Percent Disease Index

^bChiLCuV = Chilli Leaf Curl Virus

(PC₁) explained 52.67% of the total accounted for variance in which a decrease in PDI of ChiLCuV was associated with a decrease in PDI of chilli anthracnose disease but will eventually increase plant height. The second component (PC₂) explained 20.96% of the total accounted for variance in which a decrease in PDI of Chilli anthracnose disease was associated with an increase in PDI of ChiLCuV and plant height. There are no such guidelines for determining the importance of a trait coefficient for each principal component. Johnson and Wichern (1998) regard a coefficient greater than half of the coefficient, divided by the square root of the standard deviation of the eigenvalue of the respective principal component, to represent a significant difference.

The dendrogram was formed following Ward (1963) using squared Euclidean distance. There exists high diversity among chilli genotypes exhibiting strong relationships among genotypes (Fig. 3). All 26 genotypes were grouped into 6 distinct clusters based on average linkage between 2 clusters representing characteristic similarities and dissimilarities. The higher the rescaled distance joining a genotype, the higher the dissimilarities in characteristic features and vice versa. Genotypes that are close are perceived for their similarity in PCA; but the genotypes which are far apart are considered more diverse (Fig. 4). Genotypes 'Chinese Bona', 'Pant C 1', 'Bidhan Chilli 4', 'Chilli 38-Ragi', 'Srinagar', and 'BCC 1' were quantitatively dissimilar from others. The remainder of the genotypes had similar features,

Table 7: Results of principal component analysis (PCA) for quantitative characters contributing to divergence

Principal component (PC)	Eigenvalue	%Variance	%Cumulative Variance
PC1	3.16030215	52.67	52.67
PC2	1.25770058	20.96	73.63
PC3	0.67213122	11.20	84.84

^aEigenvalues and variance accounted for (%) by PCA based on the correlation matrix

Table 8: Contribution of diverse traits in the principal components of chilli

Variables	PC ₁ ^a	PC ₂	PC ₃
Factor loadings due to PCs with eigenvalues greater than 1			
PDI ^a of ChiLCuV ^b	-0.445696	0.419436	-0.046058
PDI of chilli anthracnose	-0.360723	-0.321216	0.768103
Plant height	0.464195	0.186041	0.436577

^aPC₁₋₃ = principal components 1-3

^aPDI = Percent disease index

^bChiLCuV = Chilli Leaf Curl Virus

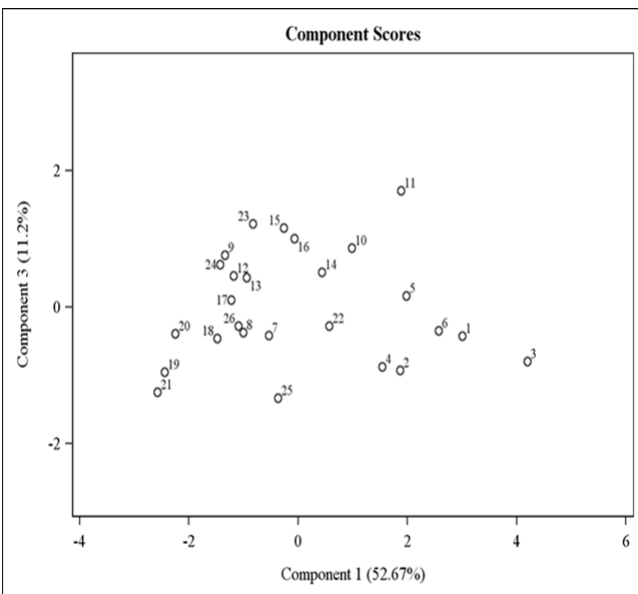


Fig. 4: Scatter diagram of regression factor scores for the first and third components as determined by principal component analysis. Points in the diagram closest to the intersection of 0 on the X- and Y-axes indicate similarity. Outliners on the X-axis, i.e. 1 = Chinese Bona, 2 = Pant C 1, 3 = Bidhan Chilli 4, 4 = Chilli 38-Ragi, 5 = Srinagar and 6 = BCC 1, indicate diversity. Numbers correspond to the names of the genotypes in Fig. 3.

forming a separate cluster. From PC₁ vs. PC₃ plot, selection may be refined considering all 3 components, with 'Bidhan Chilli 4' being the best performing genotype having high tolerance to both ChiLCuV and chilli anthracnose diseases and an optimum combination of all variables followed by

'Pant C 1', 'Chinese Bona' can be used as improved genetic material for breeding against ChiLCuV and chilli anthracnose diseases, respectively.

This was one of the few attempts to identify potential donors among *C. annuum* L. genotypes for developing multiple disease resistance (ChiLCuV and anthracnose) having preferred economic traits. Genetic divergence and geographic diversity were unrelated in chilli concerning the occurrence of ChiLCuV and anthracnose diseases. Based on D² statistics, PCA, yield potentiality, and reaction to both ChiLCuV and chilli anthracnose diseases, the genotypes, 'Bidhan Chilli 4', 'Pant C 1', and 'Chinese Bona' were identified as potential donors. The linkage between these two diseases in identified genotypes will help breeders to develop multiple disease-resistant varieties/hybrids to avoid the indiscriminate use of pesticides.

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RESEARCH ARTICLE

Evaluation of Long-Day Onion (*Allium cepa* L.) Genotypes for Yield and Quality Traits Under Temperate Conditions of Kashmir Valley in India

Geetika Malik, Rafiq A Shah, Alima Shabir, Vishal Dinkar*, Javid I Mir and Om C Sharma

Abstract

Onion (*Allium cepa* L.) is an important vegetable crop consumed throughout the world. Diverse crop germplasm is a prerequisite for the long-term success of breeding programs. We evaluated 50 long-day onion germplasm lines and two check varieties for phenotypic variation in 16 yield and quality traits. The genotype CITH-O-40 recorded the highest total yield and marketable yield, which can be attributed to the lowest bolter production, highest ABW and its fairly good polar and equatorial bulb diameter. The PCA-based hierarchical clustering divided the whole germplasm into three major clusters. Seven principal components (PC1 to PC7) explained ~77.8% of the total phenotypic variation. Polar diameter, neck thickness, percent bolters, marketable yield and total yield, constitute the PC1, the first main factor. Marketable yield showed a negative correlation with percent bolters and doubles production but had a significantly positive correlation with TSS content. Our results suggest that for increasing total bulb yield and marketable yield, the genotypes that produce a high percentage of bolters and doubles should not be considered in crop improvement programs. The current work elucidates the morphological variability present in the examined germplasm collection, which can be used for onion improvement breeding.

Keywords: *Allium cepa* L., Cluster analysis, Germplasm, Long day onion, Morphological variation, PCA.

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Introduction

Allium cepa L. is a highly valuable vegetable and spice reported to have originated in central Asia. The economic value of this crop derives from its culinary uses, nutritional benefits, and health-promoting properties (Benkeblia, 2005). Domestic and export demand is rising continuously; thus, high-yielding varieties with better quality traits is one of the primary breeding objectives. Genetic variability is essential to meet the diversified goals of plant breeding, such as higher yield, wider adaptation, desirable quality parameters, and resilience to biotic and abiotic stresses (Kushwaha *et al.*, 2021). The selection of genetically diverse parents is of immense importance for successful recombination breeding (Arunachalam, 1981; Akter *et al.*, 2015). Parents identified on the basis of the divergence analysis would be more promising in onion (Mohanty and Prusty, 2002). Knowledge of local populations can be useful in breeding location-specific cultivars since they possess balanced trait complexes suited for specific climatic and edaphic factors (Ricciardi *et al.*, 2020). These landraces and local cultivars often possess vast variability for agronomically important traits and are readily available for breeding elite cultivars without undesirable linkage drag (Swarup *et al.*, 2021). Thus, conservation and precise estimation of genetic variability within landraces are basic and continuous processes for crop improvement (Mondal, 2003; Verma *et al.*, 2008). Thus, germplasm evaluation in onion

and related *Allium spp.* is a preliminary and essential step for quantification of the potential value of germplasm line for breeding the specific trait as well for higher yield (Dhatt *et al.*, 2005; Khosa *et al.*, 2014).

Principal component analysis (PCA) is a multivariate analysis method that reduces complexity in multi-dimensional data while retaining trends and patterns and enabling an easier understanding of connections and impacts among different variables (Lever *et al.*, 2017). PCA identifies the key traits decisive for genotypic variation and differentiation (Kovacic, 1994).

The germplasm studied in this investigation belongs to the long-day category of onion, which is transplanted in November-December in the Kashmir region. Its bulbing starts in the month of April following dormancy during winters and growth resumption in March. The bulb growth and development enter well into the month of June when the day length reaches 14 hours and more. When tops wither or topple over, the bulbs are harvested by the end of June or the beginning of July. The bulb yield of long-day onions in Kashmir is higher than short-day/ intermediate day onions grown elsewhere. However, the storage life tends to be shorter due to high water content of large bulbs. To date, no public sector variety has been released/ recommended for the region, although an introduction, 'Brown Spanish' is increasingly becoming popular among onion growers due to consistent efforts by the Indian Council of Agricultural Research (ICAR)-Central Institute of Temperate Horticulture (CITH), Srinagar. ICAR-Directorate of Onion and Garlic Research (DOGR), Rajgurunagar, Pune, is also instrumental in intensifying research on long-day onion through its All-India Network Research Project on Onion and Garlic, which it operates in the region through ICAR-CITH, Srinagar. The project has tested many long-day onion entries from both the public and private sector at ICAR-CITH.

Keeping all the above facts in view, this study was undertaken to determine the nature and extent of variability in onion germplasm collection at ICAR-CITH, Srinagar, and to identify promising genotypes according to their genetic potential for their possible use in future improvement programs and the development of high-yielding cultivars of onion.

Materials and Methods

Plant material and Experimental design

The experimental material consisted of 50 germplasm collections of long-day onion (*Allium cepa* L.) along with two long-day check varieties viz., Brown Spanish and Yellow Globe. The study was conducted for three consecutive years from 2016 to 2018 at ICAR-CITH, Srinagar, Jammu & Kashmir, India. The Research Farm is situated at a latitude of 34° 05' N and a longitude of 74° 50' E, at an elevation of 1640 meters above mean sea level (MSL). The average monthly

meteorological data during the three cropping seasons of experimentation is given in Table S1.

The experiment was laid out in a randomized complete block design (RCBD). The sowing was done in August and transplantation in November/December at a spacing of 20 cm x 10 cm in 2.5 square meter plots per replication. Recommended NPK and S fertilizers were applied and appropriate plant protection measures were adopted for raising a good crop. Soil macronutrient status and other quality parameters at the experimentation block is given in Table S2. The observations were recorded on ten randomly selected plants of each germplasm line in each replication for 16 yield and quality parameters viz., plant height (cm), number of leaves/plant, neck thickness (cm), polar diameter (mm), equatorial diameter (mm), Polar diameter/Equatorial diameter (P:E ratio), percent 'A grade' bulbs, percent 'B grade' bulbs, percent 'C grade' bulbs, percent bolters, percent doubles, percent unmarketable bulbs, marketable yield (q/ha), total yield (q/ha), average bulb weight (g) and total soluble solids (%).

Plant height was measured with a scale from ground level to the top of the plant just before the crop ripeness. The number of leaves per plant was counted along with the plant height measurements. Neck thickness and polar and equatorial diameters were measured with the Vernier Caliper. Percent bolters and percent doubles were calculated on a count basis in the field and average bulb weight was calculated by dividing the total yield of the plot by a total number of bulbs. Total and marketable bulb yields (q/ha) were determined on the basis of plot yield. Based on the recommendations of ICAR-DOGR, Pune, onion bulbs were categorized into three grades on the basis of bulb size, i.e., A grade (>4.5 cm), B grade (3.5–4.5 cm) and C grade (2.5–3.5 cm). Total soluble solids (TSS) readings were taken 15–20 days after bulb harvesting by 'Hand-held Digital Refractometer' (ATAGO®, Japan). Harvesting was done in the third to fourth week of June.

Statistical analysis

The data of three years was pooled, and mean descriptive statistics were carried out using SAS statistical analysis software (SAS Institute Inc., 2017). The significant differences were detected using one-way analysis of variance (ANOVA) between the mean values of all characters recorded among the studied genotypes. The least significance difference (LSD) was calculated at 5% level of significance to test the differences between the means of tested genotypes. Pearson's correlation coefficient was calculated to analyze the correlations between the studied traits.

PCA and cluster analysis

Principal components analysis (PCA) was done to identify major traits accounting for the variation among the studied onion genotypes. The PCA was based on the correlation

matrix, and the number of significant principal components was determined based on the Kaiser criterion, retaining any component with an Eigenvalue greater than 1 (Kaiser, 1960). Following PCA, the squared cosine ($\cos^2$) was computed, which gave the quality of representation of the variables on the factor map and the total contribution of individual traits. $\cos^2$ demonstrates the importance of a component for a given observation and is important in identifying which component to make an inference on (Adu *et al.*, 2018). For a given trait, the sum of the $\cos^2$ on all dimensions is equal to 1, whereupon if the trait is perfectly represented by only two dimensions (PC1 and PC2), the sum of the $\cos^2$ on these two dimensions is 1 (Kassambara, 2017). The contribution, explaining the variations retained by two PCs (PC1 and PC2), is given by the formula (Kassambara, 2017):

$$[(C1 \times \text{Eig1}) + (C2 \times \text{Eig2})] / (\text{Eig1} + \text{Eig2})$$

Where C1 and C2 are the contributions of the variable on PC1 and PC2, respectively, and Eig1 and Eig2 are the Eigenvalues of PC1 and PC2, respectively. To identify groups of genotypes with similar key quantitative traits, cluster analysis was performed using traits identified by the PCA as key contributing variables to genetic variation. PCA and Hierarchical Clustering on Principal Components (HCPC) were performed using R software v3.6.3 (2020).

Results and Discussion

The mean values and range of 16 yield and quality traits of 50 long-day onion genotypes and two check varieties from pooled data of the three years (2016-18) is presented in Table 1. The bulb diameter (polar and equatorial) and the P:E ratio determines the shape of the bulb and its storability. The maximum polar diameter (85.78 mm) was found in the genotype CITH-O-11, while the minimum (41.64 mm) was found in CITH-O-33. The equatorial diameter ranged from 46.11 mm to 84.53 mm. The highest equatorial diameter (84.53 mm) was recorded in CITH-O-33, while the smallest (41.64 mm) in CITH-O-13. The average P:E ratio ranged from 0.60 to 1.72 cm and the highest P:E ratio (1.72) was recorded in CITH-O-13, while the smallest (0.60) in CITH-O-2. Neck thickness has a significant effect on bulb storability and quality. The onion genotype showed a wide range in neck thickness ranging from 0.69 to 3.59 cm. The highest neck thickness (3.59 cm) was observed in CITH-O-24, whereas the lowest (0.69 cm) was observed in CITH-O-5.

Marketable yield depends upon the number and weight of A, B, and C grade bulbs and will determine the value of the produce. The yield of 'A grade' bulbs during three years ranged from 18.55 to 41.55%. The maximum 'A grade' bulbs (41.55%) were found in genotype CITH-O-15, while the lowest (18.55%) was found in check variety Yellow Globe. Similarly, the yield of 'B grade' bulbs ranged between 13.94 to 37.95% and 'C grade' bulbs ranged between

9.26 to 42.43%. In onions, the production of doubles and bolter bulbs are genetically determined parameters that deteriorate marketable bulb yield and quality (Abdalla and El Hassan, 1975; Rabinowitch, 1979). Bolted bulbs show poor shelf life and cannot be stored for a longer period of time. Thus, the identification of genotypes with minimum bolting is desirable. Double bulb production in the onion crop is undesirable for the processing industry and leads to lower market value.

In our study, the percent bolters production ranged from 2.05 to 18%. The maximum percent bolters were recorded in CITH-O-20 (18%), while the minimum was recorded in CITH-O-31 (2.05%). The percent doubles ranged from 1.04 to 7.35%. The maximum percent doubles (7.35%) were recorded for genotype CITH-O-1, while minimum percent doubles were found in CITH-O-44 (1.04%). The maximum percent of unmarketable bulbs was found in CITH-O-48 (62.75%), while the minimum percent of unmarketable bulbs was recorded in genotype CITH-O-40 (11.85%). Average bulb weight (ABW) determines the marketable yield and total yield of the crop. The ABW ranged from 90.07 to 365.74 g. The maximum average bulb weight (365.74 g) was found in CITH-O-40, while the lowest (90.07 g) was found in CITH-O-11. The average marketable yield ranged from 290.47 to 1582.46 q/ha. The maximum average marketable yield (1582.46 q/ha) was recorded in CITH-O-40, while the lowest (290.47 q/ha) in CITH-O-10. The average total yield ranged from 329.05 to 1642.38 q/ha. The maximum average total yield (1642.38 q/ha) was recorded in CITH-O-40, while the lowest (329.05 q/ha) in CITH-O-10. Trivedi *et al.* (2006) have indicated that onion yield improvement could be possible by increasing the equatorial and polar diameters of bulbs, average bulb weight and percent of 'A' grade bulbs. This is evident from the data that genotype CITH-O-40 topped the list of several desirable traits analyzed in our study, such as the highest total yield and highest marketable yield, which can be attributed to the lowest bolter production, highest ABW and fairly good polar and equatorial diameter of bulb. The genotype CITH-O-45 recorded the highest total soluble solids (TSS) content, *i.e.*, 14.54%. Genotypes CITH-O-36 (14.26%) and CITH-O-44 (14.06%) also recorded very high TSS content. Thus, these three genotypes *viz.*, CITH-O-36, CITH-O-44 and CITH-O-45, can serve as breeding material for high TSS content.

Correlation analysis

Pearson's correlation between different parameters evaluated is presented in Table 2. Several studies have shown that significant genetic variation exists for yield and yield-related traits *viz.*, polar diameter, equatorial diameter, neck thickness and ABW in onion (Trivedi *et al.*, 2006; Santra *et al.*, 2017; Barakat *et al.*, 2021). In our study, total bulb yield showed a significant positive correlation with marketable yield ($r = 0.986$) and TSS ($r = 0.339$) and

Table 1: Mean values and range of 16 yield and quality traits of 50 onion genotypes and two check varieties evaluated under temperate conditions of Kashmir, India

Genotypes	Plant height (cm)	Range	No. of leaves/plant	Range	Polar diameter (mm)	Range	Equatorial diameter (mm)	Range	P:E ratio	Range	Neck thickness (cm)	Range	AGB (%)	Range	BGB (%)	Range
CITH-O-1	38	14-71	9	7-12	44	35-60	63	58-72	1	1	1	1-2	23	18-30	34	28-44
CITH-O-2	41	17-73	9	7-10	47	36-66	77	73-83	1	1	1	1-2	26	19-37	33	21-43
CITH-O-3	60	43-73	9	7-10	68	61-73	76	71-80	1	1	1	1-4	26	17-32	32	25-38
CITH-O-4	49	34-59	9	7-11	52	42-58	75	73-77	1	1	1	0-3	26	12-33	25	22-29
CITH-O-5	58	33-73	9	7-11	67	57-73	69	59-74	1	1	1	1-2	30	11-50	22	18-30
CITH-O-6	55	35-64	8	7-11	61	61-62	55	54-56	1	1	1	1-3	29	15-37	21	15-28
CITH-O-7	52	33-63	9	7-12	68	58-86	76	74-78	1	1	3	1-7	30	26-32	21	13-31
CITH-O-8	60	35-81	9	7-10	53	51-57	75	68-78	1	1	3	1-7	32	23-40	19	13-29
CITH-O-9	63	35-87	9	7-10	59	47-66	82	76-86	1	1	1	1-3	32	12-53	22	20-24
CITH-O-10	57	37-73	8	7-9	62	54-78	64	62-67	1	1	1	1-2	30	23-37	19	13-25
CITH-O-11	62	39-86	8	7-9	86	83-90	65	48-83	1	1	2	1-6	30	25-34	24	7-42
CITH-O-12	54	36-74	8	7-8	56	52-61	73	69-76	1	1	2	1-4	34	32-35	22	10-29
CITH-O-13	59	37-76	8	7-9	79	73-89	46	42-54	2	2	2	1-5	31	28-36	23	19-25
CITH-O-14	55	34-76	8	7-10	56	54-57	64	61-67	1	1	2	1-5	33	29-40	26	25-29
CITH-O-15	58	35-72	9	7-10	59	52-64	68	65-73	1	1	1	1-2	42	10-50	27	24-30
CITH-O-16	50	35-60	9	7-11	71	69-73	81	79-84	1	1	3	1-8	29	22-34	20	17-25
CITH-O-17	52	34-68	10	7-16	62	59-64	76	73-80	1	1	1	1-3	28	22-35	25	22-28
CITH-O-18	55	36-75	8	7-11	68	67-68	77	76-80	1	1	3	1-9	37	28-47	29	24-32
CITH-O-19	59	35-76	9	7-10	54	50-57	82	80-84	1	1	3	1-9	31	29-36	28	23-36
CITH-O-20	58	35-76	9	7-11	62	58-64	63	61-65	1	1	2	1-6	26	18-39	26	11-47
CITH-O-21	55	36-77	8	7-11	65	64-66	81	71-87	1	1	3	1-9	34	26-40	23	20-25
CITH-O-22	48	35-55	8	7-9	63	62-63	69	67-72	1	1	1	1-2	27	17-39	26	21-30
CITH-O-23	51	35-68	11	7-17	51	51-52	67	67-68	1	1	1	1-3	33	35-38	14	6-23
CITH-O-24	54	35-74	9	7-8	51	52-52	75	74-76	1	1	4	1-10	24	15-35	22	15-32
CITH-O-25	50	41-56	7	7-9	70	56-90	57	48-65	1	1	1	1-2	28	18-36	38	9-59
CITH-O-26	55	40-81	9	7-10	63	62-63	81	77-84	1	1	3	1-6	28	13-36	20	15-25
CITH-O-27	60	33-84	9	7-12	68	64-74	64	61-68	1	1	1	1-3	34	23-44	30	26-36
CITH-O-28	54	33-70	10	7-14	64	64-65	77	61-82	1	1	1	1-3	39	34-43	30	23-43

CITH-O-29	48	35-63	8	7-10	56	46-72	70	67-72	1	1	3	1-7	24	7-42	23	18-28
CITH-O-30	50	37-62	8	7-8	56	56-57	71	70-72	1	1	1	1-2	31	31-34	21	18-25
CITH-O-31	49	41-58	9	7-10	61	59-62	70	68-73	1	1	3	1-9	27	16-32	20	16-23
CITH-O-32	42	31-51	8	7-8	48	43-52	67	65-71	1	1	2	1-6	23	14-35	29	23-35
CITH-O-33	60	39-87	8	7-10	42	41-42	85	84-86	0	0	2	1-6	33	35-36	19	11-25
CITH-O-34	48	35-55	7	6-8	58	51-62	81	76-83	1	1	1	1-2	30	23-37	28	22-38
CITH-O-35	51	37-61	8	7-10	56	52-61	74	72-77	1	1	2	1-6	33	24-40	30	9-55
CITH-O-36	53	31-65	8	7-8	54	53-56	76	61-83	1	1	1	1-3	36	10-44	22	15-35
CITH-O-37	53	34-72	8	7-8	51	46-55	68	67-70	1	1	1	1-2	32	9-40	22	19-25
CITH-O-38	47	32-63	7	7-8	67	52-82	66	58-71	1	1	1	1-2	33	30-37	25	20-31
CITH-O-39	54	35-69	7	7-8	65	53-72	72	69-75	1	1	1	1-2	41	28-61	18	4-25
CITH-O-40	55	35-71	9	7-11	65	52-72	83	64-92	1	1	1	1-2	27	19-36	35	25-43
CITH-O-41	58	38-79	9	8-12	62	61-63	73	71-75	1	1	1	1-2	21	11-29	34	30-41
CITH-O-42	57	36-74	7	6-9	63	51-70	78	68-83	1	1	1	1-3	29	22-33	28	19-34
CITH-O-43	59	39-84	8	8-8	50	49-52	67	59-72	1	1	2	1-4	32	28-34	19	14-22
CITH-O-44	51	38-60	8	7-8	68	58-74	84	71-92	1	1	1	1-3	33	21-44	26	22-30
CITH-O-45	55	37-66	8	7-9	61	52-79	69	63-73	1	1	3	1-7	37	31-45	26	18-32
CITH-O-46	53	37-71	10	7-13	52	50-54	57	53-64	1	1	1	1-2	29	20-304	18	14-22
CITH-O-47	49	33-71	8	7-10	66	65-67	76	75-77	1	1	1	1-2	28	13-37	18	13-19
CITH-O-48	48	38-53	8	7-9	56	55-57	79	71-84	1	1	2	1-5	20	4-28	26	16-35
CITH-O-49	45	42-53	7	6-8	79	56-126	59	54-66	1	1-2	1	1-23	20	13-28	36	26-48
CITH-O-50	61	61-64	8	8-8	54	53-56	67	58-74	1	1	1	1-24	21	5-30	17	15-17
Yellow Globe	57	29-68	8	5-9	57	52-60	67	73-83	1	1	1	1-2	19	18-19	29	27-34
Brown Spanish	34	28-45	7	4-7	57	67-73	63	57-86	1	1	1	1-2	24	8-37	26	25-47
Minimum	34		7		42		46	0			1		19		14	
Maximum	63		11		86		85	2			4		42		38	
L.S.D. (at 5%)	30		3		5		11	0			1		16		14	

To be continued ...

Abbrev.: P:E ratio (Polar: Equatorial diameter ratio); ABG (A grade bulb); BGB (B grade bulb); CGB (C grade bulb); ABW (Average bulb weight); TSS (Total soluble solids).
Footnote: All the values are presented up to one place of decimal to the nearest integer of the actual value.

Genotypes	CGB (%)	Range	Bolters (%)	Range	Doubles (%)	Range	Unmarketable bulbs (%)	Range	Marketable yield	Range	Total yield (q/ha)	Range	ABW (g)	Range	TSS (%)	Range
CITH-O-1	28	8-35	6	2-15	7	2-10	28	13-70	558	192-775	742	683-814	141	129-163	8	7-10
CITH-O-2	31	19-38	6	3-11	3	2-3	27	9-14	796	192-1071	1007	683-1102	148	129-163	11	10-13
CITH-O-3	23	23-31	6	0-17	1	0-2	25	8-21	1072	494-1418	1117	548-1453	151	131-164	14	13-15
CITH-O-4	24	24-42	6	0-17	3	0-5	26	24-76	923	502-1196	964	555-1226	115	102-133	10	7-15
CITH-O-5	26	23-40	4	0-13	3	0-5	26	9-19	788	428-993	855	470-1061	214	113-279	10	6-16
CITH-O-6	29	28-33	7	1-22	2	0-5	32	28-28	761	745-763	808	790-817	191	188-196	6	6-6
CITH-O-7	32	11-38	11	2-31	4	0-7	39	10-43	556	525-629	630	629-688	125	116-136	11	7-12
CITH-O-8	31	19-32	6	3-19	5	0-5	35	16-28	811	401-1057	864	476-1083	111	106-115	12	9-15
CITH-O-9	21	20-39	4	0-10	6	3-8	31	7-25	580	523-629	630	554-687	217	136-267	13	12-14
CITH-O-10	27	0-19	12	2-30	5	2-9	40	10-45	290	97-389	329	112-454	180	134-259	13	5-20
CITH-O-11	18	6-22	13	0-39	6	2-9	31	17-54	480	303-586	516	332-610	90	80-96	9	6-14
CITH-O-12	25	7-29	11	1-33	3	0-7	30	6-50	639	580-686	665	605-714	149	141-161	9	7-12
CITH-O-13	27	15-32	10	0-26	3	1-7	32	20-36	719	592-937	765	647-1010	92	81-154	12	8-9
CITH-O-14	23	11-26	7	0-22	5	0-8	25	17-33	613	473-685	652	529-717	114	105-126	13	9-19
CITH-O-15	13	12-20	13	0-32	2	1-6	31	15-40	635	432-971	464	324-540	158	96-196	11	10-12
CITH-O-16	35	12-44	9	0-27	2	0-4	40	17-39	889	677-1015	938	726-1051	195	166-216	10	9-12
CITH-O-17	29	13-36	10	0-29	3	0-5	29	15-35	589	573-601	630	605-647	200	154-274	8	7-9
CITH-O-18	9	5-17	6	0-19	4	0-6	25	12-32	749	592-854	797	674-882	210	160-295	9	8-13

CITH-O-19	28	18-32	7	0-22	2	0-5	26	0-30	719	516-856	750	489-900	126	118-136	14	12-15
CITH-O-20	24	5-26	18	0-54	3	0-8	47	5-66	467	410-568	507	451-580	118	105-137	13	10-16
CITH-O-21	22	17-24	6	0-17	2	0-7	24	12-31	599	436-698	637	491-726	111	105-118	11	8-16
CITH-O-22	21	5-22	16	0-45	4	0-9	44	22-57	606	470-707	665	559-759	208	133-263	11	7-17
CITH-O-23	29	11-15	10	1-29	4	0-11	46	37-48	461	424-544	513	454-596	176	175-178	11	11-11
CITH-O-24	29	13-28	10	0-30	2	1-7	49	33-42	634	629-661	697	689-711	170	163-172	9	8-9
CITH-O-25	12	8-14	14	0-41	2	0-6	44	14-53	522	470-564	556	541-631	143	128-160	12	11-13
CITH-O-26	16	15-16	17	1-44	5	0-8	42	28-56	483	422-594	507	452-653	228	156-289	11	10-12
CITH-O-27	19	7-29	3	2-9	4	0-3	27	13-23	1370	362-1918	1400	436-1962	152	105-176	12	8-19
CITH-O-28	20	14-19	3	0-6	2	0-5	22	0-20	715	604-797	735	622-826	191	147-259	11	9-15
CITH-O-29	23	17-34	9	0-26	4	0-11	32	14-43	361	255-518	383	282-600	110	82-144	12	8-17
CITH-O-30	23	18-23	5	1-15	4	0-12	30	14-34	344	297-357	375	329-383	174	170-176	8	8-9
CITH-O-31	23	22-62	2	0-5	3	1-3	48	7-24	1304	570-1722	1344	616-1758	148	140-156	8	7-10
CITH-O-32	35	19-45	8	0-23	1	0-4	20	5-31	740	366-963	758	388-994	131	92-150	11	8-16
CITH-O-33	19	18-20	6	0-18	3	1-8	48	23-34	491	458-532	529	477-596	126	124-128	8	7-8
CITH-O-34	28	10-31	4	0-11	3	0-8	31	22-23	1130	546-1479	1156	561-1509	250	133-322	12	11-13
CITH-O-35	15	12-18	4	0-11	5	0-13	24	9-34	564	513-589	584	543-619	142	137-149	11	10-12
CITH-O-36	31	21-47	3	1-7	4	1-6	35	20-28	418	278-495	446	337-539	117	81-151	14	13-16
CITH-O-37	31	5-41	13	0-31	6	0-9	33	27-44	491	309-587	594	413-865	160	99-191	8	6-10

CITH-O-38	24	20-30	7	0-14	2	2-4	28	12-20	1023	425-1375	1054	464-1413	192	111-241	14	13-14
CITH-O-39	23	5-25	5	0-16	2	0-5	29	25-31	878	320-1163	935	391-1283	176	94-239	8	7-8
CITH-O-40	20	19-36	3	0-7	3	0-5	12	5-11	1582	512-2124	1642	549-2255	366	132-514	13	12-13
CITH-O-41	23	5-25	15	0-44	2	0-7	41	13-53	743	598-820	787	659-909	244	158-292	13	13-14
CITH-O-42	26	12-27	6	0-17	5	1-10	24	10-32	562	533-590	595	494-655	316	142-405	13	11-14
CITH-O-43	26	15-28	12	1-19	6	1-12	30	4-38	340	240-430	404	369-537	152	119-194	10	8-13
CITH-O-44	19	15-22	8	0-24	1	0-3	27	11-34	688	507-782	758	560-959	155	134-170	14	13-16
CITH-O-45	22	13-27	5	0-13	4	0-6	17	10-27	518	402-578	550	518-643	123	107-138	15	14-15
CITH-O-46	16	4-20	18	3-45	4	0-7	43	22-57	518	454-641	549	499-670	218	160-499	13	8-20
CITH-O-47	23	24-29	9	0-28	3	0-9	42	34-44	514	510-524	557	553-573	202	201-209	13	13-13
CITH-O-48	31	25-42	4	0-11	4	0-12	63	19-31	501	478-543	556	505-620	144	127-159	11	7-13
CITH-O-49	22	20-23	8	0-23	5	4-10	27	19-39	344	291-430	395	381-570	126	117-132	9	6-7
CITH-O-50	42	8-60	9	0-27	4	0-12	34	4-45	412	282-526	446	406-555	106	119-141	14	13-14
Yellow Globe	37	35-39	3	1-5	2	2-5	27	13-44	758	561-963	914	537-1117	250	129-299	12	10-12
Brown Spanish	34	21-40	2	0-2	2	0-2	29	5-35	666	366-1480	698	372-1760	132	128-374	9	10-13
Minimum	9		2		1		12		290		329		90		6	
Maximum	42		18		7		63		1582		1642		366		15	
L.S.D. (at 5%)	12		23		6		32		493		507		93		5	

Table 2: Pearson's correlation between different yield and quality traits studied in onion germplasm under the temperate conditions of Kashmir, India

Parameters	Plant height (cm)	No. of leaves per plant	Polar diameter (mm)	Equatorial diameter (mm)	P:E ratio	Neck thickness (cm)	AGB (%)	BGB (%)	CGB (%)	Bolters (%)	Doubles (%)	Unmarketable (%)	Marketable yield (q/ha)	Total yield (q/ha)	TSS (%)
No. of leaves per plant	ns	1													
Polar diameter (mm)	ns	ns	1												
Equatorial diameter (mm)	ns	ns	ns	1											
P:E ratio	ns	ns	0.806**	-0.684**	1										
Neck thickness (cm)	ns	ns	ns	0.241*	ns	1									
AGB (%)	0.327**	ns	ns	ns	ns	ns	1								
BGB (%)	-0.253**	ns	ns	ns	ns	ns	-0.265**	1							
CGB (%)	ns	ns	-0.305*	ns	-0.232*	ns	-0.460**	-0.239*	1						
Bolters (%)	ns	ns	ns	-0.285**	0.260**	ns	ns	ns	ns	1					
Doubles (%)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	1				
Unmarketable (%)	ns	ns	ns	ns	ns	ns	-0.264*	ns	ns	0.423**	ns	1			
Marketable yield	ns	ns	ns	ns	ns	ns	ns	0.281*	ns	-0.438**	-0.391**	-0.286*	1		
Total yield (q/ha)	ns	ns	ns	ns	ns	ns	ns	0.298*	ns	-0.452**	-0.339**	-0.281*	0.986**	1	
TSS (%)	ns	ns	ns	0.292*	ns	-0.331**	ns	ns	ns	ns	ns	ns	0.344**	0.339**	1
ABW (g)	0.266*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Abbrev.: P:E ratio (Polar: Equatorial diameter ratio); AGB (A grade bulb); BGB (B grade bulb); CGB (C grade bulb); ABW (Average bulb weight); TSS (Total soluble solids).

* Correlation is significant at the 0.05 level. ** Correlation is significant at the 0.01 level. ns: non-significant

a significant negative correlation with percent bolters ($r = -0.452$) and percent doubles ($r = -0.339$). Concurrently, marketable yield showed a significant negative correlation with percent bolters ($r = -0.438$) and percent doubles ($r = -0.391$). However, marketable yield showed a significant positive correlation with TSS ($r = 0.344$). Previously, Arya *et al.* (2017), while studying twenty-six onion accessions, found a significant negative correlation between percent doubles, percent bolters and marketable yield and a positive correlation with TSS. Therefore, for increasing total bulb yield and marketable yield, the genotypes that produce a high percentage of bolters and doubles should not be considered in crop improvement programs. Onion TSS content determines the suitability to storage and recovery of dehydrated onion. TSS content is affected by various factors such as nutrient application, row spacing, regional condition and storage conditions (Tekle, 2015; Lee *et al.*, 2016; Bekele *et al.*, 2018; Islam *et al.*, 2019). Studies regarding the association of plant height and biomass accumulation with TSS content are scanty. In our study, TSS content and neck thickness are negatively associated. This is obvious because the wider neck of onion bulbs is disadvantageous as it leads to post-harvest moisture loss and, thus, decreases the TSS content. TSS content was found to be positively correlated with equatorial diameter and interestingly, in turn, equatorial diameter is negatively associated with bolters production, which is an undesirable feature. Thus, the genetic basis of such associations needs to be investigated.

Principal Component Analysis (PCA)

The aim of factor analysis is to reduce the number of effective parameters to discriminate genotypes. PCA reduces the dimensionality of the data by transforming initial variables into a new small set of variables without losing the important information in the original variables. Seven principal components, PC1 to PC7, were extracted from the original data, which had latent roots >1 , and accounted for nearly 77.8% of the total variation. Results of the PCA analysis. *i.e.*, Eigenvalues, the proportion of variance explained and the cumulative proportion for the extracted PCs, is presented in Table 3. PCA showed that 11 of the studied parameters accounted for 47.3% of the variance as the three main factors, with the other 5 parameters scattered within four factors determined as 77.8% of the total variance. PCA of quantitative traits in onion genotypes revealed maximum variation and the first seven PCs showed Eigenvalues >1 , where PC1 possessed the highest Eigenvalue (3.12). Out of seven major PCs extracted, the PC1 showed significant factor loadings for morphological traits *viz.*, polar diameter (0.537), percent bolters (0.356), no. of leaves/plant (0.347) and neck thickness (0.275). Arya *et al.* (2017) observed that high positive loading from onion average bulb weight, bulb yield and high negative loading from leaf length, double/deformed bulb in PC1 contributed more towards

differentiating the clusters. Similarly, Singh *et al.* (2013) observed that in the first principal component for onion plant height, marketable yield, bulb polar diameter and bulb neck thickness were the major contributors. These findings show that our results are in agreement with previous findings. Hanci and Gokce (2016) reported that the characters contributing more positively to PC1 were bulb weight and diameter of the pseudo stem.

The first two principal coordinates were used to create an outline of the differences and relationships between the genotypes by measuring the quality of representation ($\cos^2$) of individuals on the factor map (Fig. 1). The first factor separated genotypes CITH-O-40, CITH-O-34, and CITH-O-27 and CITH-O-3 on the side of factor plot that has the highest number of traits like marketable yield, total yield and polar diameter and were the most prominent genotypes contributing towards PCA. Also, the genotypes CITH-O-11 and CITH-O-13 settled in the upside of factor plot that have lower amounts for these traits and were the least contributing individual factors. Dangi *et al.* (2018), on the basis of squared cosine value ($\cos^2$) for individual factors on the first two principal coordinates, also found the most prominent genotypes contributing towards PCA as well as the least contributing individual factors.

Hierarchical clustering on principal components (HCPC)

In the present investigation, hierarchical clustering was performed by using Ward's criterion on the selected principal components. Ward's criterion is based on multi-dimensional variance like PCA. The PCA step is considered a denoising step, which can lead to a more stable clustering to identify groups (*i.e.*, clusters) of similar objects within a data set of interest. Our PCA results showed that PC1, PC2 and PC3 explained 19.56, 15.46, and 12.27% variability, respectively and contributed 47.3% of the total variation in the data sets, suggesting these PC scores might be used to summarize the original 16 variables in any further analysis of the data. Characters with the largest absolute value closer to unity within the first PC influence the clustering more than those with lower absolute value closer to zero. Therefore, in the present study, the first three principal components were taken because of the relatively high contribution of these components rather than the small contribution from each principal component. The cluster analysis performed on the PCA results suggested a three-cluster solution (Fig. 2). The partitioning into three clusters is represented on the factor map produced by the first two principal components and the individuals are colored according to their clusters (Fig. 3). The barycentre of each cluster is also represented by triangle, circle and square. The graph shows that the three clusters are well-separated on the first two principal components. Superimposing the individuals on the principal component map suggested that cluster

Table 3: Principal components analysis for 16 traits in onion genotypes evaluated under temperate conditions of Kashmir, India

PCA	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Eigenvalues	3.126	2.481	1.975	1.424	1.296	1.134	1.014
Proportion of variance	0.1956	0.1546	0.1227	0.0872	0.0829	0.072	0.063
Cumulative Proportion	0.196	0.350	0.473	0.560	0.643	0.715	0.778
Variables	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7
Plant height (cm)	0.198	0.489	0.28	0.251	0.253	0.158	0.411
No. of leaves/plant	0.347	0.449	-0.189	0.584	0.312	-0.41	-
Polar diameter (mm)	0.537	0.11	0.101	-0.347	0.229	-0.458	-
Equatorial diameter (mm)	-0.226	-0.333	0.334	-0.129	0.101	0.129	-0.29
P:E ratio	0.106	0.585	-0.124	0.112	-0.155	0.115	-
Neck thickness (cm)	0.275	-0.571	0.197	-0.455	0.24	0.111	0.246
%A grade bulb	0.135	0.458	-0.412	-0.26	0.227	-0.315	-0.161
%B grade bulb	-0.262	0.18	-0.31	0.192	-0.525	0.32	0.125
%C grade bulb	-0.317	-0.3	0.146	-0.184	0.371	0.467	0.145
%Bolters	0.356	0.158	0.122	0.337	0.254	-0.191	-0.115
%Doubles	0.257	-0.262	0.167	-0.158	0.795	0.132	-0.324
%Unmarketable	0.294	-0.137	0.495	-0.163	-0.317	-0.469	-0.187
Marketable yield(q/ha)	-0.499	0.226	-0.172	-0.279	0.127	-	-
Total yield (q/ha)	-0.496	0.231	-0.173	0.16	-0.285	-	-
TSS (%)	-0.271	0.191	0.565	-0.108	0.182	-0.395	0.175
ABW (g)	0.122	0.27	0.745	-0.132	0.234	-0.402	-0.192

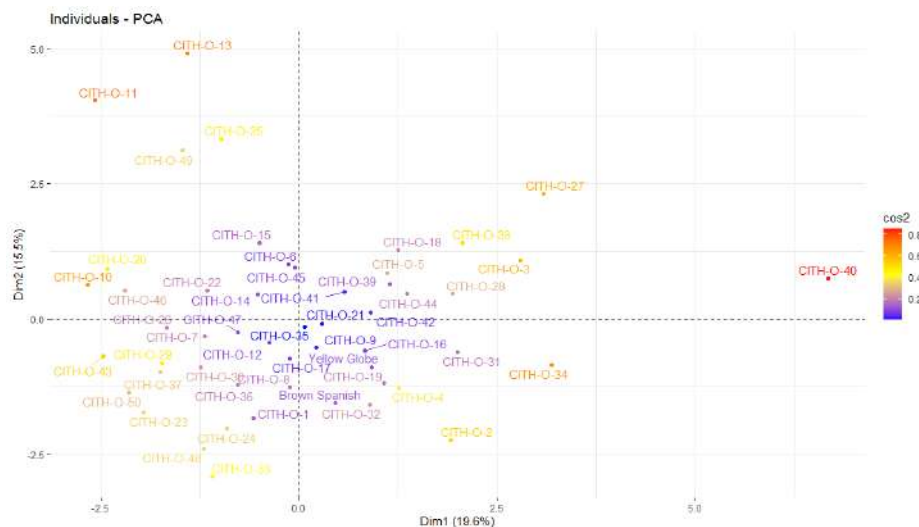


Fig. 1: Contribution of 52 onion accessions towards PCA based on $\cos^2$ value

1 is largely negative to PC1; cluster 2 is largely negative to PC2 and cluster 3 is mainly positive to PC1. Therefore, individuals in clusters 1, 2 and 3 are associated with the variables that are mapped to the respective quadrants of the factor map. The variables P:E ratio, polar diameter, % B grade bulb, number of leaves/plant, ABW, and equatorial

diameter were most significantly associated with cluster 1. Cluster 2 was significantly associated with variables such as percent unmarketable bulbs, C grade bulb, %doubles, P:E ratio, percent B grade bulb, total yield, marketable yield and polar diameter. Cluster 3 was significantly associated with variables such as unmarketable bulbs, %A grade bulb,

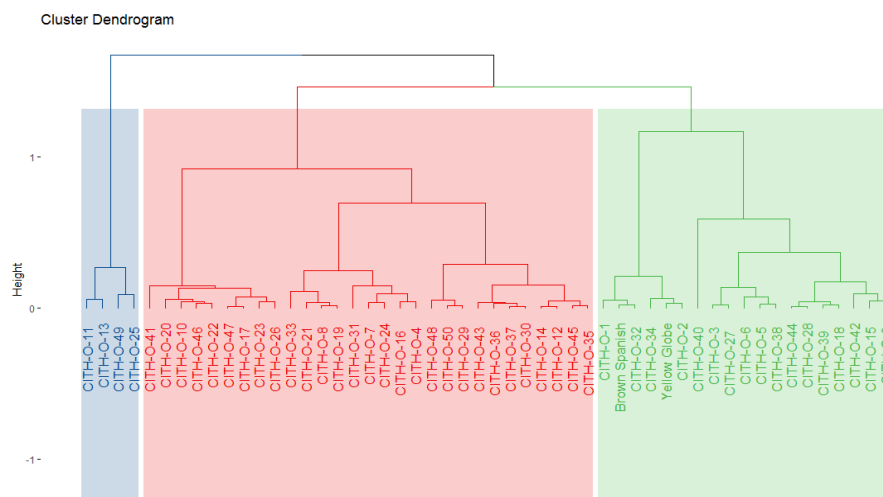


Fig. 2: Hierarchical clustering on principal components (HPCP) showing clustering patterns of 52 onion collections based on 16 parameters

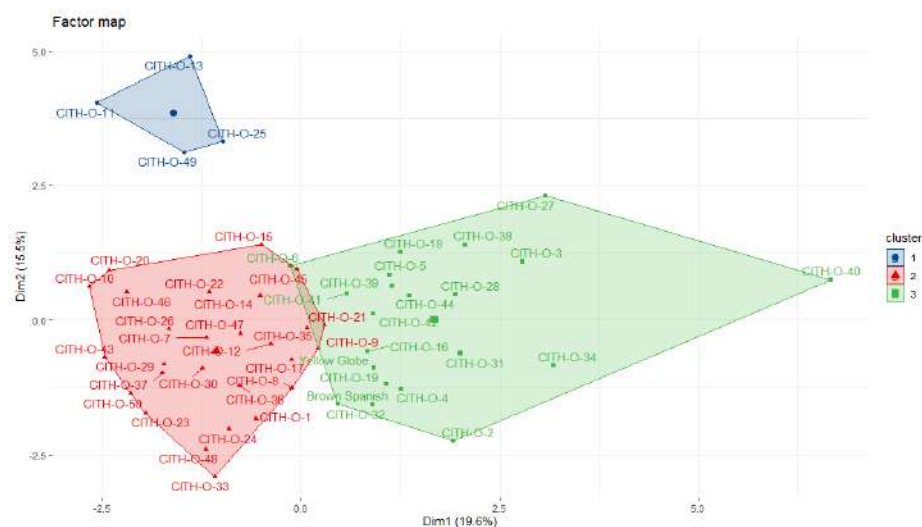


Fig. 3: Factor map representation of three clusters produced by the first two PCs (Individuals are coloured according to their cluster)

%doubles, %bolters, P:E ratio, A grade bulbs, total yield, marketable yield and equatorial diameter. For each cluster, the top 4 closest individuals to the cluster center are shown in Fig. 3. For example, representative individuals for cluster 1 include CITH-O-25, CITH-O-13, CITH-O-11 and CITH-O-49, representatives of cluster 2 include CITH-O-30, CITH-O-36, CITH-O-47 and CITH-O-29, and cluster 3 include CITH-O-44, CITH-O-39, CITH-O-5, CITH-O-31 and CITH-O-42. Although the 52 genotypes have the same geographic origin except for Yellow Globe and Brown Spanish still, they were grouped into different clusters, indicating the presence of significant phenotypic diversity. Nevertheless, the results in the present study highlighted genotype groups that have similar or contrasting features or contribute strongly to the quality of representation on the factor map or to variation and could accordingly be explored to identify the genetic basis of specific traits.

Conclusion

In a nutshell, the most important traits contributing to variation in the onion genotypes included plant height, number of leaves, polar diameter, equatorial diameter and neck thickness. Besides this, traits like average bulb weight, total yield and marketable yield could be used to select onion genotypes for a notable improvement in cultivation in changing environments. The PCA-based hierarchical clustering divided the whole germplasm into three major clusters. The first seven principal components explained ~77.8%, and the first three principal components explained 47.3% of the total phenotypic variation. Polar diameter, neck thickness, percent bolters, marketable yield and total yield, constitute the PC1, the first main factor. This study also offers a path to rapid screening of large onion genotypes in field experiments for crop improvement purposes like crossing the genotypes from different clusters. For example,

Cluster I and Cluster II could result in heterotic expression and bring large variability in the segregating generation. Identification of key phenotypic parameters will reduce the costs associated with phenotyping of all traits. For instance, the genotype CITH-O-40 recorded the highest total yield and marketable yield, which can be attributed to the lowest bolter production, highest ABW and its fairly good polar and equatorial bulb diameter. In fact, we observed that marketable yield showed a significant negative correlation with percent bolters and doubles production, but it had a significantly positive correlation with TSS content. Furthermore, the quantitative approach employed here can underpin further well-targeted and in-depth field-based studies of traits to support onion breeding programs.

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Declarations of interest

There is no conflict of interest.

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Table S1: Monthly average meteorological data for crop seasons of the year 2016-2018 at Srinagar

Day	Average temperature (°C)		Average rainfall (mm)
	Max.	Min.	
Year 2016			
January	11	-2	64
February	15	1	28
March	16	4	140
April	20	8	82
May	26	12	44
June	30	17	18
July	30	18	55
August	28	17	44
September	28	14	2
October	25	6	1
November	17	0	0
December	11	-3	0
Year 2017			
January	4.7	-1.1	134
February	10.9	1.4	87
March	15.6	4.1	58
April	21.2	7.8	169
May	24.7	12.4	23
June	27.7	15	66
July	29.9	18.8	31
August	30.1	17.6	22
September	28.9	13.2	11
October	24.8	6	0
November	16.3	1.1	10
December	11.3	-2.1	30
Year 2018			
January	7.0	-2.0	60
February	8.2	-0.7	70
March	14.1	3.4	100
April	20.5	7.9	80
May	24.5	10.8	80
June	29.6	14.9	50
July	30.1	18.1	70

August	29.6	17.5	60
September	27.4	12.1	30
October	22.4	5.8	20
November	15.1	0.9	20
December	8.2	-1.5	50

Table S2: Soil status and available macronutrients at Vegetable block, ICAR-CITH before transplanting of Onion crop.

Soil parameters	2016	2017	2018
Soil texture	Silt Loam		
Soil pH	7.89	7.58	7.1
Electrical conductivity (ds/m)	1.11	0.525	0.823
Soil organic Carbon (%)	0.50	0.47	0.48
Available N (kg/ha)	313.6	324.1	329.7
Available P (kg/ha)	11.72	11.92	13.01
Available K (kg/ha)	254.35	259.2	258.71
Available Ca (Cmol/kg)	3.8	4.2	3.7
Available Mg (Cmol/kg)	2.1	2.4	2.5
Available S (ppm)	12	13.9	15.1

RESEARCH ARTICLE

Seeds of Harmony: Conservation of Landraces by Farmers, Communities and NGOs in the Western Ghats, a Case Study

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Abstract

India is a megadiverse country and holds a vast diversity of different crops being conserved on-farm, including landraces. In the present study conducted in the Western Ghats region of the country, efforts were made to collect information from three different stakeholders, viz., individual custodian farmers, communities and non-governmental organizations (NGOs) working through different ways with a common aim to protect the indigenous cultivars. A custodian farmer named Mr. B K Deva Rao, one local community named *Kalsubai Parisar Biyanee Samvardhan Samajik Sanstha* and an NGO entitled Bharatiya Agro Industries Foundation (BAIF) were selected and the basic framework for the conservation of landraces was recorded. The roles played by all three stakeholders are interlinked and connected by supporting each other to save the seeds of the traditional cultivars. Additionally, the collaboration with government organizations promotes landrace utilization and sustainable management for crop improvement initiatives. The integration of formal and informal conservation approaches is essential for holistic plant genetic resource conservation, addressing both the scientific and socio-cultural aspects.

Keywords: Landraces, Custodian Farmers, Conservation, On-farm, Farmer Community.

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Introduction

India is a country of villages and farmers, where more than 60 percent of its population lives in rural areas. Livelihood conditions differ considerably across the country. India has many agro-climatic regions and the Western Ghats are one of them. The Western Ghats are a chain of mountains lying along the western coast of peninsular India, covering Gujarat, Maharashtra, Goa, Karnataka, Kerala and Tamil Nadu from the hills south of the Tapti River in the north to Kanyakumari in the south (Scaria, 2023). Meanwhile, the Western Ghats house vast genetic resources of different flora and fauna. People are dependent on them for their livelihood purposes. Historically, farmers around the world have been and continue to be the stewards of most of the resources, maintaining, refining and trading them to suit their needs. Thus, they are key actors in the conservation and enhancement of agro-biodiversity and their genetic resources.

Landraces serve as sources for the productivity, resilience and adaptability of agricultural systems. The conservation of this diversity is primarily facilitated through farmers' cultivation and selection methods. Local exchange and gene flow among landraces play a crucial role in promoting genetic variation, while continued cultivation fosters local adaptation (Bellon, 1996; Louette *et al.*, 1997; Mercer and Perales, 2010).

Landrace conservation is a collaborative effort that often involves both formal and informal conservation through the

combined contributions of individual custodian farmers, local communities and government and non-governmental organizations (NGOs). Each of these stakeholders plays a unique and crucial role in safeguarding traditional crop varieties and preserving agricultural biodiversity (Joshi and Upadhyaya, 2019). For several generations, farmers or farming communities have been conserving and growing landraces (traditional crop varieties) that have been suited to the local environment. Thus, the paper discusses the importance and role played by a custodian farmer, a local farming community and an NGO in conserving, reviving, maintaining and cultivating the landrace diversity in the Western Ghats region as a case study.

Materials and Methods

Study Area

The entire Western Ghats biogeographic region is a major genetic estate with an enormous biodiversity of ancient lineages (Daniels, 2003). The Western Ghats form one of the 34 biodiversity hotspots in the world (Myers, 2000). It consists of 6% of the total land area of India, but has >30% of the biodiversity of India. Nearly 5800 species of flowering plants occur here, of which 56 genera and 2100 species are endemic. In addition to diverse flora and fauna, the Western Ghats are also home to diverse social, religious, cultural and linguistic groups. The Western Ghats consist of a rich diversity of crops and their wild relatives, *viz.*, rice, sorghum, finger millet, foxtail millet, little millet, green gram, horse gram, pigeon pea, *Dolichos* bean, rice bean, taro, yams, banana, mango, jackfruit, ginger, turmeric, chili, sugarcane, coconut, cotton and many more (Arora, 1988; Rohini *et al.*, 2015).

Data Collection

Initially, a comprehensive review of the literature was done concerning the conservation efforts carried out on-farm by individual custodian farmers, communities and NGOs. This information was obtained from the Bioversity International regional station (NASC complex, New Delhi), ICAR-National Bureau of Plant Genetic Resources (NBPGR) regional station (Akola, Maharashtra), the crop-based research institute (ICAR-Directorate of Cashew Research, Puttur, Karnataka), NGOs (*Sahaja Samruddha*, Bengaluru, Karnataka and BAIF, Pune, Maharashtra), communities (*Siddharudha Savayava Krushikara Balaga*, Khanapur, Karnataka) and co-operatives (*Savayava Krishi Pariwara*, Thirthahalli, Karnataka).

Based on the preliminary information gathered, a custodian farmer, one local community and an NGO were chosen to comprehend their respective roles and efforts in relation to on-farm conservation of landraces (Fig. 1). A detailed amount of information was collected from these entities on various aspects, from basic details to marketing aspects, including landrace diversity, livelihood options, etc.



Fig. 1: Details of the different stakeholders under study

Results and Discussion

Seed is the soul of agriculture. It has taken many years of dedicated efforts by the farmers to develop and conserve crop races suitable for local agro-climatic conditions. However, in recent years, agriculture has been practiced in a high external input mode with hybrids every season and surely farmers will have to go back to the market to buy the seeds. Over time, custodian farmers have assumed a crucial role in preserving agro-biodiversity, particularly traditional varieties and landraces, whether through direct or indirect conservation methods. Additionally, some farming and tribal communities, with the help of a few NGOs, have preserved many indigenous seeds.

Individual Farmer-led Conservation

The present study gathered detailed insights from Mr. B.K. Deva Rao, a custodian farmer residing in Belthangady, Dakshina Kannada, Karnataka, India, who has been actively engaged in on-farm conservation of landraces for the past few decades. B.K. Deva Rao's dedication to on-farm conservation has inspired his son, Mr. Parameshwara Rao (54 years old) and daughter-in-law, Mrs. Vasanthalaxmi (42 years old), to adopt similar practices on approximately 30 acres of land in the remote village of Mithabagilu in the foothills of the Western Ghats near the coastal plains of Karnataka (Figs 2a-b). For the past 50 years, Mr. B.K. Deva Rao has been deeply immersed in on-farm conservation efforts aimed at preserving agrobiodiversity alongside various other farming activities, including livestock management (Figs 2c-f).

To ensure sustainable income, Mr. Parameshwara Rao initiated an innovative venture known as "*Swasti Farms Kukkavu*," a local visiting farm, a few years ago. Through these activities, they aim to promote traditional knowledge, local cuisines and farming methods. Mr. Parameshwara Rao, as a farmer breeder, selected and developed a rice variety called "*Swasti*" with high yield potential, adaptability to local climatic conditions and resistance to pests and diseases (Figs 3a). They actively engage in seed exchanges with farmers from various regions to increase and preserve diversity for future generations. They are focusing on a diverse array of rice landraces (>140), along with cucumber (> 10), cowpea (6), brinjal (3), ivy gourd (3), jackfruit (> 50), mango (80) and various root crops (10). Locally grown varieties such as *Raja Kayime*, *Kutti Kayime* and *Suggi Kayime* hold



Fig. 2: Custodian farmer (a-b) B.K. Deva Rao with his son Mr. Parameshwara Rao; (c-f) PGR and livestock activities

special importance for them, passed down through many generations and sourced from different farmers (Figs 3b-d) (Table 1).

Their knowledge of the traditional practices that enhance the cultivation of various crops on the farm was unmatched. They were well aware of the benefits of the landraces, like nutritional value, medicinal value and cultural value, which they promoted among the fellow farmers. They themselves pray for the gods during festivals by offering a variety of products and dishes made by the native varieties (*Suggi Kayime*, *Ibbudla*, *Kashi Gumbala* and *Virupa Kayime*). They greet the guests in a traditional way by offering well water along with pieces of jaggery. Thus, maintaining the traditional practices associated with the landraces enhances their conservation as well. They also reap the medicinal benefits offered by the native varieties, which enhance their lives (healthy diet) and livelihoods (more price for the native varieties products, such as red rice, brown rice, pickles, etc.).

They grow the landraces of different crops with scientific and traditional knowledge that they got from their forefathers. They have different varieties for both the *rabi* and *kharif* seasons, which sustains the cultivation throughout the year. They know the unique traits and value of each and every variety they grow. Despite their dedication, they acknowledge the loss of several landraces from their farm due to natural occurrences such as landslides, as well as challenges posed by wildlife like monkeys and wild boars during harvesting seasons and crop stand periods. Recognizing his outstanding achievements in agriculture, Mr. B.K. Deva Rao has been honoured with the *Karnataka Rajyotsava Award* by the Karnataka Government. Recently,



Fig. 3: (a) *Swasti*, a farmer rice variety by Mr. Parameshwara Rao; (b-d) Representative images of landraces conserved by the family

Table 1: Representative landrace diversity conserved by Mr. B.K. Deva Rao

Crop	Landraces
Cucumber	<i>Mullu Southe</i> , <i>Sanna Southe</i> , <i>Dodda Mullu Southe</i> , <i>Aanemottu/Hegge Southe</i> , <i>Aati Southe</i> , <i>Oddu Southe</i> , <i>Garbeeja</i> , <i>Ibbudla</i>
Cowpea	<i>Kempu Halasande</i> , <i>Bannada Halasande</i> , <i>Gidda Halasande</i> , <i>Bijada Halasande</i> , <i>Bagade</i>
Brinjal	<i>Mottu Gulla</i> , <i>Musuku Badane</i> , <i>Keddasa</i>
Lab-Lab bean	<i>Kathi Avare</i> , <i>Chaturbuja Avare</i>
Jackfruit	<i>Nar Thuluva</i> , <i>Thuluva</i> , <i>Barikke</i> , <i>Aati Variety</i> , <i>Rudrakshi</i> , <i>Happala Sonte</i> , <i>Uppada Pacheeru</i>
Mango	<i>Battala Mavu</i> , <i>Neeru Kuku</i> , <i>Sanna Mavu</i> , <i>Dodmane Mavu</i> , <i>Nekkare Mavu</i>
Banana	<i>Avande Baale</i> , <i>Kadhali Baale</i> , <i>Sakkare Baale</i> , <i>Boodu Baale</i> , <i>Kallu Baale</i>
Ridge gourd	<i>Pundi Heere</i> , <i>Daare Illada Heere</i> , <i>Neera Peere</i>
Ladies finger	<i>Bahuvashika Bhendi</i> , <i>Thoora Uda/Ane Kombu Bhendi</i> , <i>Bili Bhendi</i>
Ash gourd	<i>Kashi Gumbala</i> , <i>Dodda Gumbala</i> , <i>Sanna Gumbala</i>
Rice	<i>Raja Kayime</i> , <i>Kutti Kayime</i> , <i>Suggi Kayime</i> , <i>Neerambade</i> , <i>Virupa Kayime</i> , <i>Ajippa</i> , <i>Swasti</i> , <i>Jeerige Sanna</i> , <i>Mysuru Mallige</i> , <i>Padma Rekha</i> , <i>Soma Saale</i>

in 2023, Mr. B.K. Deva Rao received the Protection of Plant Varieties and Farmers' Rights Authority's 'Plant Genome Saviour Farmers Reward, 2020–21' in New Delhi.

Custodian farmers play a critical role in conserving the genetic diversity and adaptability of landraces, ensuring their availability for future generations. Custodian farmers are often recognized and respected for their knowledge and expertise and they are also recognized by the local communities for their immense contribution towards



Fig. 4: (a-c) Farmers of the community in their respective *rabi* crop field; (d) Seed marketing from farmers to market; (e, f) Seed storage systems of different farmers of the community; (g) Community seed gene bank, *Kalsubai Parisar Biyane Samvardhan Samajik Sanstha*, Khirvire, Akole, Ahmednagar, Maharashtra

landrace conservation. These findings resonate with the conclusions drawn by Gajanana *et al.* (2015) regarding mango landrace conservation in different regions of India. Additionally, Puneeth *et al.* (2024) survey on custodian farmers in the Western Ghats of Karnataka reveals the same results. Furthermore, Sthapit *et al.* (2013) documented various case studies across India, Malaysia, Indonesia and Nepal, shedding light on the role, motivations, methods of seed dissemination and connections with formal seed systems in landrace conservation efforts.

Community-led Conservation

The conservation of landraces by local communities represents a critical and sustainable approach to preserving traditional crop varieties and agricultural practices. In the present study, a survey was conducted to gather insights into the functioning and management of the *Kalsubai Parisar Biyane Samvardhan Samajik Sanstha* in Akole, Ahmednagar district, Maharashtra, focusing on on-farm conservation of landraces. The study revealed that the community maintains a register to record germplasm collection site details, indigenous knowledge associated with these landraces and their on-farm maintenance (Patil *et al.*, 2015). This community was also working in 20 tribal villages in the Akole block of the Ahmednagar district of Maharashtra for the conservation and promotion of indigenous crop cultivars (landraces) in different crops in the region. They engage

in seed selection, adaptation and exchange, ensuring the continuous availability of diverse varieties (Brush, 2000; Subedi *et al.*, 2003).

Since June 2016, the community has been involved in seed production for rice, hyacinth bean, little millet, horse gram, green gram and other crops. A total of 615 individual members, including 155 men and 460 women, are engaged in seed production, highlighting the active participation of women in seed production and conservation efforts. The motivation to start seed production stemmed from the establishment of *in situ* conservation centers through the Bharatiya Agro Industries Foundation (BAIF) program (Fig. 4a). Through field visits by knowledgeable farmers, including women and elders, unique crop cultivars were identified for promotion through participatory varietal selection. This process generated significant interest among farmers in selecting crop cultivars in the field itself at the maturity stage. For promotion of on-farm conservation of vegetable landraces, the community started a campaign for an indigenous vegetable cultivar nutrition garden, from which 10,000 families benefited (Figs 4b-d).

In the present study, farmers have identified superior landraces in different crops that are well-adapted to specific agro-ecological conditions and used in large-scale seed production for marketing purposes (Supplementary Table 1). The community in the present study has a seed bank

with 95 accessions of different crops (rice-13, horse gram-4, cowpea-5, hyacinth bean-18 and other pulses), oil seeds and local vegetables. The conservation of all these crop cultivars is done in ten *in situ* conservation centers in 20 villages, through kitchen gardens and through storage in the community seed bank (Figs 4e–g). Local communities establish and manage community seed banks, preserving landraces and ensuring their availability during times of need (Witcombe *et al.*, 1996).

Local communities ensure the survival of rare and unique traits that might be lost in modern agricultural systems (Perales and Golicher, 2014). Communities contribute to the improvement of landraces through participatory plant breeding programs, combining traditional knowledge with modern breeding techniques. Several successful examples of landrace conservation by local communities exist worldwide. One such initiative is the “Territorial Seeds” project in Peru, where indigenous communities in the Andes have been safeguarding traditional crop varieties like quinoa and potatoes for generations (Pimbert, 2009). This project highlights the importance of collective action and community-driven efforts in preserving agrobiodiversity. In the same way, the Seed Savers Group community in the Jawhar block of Maharashtra is working on the conservation of indigenous crop varieties of different fruit crops (Chothe *et al.*, 2014).

The members of this community were also conferred with several awards and recognitions for their immense contribution (Table 2). The community also participated in different events, workshops and trainings and shared crop cultivars of rice, hyacinth bean, millets and vegetables. Exposure visits by farmers, students and NGOs to the seed bank and *in situ* conservation and demonstration centers reached more than 3500 farmers, students, NGO representatives, etc.

Non-government organization (NGO)-led conservation

By engaging with local communities, farmers and various stakeholders, NGOs undertake the crucial task of safeguarding and promoting the cultivation of invaluable landrace genetic resources. Their endeavours encompass a wide range of activities, including research, guidance, capacity building and the implementation of conservation techniques aimed at empowering communities and conserving agricultural biodiversity. Through their interventions, NGOs facilitate the sustainable utilization and management of landraces, ensuring the preservation of unique local crop varieties, their continued cultivation and their integration into modern agricultural practices.

The present study sought insights from the Bharatiya Agro Industries Foundation (BAIF) Development Research Foundation, Pune, Maharashtra, regarding the role and significance of NGOs in on-farm conservation. Established in

1967 by the late Dr. Manibhai Desai, a follower of Gandhi, the BAIF Development Research Foundation has been dedicated to promoting sustainable livelihoods and enhancing the quality of life for tribal and rural communities (Table 3).

In 2008, BAIF initiated a community-led conservation and management program for indigenous crop cultivars and forest food resources in Maharashtra. The primary objective was to conserve and revive crop diversity along with associated knowledge. The program involved scientific studies focusing on morphological and nutritional aspects, the establishment of community seed banks and community-level seed production (Figs 5a–d). Starting in Jawahar (Palghar), the program has received the support of the government of Maharashtra in expanding its reach to other blocks in Ahmednagar, Pune, Nandurbar, Gadchiroli and Sindhudurg districts of Maharashtra. This program has also been scaled up since July 2021 with its implementation at Champawat (Uttarakhand), Dangs (Gujarat) and Sambalpur (Odisha).

The Maharashtra Gene Bank project was conceptualized and implemented across various agro-climatic zones of the state, with a focus on conserving and managing bio-resources with active community participation. Under this initiative, BAIF’s program emphasized participatory *in situ* and *ex situ* conservation, management of local bio-resources, including crop cultivars, livestock breeds and forestry species, as well as habitat eco-restoration in eight clusters spanning diverse agro-climatic zones and covering 94 villages in Maharashtra. BAIF also established a women-led enterprise for the production and sale of vegetable crop cultivars for kitchen gardens, with 10,000 packets prepared, each containing 12 different vegetable crops. The importance of farmers and NGOs in seed exchange with an aim to conserve rice landraces was also reported by Muralikrishnan *et al.* (2021). ICAR-NBPGR has a Memorandum of Undertaking with BAIF for landrace collection, conservation and characterization. They also facilitate the exchange of seeds among farmers, helping to prevent the erosion of genetic diversity. Established *ex situ* gene bank at BAIF Central Research Station, Urulikanchan (Pune), Maharashtra (Figs 5e–i). Another notable example is the “Navdanya” movement in India, started by environmentalist Dr. Vandana Shiva to promote native seed-saving practices, thereby conserving numerous landraces and promoting sustainable agriculture (Vandana, 1991).

Deciphering Conservation Dynamics: Farmer, Community and NGO-Led conservation

From the present study, it is evident that individual custodian farmers, communities and NGOs have a common goal, *i.e.*, “protection of indigenous cultivars for future generations,” but are working in different ways to achieve this goal

Table 2: Members of the community conferred with recognitions or awards

S. No.	Name of Recognition/Reward	Year	Name of the awardee
1	Narishakti Award (2018) by the Ministry of Women and Child Development, Government of India, from the President of India.	2019	Srimati Rahibai Popere
2	Felicitation by the Honourable Governor of Himachal Pradesh during the National Seed Conference.	2019	Kalsubai Parisar Biyane Savardhan Samajik Sanstha
3	Vasundhara Kirloskar Award	2018	Srimati Raibai Popere
4	Felicitation by the Department of Animal Husbandry, Government of Maharashtra, at Jalna, Maharashtra	2018	Kalsubai Parisar Biyane Savardhan Samajik Sanstha
5	BAIF's Innovative Farmer Award	2016	Srimati Mamtabai Bhangre
6	BAIF's Innovative Farmer Award	2017	Srimati Rahibai Popere
7	SRISTI SANMAN Award 2017 by SRISTI, Honey Bee Network and NIF, Ahmedabad	2017	Srimati Rahibai Popere
8	Padmashree Award	2021	Srimati Rahibai Popere

Table 3: Details of the Non-Government Organization (BAIF)

Name of the NGO	Bharatiya Agro Industries Foundation (BAIF) Development Research Foundation, Pune, Maharashtra.
Location	Jawhar (Palghar), Akole (Ahmednagar), Junner (Pune), Dhadgaon (Nandurbar), Etapalli (Gadchiroli), Kudal (Sindudurg) blocks of Maharashtra also in Champawat (Uttarakhand), Dangs (Gujarat) and Sambalpur (Odisha).
Activities	Documentation of crop diversity and associated knowledge in diverse agro-climatic zones. Germplasm collection, characterization, evaluation and participatory seed production. <i>In situ</i> conservation with the active involvement of the local community. Community-level processes include networking, establishing community seed banks and participatory events.
Focused crops	Local cultivars of paddy, millets (foxtail, finger, little and barnyard millet), beans, pulses, maize, sorghum, wild vegetables and tuber crops.

(Table 4). The synergy between NGOs, local communities and custodian farmers strengthens landrace conservation efforts. Though NGOs are integrating both community and custodian farmers together to achieve on-farm conservation, there is a need to integrate with government organizations. By combining scientific research, external support and traditional wisdom, these stakeholders collectively contribute to the sustainable management of agricultural biodiversity. The involvement of all entities fosters community ownership, cultural preservation and a sense of responsibility towards safeguarding landraces, ultimately leading to more resilient and diverse agricultural systems in the face of global challenges such as climate change and food security.

Conclusion

Throughout the history of agriculture, landrace conservation has been practiced both in formal and informal ways. The farmers conserve and exchange landraces to meet diverse requirements such as sustenance, animal feed, energy, textiles and medicinal purposes. From the present study, it is evident that landrace conservation is being practiced at different levels among farmers, communities and NGOs towards a common goal of conserving landraces for present and future sustainability. However, many farmers and communities are engaged with relative organizations in conservation and promotional activities. Due poor

**Fig. 5:** (a-d) Seed production in farmers' fields; (e-i) community seed bank at Jawhar block of Palghar, Maharashtra

Table 4: Comparison of custodian farmer, community and NGO towards on-farm conservation of landraces

Features	Individual farmer-led conservation	Community-led conservation	NGO led conservation
Conservation	Mostly short-term	Medium to long-term	Medium to long-term
Shared responsibility towards landraces conservation	Low	High	High
Landraces seed exchange	Low	High	High
Landraces cultivation scale	Small to medium	Medium to large	Medium to large
Knowledge sharing	Inherent sharing of knowledge with future generations of the family. Often with other members of the community.	Knowledge sharing among the members of the community	Knowledge sharing among the members of the community
Safety duplication	Very rare	The same landraces were conserved by different farmers in the community	The same landraces were conserved by several communities
Connection with NPGRS	Only few custodian farmers connected with NPGRS	Few communities linked with NPGRS	Mostly connected with NPGRS
Scientific support	Rare	Often	Most of the time
Funding	Very rare or nil	Few have that opportunity	NGOs get funding from government and other agencies
Landrace crop diversity	Few crops	More crops	More crops
Purpose of conservation	Hobby, reputation and social recognition, conservation (few)	Community benefit, conservation	Conservation, financial benefit

identification and insufficient documentation, the role of these stakeholders go unnoticed. Thus, there is a need for the integration of all the guardians of landraces with government organizations and institutions. It supports to bridge the gap by fostering collaboration and knowledge exchange among researchers, policymakers, farmers and other stakeholders in conserving the vast traditional knowledge of the native varieties.

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Author Contribution

SA and GMP designed the study. GMP carried out the study with the assistance of SP and SA. GMP and RG wrote the manuscript. SA, SP and RV edited and corrected the manuscript. All authors reviewed the manuscript.

Data Availability

The data that were generated during the study as well as those that support the findings are included in the paper.

Statements and Declarations

The authors declare no competing interest

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Supplementary Table 1: Selected crop cultivars under seed production by the community

Crop and landrace	Specific Traits
<i>Hyacinth Bean</i>	
<i>Kadu Wal</i>	Growth period 7-8 months, short height, bushy, season- <i>rabi</i> , grows on residual soil moisture, resistant to pests and diseases, water stress tolerant, round shaped seed, good market value
<i>God Wal</i>	Growth period 5-6 months, bushy, short height, grows well in shallow soil, pest and disease resistant, good for <i>dal</i> preparation
<i>Gavati Ghevda</i>	Climber type, perennial landraces (3-4 years), pod yield- 30-40 kg/plant in season, good market value, water stress tolerant
<i>Butka Ghevda</i>	Perennial, 25-30 kg/plant/year, bush type, pods are small and bold, grain dried and used in food scarcity period in tribal areas, good storage value, good market value
<i>Lal Ghevda</i>	Perennial, grain colour-blackish purple, used for <i>dal</i> purpose, good keeping quality, pods for short bold, grows on residual moisture
<i>Sharavan Ghevda</i>	Early maturity, season- <i>kharif</i> , short height, bushy, grows well in shallow soil, immature pods used as vegetable purpose, good yielding, long pod
<i>Patadya Ghevda</i>	<i>Patadya</i> means more width of pod, young pods used as fresh vegetable, dried seed grains used for curry and also eaten after boiling, perennial (at least 3 years), cultivated in upland, pest and disease resistant, yield about 60-70 kg per plant
<i>Vatana Ghevda</i>	Pod size is small, seeds are as like green pea, good taste, used as fresh vegetable, dried grains for curry preparation, perennial (at least 3 years),cultivated in dry and upland area, yield-20-25 kg per plant
<i>Val Ghevda</i>	Perennial, outer kernel is thick, used as fresh vegetable, curry, <i>dal</i> preparation, dry upland land type
<i>Black gram</i>	
<i>Udadi</i>	Dark black coloured seeds, small grain size, dry upland, low water requirement, tasty and nutritious, yield- 2-3 quintal/acre
<i>Udid</i>	Bigger grain size compared to udadi, used for dal, papad, plant height-1 to 1.5 ft, dry upland, grain yield-3-3.5 quintal/acre, crop period-4 months
<i>Cow pea</i>	

<i>Garvi Chavali</i>	Late maturing, reddish coloured grains, crop period-8 months
<i>Halki Chavali</i>	White coloured grains, large pod size, good storage life, maturity period-4 months
Niger	
<i>Gavati Khusani</i>	Dry upland, oil used for fracture recovery, pest and disease resistant oil used for daily cooking, cultivated on farm bunds, cake used as feed to animals, grain yield-2-3 quintals/acre
Groundnut	
<i>Khandwa</i>	Growth period - 3-4 months, nut size is bigger, grain colour is deep pink, cultivated in dry upland, grows well in shallow soil, grows in upward direction, more oil percent
<i>Ghungrya-Early</i>	Early maturity (3 months and 15 days), mature nuts sounds well, small nut size, seeds are small, seed colour-white, highest market value, good for taste
<i>Ghungrya-Late</i>	Late maturing (4 months), reddish coloured grains, good for oil purpose, nut size-big, seed size-bold
<i>Gavati Pundya</i>	Late maturity (4 months), spreading habit, less oily, good for direct consumption
Rice	
<i>Kolpi (Early)</i>	Growth period 90-100 days, tall, short fine rice, good for fodder yield and fodder quality, grows well in shallow soil, lodging landrace, non-scented
<i>Kolpi (Late)</i>	Growth period 110-120 days, tall, medium fine rice, non-scented, grows well in shallow soil, good fodder quality, good market value
<i>Ambe Mohor</i>	Growth period 110-120 days, scented, short fine rice, responds well on organic inputs, medium yielding, high market value
<i>Manur</i>	Growth period 90-100 days, short panicle, scented, tall growing, good fodder quality, small fine rice
<i>Raibhog</i>	Growth period 110-120 days, tall, long fine rice, scented, grows well in shallow soil, good fodder quality, good market value, seed colour -brownish
<i>Kalbhat</i>	Growth period 100-110 days, scented, stickiness, long and medium bold, seed colour- blackish violet, good market value, long panicle
<i>Dhavul</i>	Growth period 90-100 days, seed colour-red, grain colour-short panicle, scented, tall growing, good fodder quality, small fine rice
Finger millet	
<i>Nachani (Early) Red</i>	Growth period - 3.5 months, panicle is close shape, grain colour-red, medium height, grows well in shallow and sloppy land, dwarf landrace
<i>Nachnai (Early) white</i>	Growth period - 3.5 months, panicle is close shape, grain colour-white, medium height, grows well in shallow and sloppy land, dwarf landrace
<i>Nachani (Late) Red</i>	Growth period - 4-4.5 months, panicle is open, medium tall, grain colour-red
Green pea	
<i>Kala Vatana</i>	Growth period - 3.5-4 months, <i>rabi</i> , grain size-small, round shape, grain colour-blackish, dwarf, grows well on residual moisture, small pods
<i>Gavati Safed Vatana</i>	Growth period - 3.5 months, <i>rabi</i> , grain size-small, round shape, small pods, grain colour-white, grows on shallow and poor soil
Horse gram	
<i>Lal Hulga</i>	Growth period - 4 months, kharif season, grain colour-light red, good taste, end use- <i>vada</i> , spicy <i>jelabi</i> , sprouts as vegetable, shallow soil, Rainfed crop
<i>Safed Hulga (Early)</i>	Growth period - 3.5 months, <i>kharif</i> season, grain colour white, medium light soil, Rainfed crop
<i>Gabra Hulga</i>	Growth period - 3.5 months, grain-bold, <i>kharif</i> season, grain colour-light black, medium light soil, Rainfed crop

RESEARCH ARTICLE

Molecular Characterization of Grapes (*Vitis* spp.) Using EST-derived Simple Sequence Repeat (SSR) Markers

Sharmistha Naik¹, Jagesh K Tiwari², Rasna Zinta², Narender Negi³, Rajnish Sharma¹, Gopal Singh¹ and DP Sharma^{1*}

Abstract

The aim of this study was molecular characterization of 27 grape accessions maintained at the ICAR – National Bureau of Plant Genetic Resources, Regional Station, Shimla, Himachal Pradesh, India using EST-derived five SSR primer pairs, and also to study the transferability of EST-SSRs across different species, interspecific hybrids, and one non-*Vitis* accession *Parthenocissus quinquefolia*. SSR bands were analyzed on ethidium bromide-stained 2.5% high resolution agarose gels. A total of 31 distinct alleles were scored in 27 grape accessions, and the average number of alleles per locus was 6.2. The mean observed and expected heterozygosity values were 0.103 and 0.749, respectively. The number of effective alleles ranged from 3.415 to 4.766 with an average of 4.050. The polymorphism information content (PIC) ranged from 0.7072 to 0.8064 with a mean of 0.7650. The clustering dendrogram divided 27 grape accessions into 7 clusters, and suggested complex origins of some of the accessions. A good level of transferability of EST-SSRs across different species and interspecific hybrids was observed except in 2 grape accessions viz., *Vitis arizonica*/DVIT1269 and *Parthenocissus quinquefolia*/DVIT2400. A database of 5 EST-SSR markers was developed for future use in breeding and molecular studies.

Keywords: EST-SSR, Genetic resources, Microsatellite, Molecular characterization, *Vitis vinifera*.

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Introduction

Vitis vinifera L., the commonly cultivated grapevine, is one of the most widely grown fruits in the world (Vivier and Pretorius, 2002). Availability of adequate genetic resources and knowledge about their genetic diversity and relationships is important for recognizing gene pools, and developing effective conservation and management strategies (Khadivi *et al.*, 2019). Traditionally, the morphological method is used to characterize grape genetic resources. The history of grape morphological studies dates back to the 1st century and is still considered an efficient method for characterization (Cangi *et al.*, 2006). Morphological evaluations are direct, inexpensive, easy, and do not require expensive technology. A special term “Ampelography” is used for the morphological characterization of grapes. Ampelographic characterization is a scientific method of morphological characterization of grapes and involves phenological, pomological, and morphological characteristics (Ates *et al.*, 2011). The major disadvantages of morphological markers are that they are limited in number, influenced by the plant growth stages, and influenced by various environmental factors (Dettweiler-Miinch, 1993).

With the advent of DNA markers, the characterization of grape varieties and genetic resources using molecular markers has become more popular. Molecular markers are sequences of DNA, which are located at a specific position on the chromosomes or genes, and their presence or absence can easily be used to identify

differences among individuals (Kumar, 1999). Molecular markers display polymorphism, which may arise due to alteration of nucleotide(s) or mutation in the genome loci and make it possible to identify genetic differences between individual organisms or species (Collard *et al.*, 2005). In contrast to ampelography, molecular markers are not influenced by the environmental conditions, growth stage of the plant, pleiotropy, and epistasis. They are highly polymorphic, not limited in numbers, and the results are reproducible. Therefore, molecular characterization is widely accepted for the identification of grape varieties and genetic diversity studies (Thomas *et al.*, 1994; Bowers *et al.*, 1996). Several types of molecular markers are available now and broadly categorized into two classes viz., i) hybridization-based markers, and ii) polymerase chain reaction (PCR)-based markers (Garrido-Cardenas *et al.*, 2018). Among them, simple sequence repeats (SSRs) or microsatellites are markers of choice for the molecular characterization of grapes because of their procedural ease, robustness, polymorphism, reproducibility, and codominant nature (Sefc *et al.*, 2001; This *et al.*, 2004).

De novo development of SSRs is a costly and time-consuming endeavor. Besides, the SSR primers are frequently species-specific; therefore, SSR markers of one taxon cannot be readily used to analyze another species. This lack of transferability makes interspecific comparisons difficult. To address these associated issues with SSRs, researchers have developed inexpensive gene-based SSR markers from available genomic resources that are more likely to be transferable across taxonomic boundaries. This rapid and inexpensive development of SSRs from expressed sequence tags (ESTs) databases has been shown to be a feasible option for obtaining high-quality EST-derived SSR markers (Gupta *et al.*, 2003; Chagne *et al.*, 2004). Recent advancements in molecular biology have resulted in the development of large amounts of DNA sequence data and ESTs from several plant species from which EST-SSRs can be quickly developed. (Scott *et al.*, 2000) reported the characterization of the first ten EST-SSR loci and their transferability among grape species. (Decroocq *et al.*, (2003) reported other eight EST-SSR loci developed from an enriched cDNA library from the grape root. There are several reports that confirm the significant transferability of EST-SSRs across taxonomic boundaries than traditional SSRs in grapes (Scott *et al.*, 2000; Arnold *et al.*, 2002) as well as in other plant species (Chagne *et al.*, 2004). In view of their potential use, EST-SSRs are being developed in several crop plants (Vidya *et al.*, 2021; Li *et al.*, 2022; Sun *et al.*, 2022).

The objective of the present study was to utilize the known grape EST-SSRs for molecular characterization of grape accessions maintained at the ICAR - National Bureau of Plant Genetic Resources, Regional Station (ICAR-NBPGR-RS), Shimla and to confirm their transferability across different grape species.

Materials and Methods

Young, uniformly-sized and healthy fresh leaves were collected from 27 grape accessions maintained at the ICAR-NBPGR-RS, Shimla, Himachal Pradesh, India (31.097786 N; 77.160409 E; 1876 msl). The leaf samples were packed in aluminium foil and stored in zipper bags at -20 °C until further use. The accessions contained 16 *Vitis vinifera* cultivars, 6 non-*vinifera* species and their cultivars, 4 inter-specific hybrids, and 1 non-*Vitis* genus (*Parthenocissus quinquefolia*) from within the Vitaceae family (Table 1).

DNA was extracted from the leaf tissues based on a modified CTAB-dichloromethane protocol described by Saghai-Marooof *et al.* (1984). The yield and purity of extracted DNA were checked by running it on 1% (W/V) agarose gel. Details of the EST-derived SSR markers are given in Table 2. PCR was performed in 25 µl reaction volume containing 2.5 µl of 10x PCR buffer (without MgCl₂), 1.5 µl of 25 mM MgCl₂, 0.5 µl of 10 µM of each forward and reverse primer, 0.5 µl of 10mM dNTP, 0.2 µl of 5 U/µl of Taq polymerase (Qiagen, Venlo, Limburg, Netherlands), about 50 ng of template genomic DNA and Milli-Q water to make reaction volume to 25 µl. The PCR mixtures were cycled at 94°C for 1 min, 50-55 °C (depending on primer) for 1 min, and 72°C for 1 min repeated for 35 cycles on a Veriti Thermal Cycler (Life Technologies, Carlsbad, CA, USA). The resulting products were resolved and scored manually on ethidium bromide-stained 2.5% high resolution agarose gels. The gels were then visualized and documented on gel doc unit (AlphaImager®HP, USA).

The bands were scored using AlphaImager EC version 3.2.2 (Cell Biosciences, Inc) for data analysis. GenAEx 6.5 (Peakall and Smouse, 2012) and DARwin 6.0.21 (Perrier *et al.*, 2003) software were used for data analysis. Number of alleles, allele size, absolute frequencies and polymorphic information content (PIC) of markers were calculated for 27 samples. The PIC value of SSR markers was calculated according to the formula: $PIC = 1 - \sum (P_i^2)$, where P_i is the frequency of the i^{th} allele of a marker detected in accessions (Nei, 1973). Simple matching coefficient of dissimilarity, 100 bootstraps and weighted neighbor joining were used to construct radial tree using DARwin 6.0.21 (Perrier *et al.*, 2003). Factorial analysis was also done using DARwin 6.0.21.

Results and Discussion

Five known EST-SSR primers were used to reveal the allelic composition of 27 grape accessions. Amplified EST-SSR products were recorded in all the accessions except 2 accessions namely PGR-10 (*Vitis arizonica*/DVIT 1269) and PGR-19 (*Parthenocissus quinquefolia*/DVIT2400) in which no amplification was noticed. Representative amplified products from 3 different EST-SSR markers are shown in Fig. 1. All EST-SSRs were polymorphic and the EST-SSR amplification products were detected on agarose gel in

Table 1: Grape accessions used in the study for SSR analysis

S. No.	Genotype code	Accession Number	Name of the accession
1	PGR-01	EC772083	<i>Vitis riparia</i> x (<i>V. cordifolia</i> x <i>V. rupestris</i>)/ Malegue 44-53
2	PGR-02	EC772100	<i>Vitisvinifera</i> / Mauzac
3	PGR-03	EC772085	<i>Vitisvinifera</i> / Admirable de Curtiller
4	PGR-04	EC772082	<i>Vitis riparia</i> x <i>V. rupestris</i> /Couderc 3309
5	PGR-05	EC732195	<i>Vitis interspecific cross</i> / Black Fredonia Bunch
6	PGR-06	EC772103	<i>Vitis vinifera</i> / Muscat A Petits Grains Blancs
7	PGR-07	EC772092	<i>Vitis vinifera</i> / Chardonnay
8	PGR-08	EC452213	<i>Vitisamurensis</i> / DVIT2005.5
9	PGR-09	EC772096	<i>Vitis vinifera</i> / Hans
10	PGR-10	EC452207	<i>Vitisarizonica</i> / DVIT1269
11	PGR-11	EC452206	<i>Vitisfificifolia</i> /DVIT1106
12	PGR-12	EC452209	<i>Vitisberlandieri</i>
13	PGR-13	EC772086	<i>Vitisvinifera</i> / Aghiorghitiko
14	PGR-14	EC772094	<i>Vitis vinifera</i> / Furmint
15	PGR-15	EC772087	<i>Vitisvinifera</i> / Alvarelhao
16	PGR-16	EC772106	<i>Vitisvinifera</i> / Touriga Nacional
17	PGR-17	EC772095	<i>Vitisrupestris</i> / Goethe 9
18	PGR-18	EC772098	<i>Vitis vinifera</i> / Madeleine Royale
19	PGR-19	EC452215	<i>Parthenocissus quinquefolia</i> / DVIT2400
20	PGR-20	EC772108	<i>Vitisvinifera</i> / Savagnin Rose
21	PGR-21	EC772090	<i>Vitis vinifera</i> / Cabernet Sauvignon
22	PGR-22	EC772101	<i>Vitis vinifera</i> / Merlot
23	PGR-23	EC732197	<i>Vitis labrusca</i> / Niagara Bunch
24	PGR-24	EC772080	<i>Vitislongii</i> x <i>V. riparia</i> / Couderc 1616
25	PGR-25	IC566150	<i>Vitis vinifera</i> / Unknown variety
26	PGR-26	EC772099	<i>Vitisvinifera</i> / Mancin
27	PGR-27	EC772107	<i>Vitisvinifera</i> / Ugni Blanc

Table 2: Information on EST-SSRs used in this study including marker name, repeat, primer sequences, annealing temperature (TA), and expected length

S. No.	Marker	Repeat	Forward (F) and Reverse (R) Primer sequences (5' -3')	TA (°C)	Expected length (bp)
1	scu05vv	(AT) ₁₃	F: CAAGCAGTTATTGAAGCTGCAAGG R: TCATCCATCACACAGGAAACAGTG	51.2	174
2	scu06vv	(AT) ₈	F: CCTAATGCCAGGAAGGTTGC R: CCCTAGTCTCTCTACCTATCCATG	49.7	171
3	scu08vv	(GGT) ₅	F: CGAGACCCAGCATCGTTTCAAG R: GCAAAATCCTCCCGTACAAGTC	57.7	180
4	scu11vv	(CTT) ₈	F: AATTGATAGTGCCACGTTCTCGCC R: AACGCCGACAAGAATCCCAAGG	57.3	248
5	scu15vv	(GAA) ₆	F: GCCTATGTGCCAGACCAAAAAC R: TTGGAAGTAGCCAGCCCAACCTTC	53.5	195

Source: Scott et al., 2000

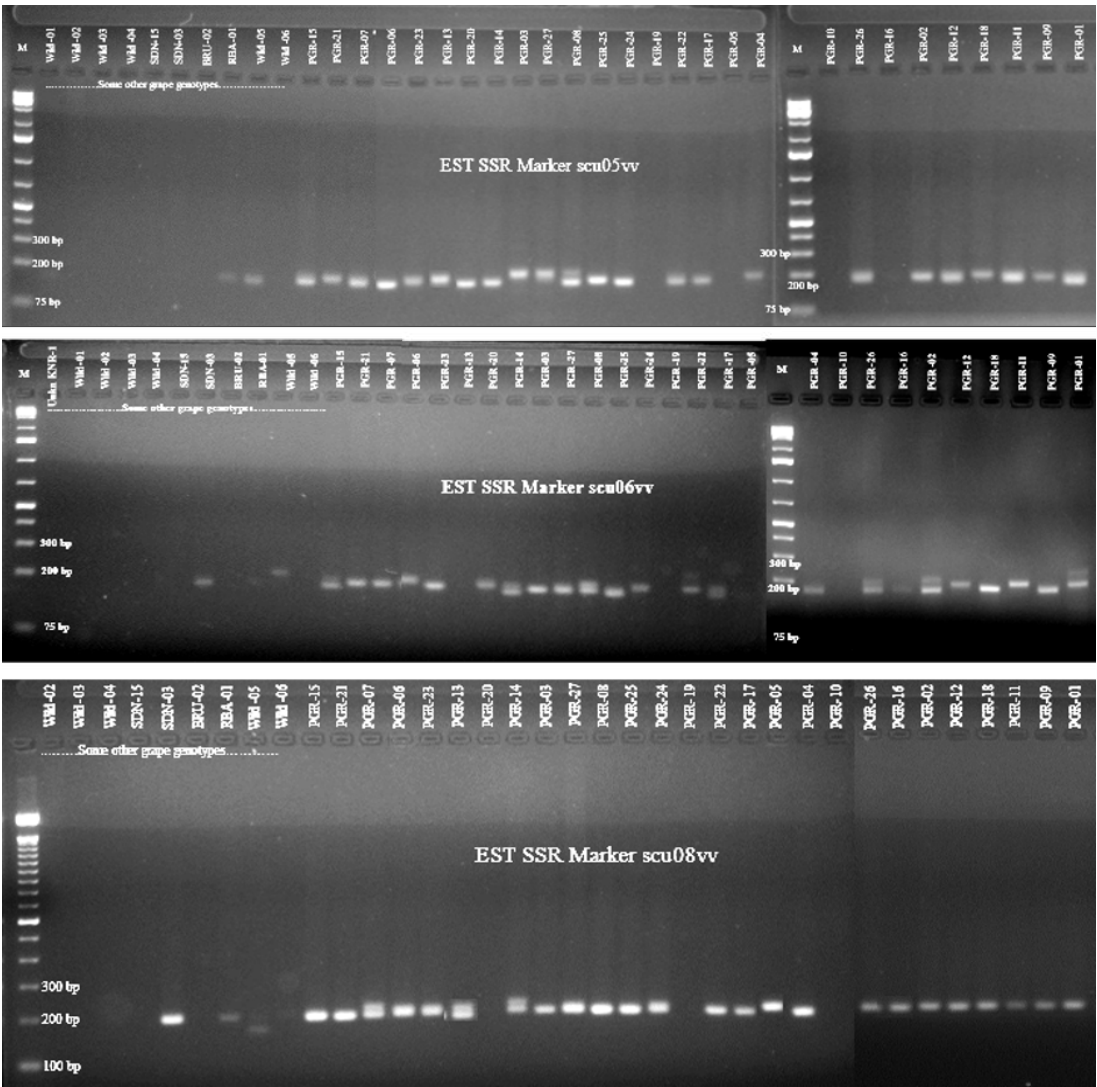


Fig. 1: Amplification of EST-SSR markers (scu05vv, scu06vv and scu08vv) in studied grape accessions. M = DNA ladder. EST-SSR marker names are shown on respective images

Table 3: Allele variations and polymorphic information content of EST-SSR markers used in the analysis of grape accessions

S. No.	Primer name ^a	Est-ssr alleles		Pic ^c
		Number	Size (bp) ^b	
1	Scu05vv	8	120(6), 142(6), 160(1), 169(3), 183(1), 188(1), 189(1), 193(6)	0.8064
2	Scu06vv	6	156(7), 171(8), 177(3), 186(2), 192(4), 196(3)	0.7929
3	Scu08vv	5	190(1), 200(5), 212(8), 226(9), 233(4)	0.7435
4	Scu11vv	8	227(4), 248(10), 252(5), 260(2), 263(2), 270(1), 274(1), 284(1)	0.7751
5	Scu15vv	4	194(5), 200(10), 210(7), 214(3)	0.7072
	Total	31		3.8251
	Average	6.2		0.7650

^aSource of EST-SSR markers: Scott et al. (2000)
^bValue in parentheses represents the actual number of accessions (out of total 27) where allele amplified.
^cPIC: Polymorphic Information Content

Table 4: A database of summary of EST-SSR allelic variations in 27 grape genotypes

S. No.	Genotype	EST-SSR marker and allele size (bp)					Total allelic frequency
		scu05vv	scu06vv	scu08vv	scu11vv	scu15vv	
1	PGR-01	183, 189	192, 196	212	248	210	7 (5.38%)
2	PGR-02	193	171, 196	200	248	210	6 (4.62%)
3	PGR-03	169	156	226	248	200	5 (3.85%)
4	PGR-04	160	177	226	227	210	5 (3.85%)
5	PGR-05	0	0	212	0	194	2 (1.54%)
6	PGR-06	120	186	226	252	200	5 (3.85%)
7	PGR-07	120	171	212, 233	263	200	6 (4.62%)
8	PGR-08	120, 169	156, 171	226	248, 270	200	8 (6.15%)
9	PGR-09	193	171	212	248	210	5 (3.85%)
10	PGR-10	0	0	0	0	0	0 (0.00%)
11	PGR-11	193	192	212	227	210	5 (3.85%)
12	PGR-12	193	192	200	248	210	5 (3.85%)
13	PGR-13	188	0	190, 200	252	214	5 (3.85%)
14	PGR-14	120	156	226, 233	252	200	6 (4.62%)
15	PGR-15	142	171	212	260, 284	200	6 (4.62%)
16	PGR-16	0	186	200	0	194	3 (2.31%)
17	PGR-17	142	156	226	227	194	5 (3.85%)
18	PGR-18	193	177	212	248	210	5 (3.85%)
19	PGR-19	0	0	0	0	0	0 (0.00%)
20	PGR-20	120	171	0	252	200	4 (3.08%)
21	PGR-21	142	171	212	263	200	5 (3.85%)
22	PGR-22	142	192	226	227	194	5 (3.85%)
23	PGR-23	142	171	226	260, 274	200	6 (4.62%)
24	PGR-24	120	156	233	248	214	5 (3.85%)
25	PGR-25	142	156	226	248	214	5 (3.85%)
26	PGR-26	193	177, 196	200	248	194	6 (4.62%)
27	PGR-27	169	156	233	252	200	5 (3.85%)
Total allelic frequency		25 (19.23%)	27 (20.77%)	27 (20.77%)	26 (20.00%)	25 (19.23%)	130 (100.00%)

25 out of 27 grape accessions indicating that there was a good level of interspecific transferability of the EST-SSRs. The observed amplification in genetically distant grape accessions was within the arbitrary expected size range that is 100 bp above or below the original *V. vinifera* fragment (Arnold *et al.*, 2002). Non-detection of EST-SSR products in 2 genotypes on agarose gels may be due to non-amplification of DNA or the DNA bands were too faint to be scored. It has also been reported by Arnold (2002) that the average EST-SSR cross-transferability is about 51.1% within Vitaceae. Thus, it may be possible that the used *V. vinifera*-derived EST-SSRs are not transferable in 2 distant relatives *viz.*,

PGR-10 (*Vitis arizonica*/DVIT 1269) and PGR-19 (*Parthenocissus quinquefolia*/DVIT2400). Secondly, in EST-SSRs, the primers flanking them are derived from relatively conserved DNA sequences, however, if the cDNA from which EST-SSRs are derived lacks introns, unrecognized intron splice sites disrupt priming sites resulting in failed amplification (Rungis *et al.*, 2004).

A summary of EST-SSR polymorphism including allele number, size, absolute allele numbers, and PIC values is presented in Table 3. EST-SSR analysis showed 31 alleles ranging from 4 alleles per locus in scu15vv to 8 alleles per locus in scu05vv and scu11vv with average of 6.2 alleles per

Table 5: Summary of genetic variation statistics for EST-SSR loci in 27 grape accessions

EST-SSR locus	Allelic parameters					
	Number of accessions (N)	No. of Alleles (Na)	No. of Effective Alleles (Ne)	Observed Heterozygosity (Ho)	Expected Heterozygosity (He)	Shannon's Information Index (I)
scu05vv	23	8.000	4.766	0.087	0.790	1.724
scu06vv	23	6.000	4.560	0.174	0.781	1.637
scu08vv	24	5.000	3.646	0.125	0.726	1.386
scu11vv	23	8.000	3.861	0.130	0.741	1.600
scu15vv	25	4.000	3.415	0.000	0.707	1.299
Mean	-	6.200	4.050	0.103	0.749	1.529
SE	-	0.800	0.262	0.029	0.016	0.080

SE: Standard Error

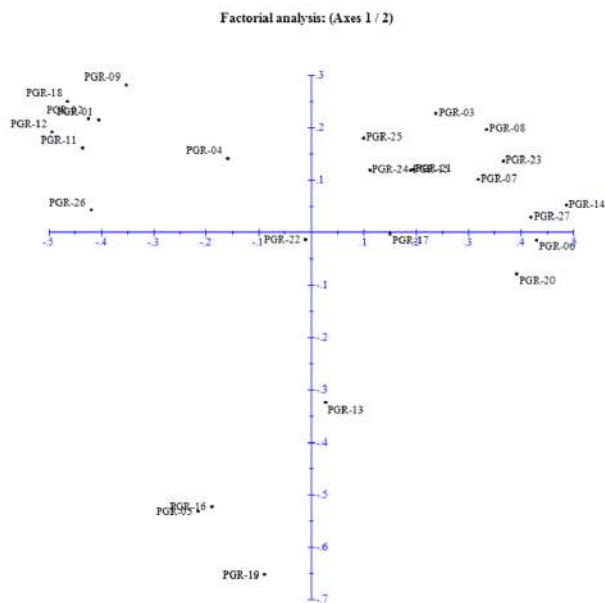


Fig. 2: Factorial analysis of 27 grape accessions using EST-SSR profiles and DARwin software

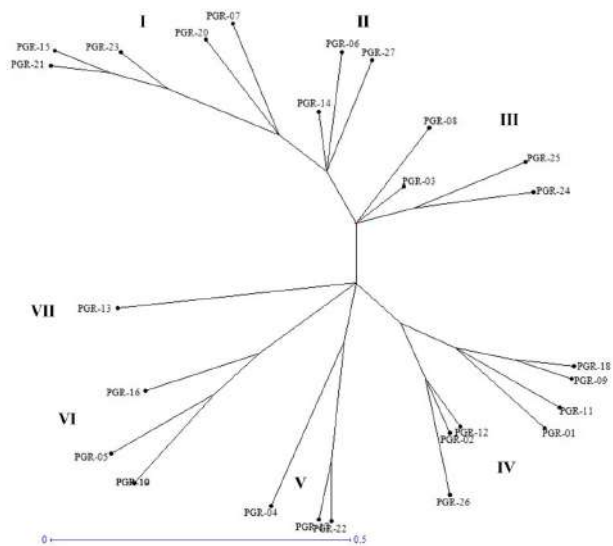


Fig. 3: Diversity analysis of 27 grape accessions using DARwin software and bootstrap value of 100

locus. The number of alleles amplified by each of the EST-SSR in our study was more than those reported by Scott *et al.* (2000) in same EST-SSRs. The number of alleles per locus in EST-SSRs in the present study was, however, lower than those obtained with traditional SSRs in the same grape accessions (unpublished). Similar results were obtained by Tabbasam *et al.* (2014), who reported that the traditional SSRs amplified a higher number of alleles compared to the EST-SSRs in cotton. This type of amplification behaviour of these markers can be attributed to their origin. SSR markers are distributed throughout the genome, while, EST-SSRs are specific and located within functional genes only. The PIC value indicates the informativeness of the SSR locus and its potential in detecting the differences among the genotypes

based on genetic relationships. The PIC values in our study ranged from 0.7072 in scu15vv to 0.8064 in scu05vv with a mean of 0.7650 (Table 3). Relatively high PIC values obtained in the present investigation demonstrated that the used EST-SSRs can be useful for grape germplasm characterization. Based on PIC values, the most informative and polymorphic locus was scu05vv.

Absolute allele numbers varied from a minimum of 1 in scu05vv (160, 183, 188 and 189 bp), scu08vv (190 bp), and scu11vv (270, 274 and 284 bp) to a maximum of 10 in scu15vv (200 bp) and scu11vv (248 bp) (Table 3). A total of 130 allelic bands were detected in 27 grape accessions that ranged from 0 (in PGR-10 and PGR-19) to 8 (in PGR-08). A database of EST-SSR markers prepared based on the

scored PCR amplification products is summarized in Table 4. Marker-wise total allele numbers in descending order were: 27 (20.77%) in scu06vv and scu08vv; 26 (20.00%) in scu11vv; and 25 (19.23%) in scu05vv and scu15vv. A high genetic variation in terms of number of alleles (Na), number of effective alleles (Ne), observed heterozygosity (Ho), expected heterozygosity (He), and Shannon's Information Index (I) was evident in 27 grape accessions (Table 5). The factorial analysis conducted using DARwin software indicated that 51.37% of the total variance was explained by the first three components 22.22%, 16.81%, and 12.34%, respectively. A factorial graph depicting the distribution of grape accessions is shown in (Fig. 2). Significant genetic diversity among studied grape accessions was also exhibited by the dendrogram generated using DARwin software (Fig. 3). Except for PGR-10 and PGR-19, in which no amplification was achieved, all other 25 accessions could be successfully discriminated. The dendrogram exhibited 7 clusters. Cluster I, II, and VII contained nine *V. vinifera* cultivars; cluster III had three *V. vinifera* cultivars and *V. amurensis*; cluster IV contained four *V. vinifera* cultivars, *V. ficifolia*, *V. berlandieri*, and one interspecific hybrid; cluster V was comprised in one *V. vinifera* cultivar, *V. rupestris*, and one interspecific hybrid; while, cluster VI contained two *V. vinifera* cultivars, and 2 accessions (*V. arizonica*, and *Parthenocissus quinquefolia*) in which no amplification was observed. Though the findings indicate relative relationships among studied grape accessions, they also suggest complex origins of some of the cultivars, for which further research is needed.

In conclusion, the polymorphic and highly transferable EST-SSRs are a viable approach for molecular characterization in grape. Although only 5 known EST-SSRs have been tested in present study, their potential utility has been illustrated. There is a need to discover more such EST markers for extensive molecular characterization and phylogenetic studies in grape genetic resources.

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RESEARCH ARTICLE

Restoration reaction in the F_1 hybrids derived from F_3 *maldandi* restorers on different cytoplasm in *rabi* sorghum [*Sorghum bicolor* (L.) Moench]

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Abstract

In this study, a reported restorer on *maldandi* cytoplasm DSMR 8 was used to identify restorer variants in early segregating generation. Large F_2 population of the cross M31-2A $\times$ DSMR 8 were grown. Among them, fifteen superior restoring plants were selected and forwarded to F_3 generation. These fifteen F_3 plants were crossed to three male sterile lines having both *milo* and *maldandi* cytoplasmic sources (M31-2A, 401A and 104A) in $L \times T$ design. The results revealed that all the obtained hybrids on both *milo* and *maldandi* cytoplasm were fertile and conferred that *maldandi* restorers work well on the *milo* cytoplasm because the pollen parents (F_3 lines) used had *maldandi* restorer gene. Hybrids having parent's MR 4 (F_3 *Maldandi* Restorer line), MR 12, MR 1, MR 14 and MR 2 were found to be good *per se* performers for grain yield and restoration on. Hybrids having parent MR 1 were found to be the best performers for grain yield and restoration across all cytoplasm (M31-2A, 104A and 401 A). Through early generation combining ability it was possible to identify promising restorers with superior agronomical traits on *maldandi* as well as *milo* cytoplasm.

Keywords: Restoration reaction, early segregating generation, *maldandi* cytoplasm.

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Introduction

In the era of climate change, C_4 plants have their own role in combating global warming. C_4 plants require optimal temperature and possess higher photosynthetic efficiency which will make them mandatory crops to grow in the future as India committed to becoming a carbon-neutral state by 2070 to escape from the irreversible effects of climate change. Sorghum [*Sorghum bicolor* (L.) Moench] is a C_4 plant with higher photosynthetic efficiency and abiotic stress tolerance (Reddy *et al.*, 2009). It adapted to a diverse set of environments ranging from arid and semiarid to tropical regions throughout the world. On a global basis, sorghum is the fifth major cereal crop in the extent of production following wheat, rice, maize, and barley. Along with providing food and fodder, it plays a vital role in providing micronutrients at a low cost (Parthasarathy Rao *et al.*, 2010). This is necessary for a country like India, where 25% of the population lives in poverty. Recently, India stood in the 'Serious' category in the Global Hunger Index 2022, positioning 107th place out of 121 countries. So, this crop addresses the issues of climate change, malnourishment and, to some extent, poverty through its sustainable "low cost and more micronutrient" phenomenon. To update and revive these crops, FAO announced 2023 year as the "International Year of Millets". Out of the several cytoplasm available in sorghum, only A_1 (*milo*) CMS

system is predominantly used for commercial production of hybrids (Reddy *et al.* 2018), though it lacks good grain quality. In addition to the A_1 cytoplasm several other CMS sources like A_2 , A_3 , A_4 , Indian A_4 , A_5 , A_6 , 9E, and KS were described in sorghum. Among these *maldandi* (A_4) cytoplasm is known for its grain quality with shoot fly resistance. There has been a lack of good restorers in the *maldandi* cytoplasm. Identifying and utilizing the breeding program would be a win-win situation for quality and insect resistance. An identified restorer on *maldandi* DSRM 8 was used as a parent in basic crossing. In this study, an effort has been made to identify restorers on *maldandi* cytoplasm along with superior agronomical traits. Further, an attempt was made to validate the reaction of early segregating *maldandi* restorers on *milo* cytoplasm.

Materials and Methods

The identified restorer DSRM 8 was crossed with *maldandi* male sterile line. The obtained hybrids were forwarded to F_2 in *summer* of 2019. Around 300 to 400 F_2 plants were grown and selfed. Among them, the top 15 plants which are presumed to be restorer and agronomically superior were selected randomly and forwarded to F_3 in *rabi* 2019. Further, these 15 plants were used as pollen parents and crossed with male sterile lines *viz.*, M31-2A, 104 A and 401 A in LxT design during *Summer* 2020. Obtained 45 hybrids were evaluated in replicated trials during *rabi* 2020. The F_3 plants of the cross M31-2A x DSRM 8 were named as MR (*maldandi* restorer). All the necessary agronomical practices were followed. The detailed flow of the work is mentioned in Fig 1.

Results and Discussion

Restoration reaction

Carefully chosen 15 MR plants had restorer gene which was contributed by one of its strong restoring parents (DSRM

8). Upon crossing with three male sterile lines, 45 hybrids were able to set the seeds irrespective of the cytoplasmic background. Though MR lines were bearing *maldandi* restorer gene, when crossed with *milo* cytoplasm (104 A and 401 A) they were possible to set seed. This was logically evident from the current study that *maldandi* restorers work well on the *milo* cytoplasm as well, but the inverse need not be true (Arunkumar *et al.*, 2004 and Verma *et al.*, 2022)

Further all 45 hybrids developed on *milo* (104 A and 401 A) and *maldandi* were evaluated for agronomic performance in replicated trials. The top hybrids for yield and yield-attributing traits were depicted in Table 1. On *maldandi* cytoplasm MR 4, MR 12, MR 1, MR 14 and MR 2 came out to be top hybrid parents for grain yield (Fig 2). Similarly, on *milo* (104 A) cytoplasm MR 2, MR 8, MR 1, MR 5 and MR 15 were superior while, on another *milo* (401 A) cytoplasm MR 11, MR 10, MR 5, MR 3 and MR1 came out to be best hybrid parents for grain yield traits (Table 2).

To be an ideal restorer, the genotype should have a tall plant height in order to shed pollen from the top. Similarly, better panicle length, weight and panicle width to generate and disseminate sufficient pollen. In the present investigation, hybrid M31-2A x MR 14 had shown desirable traits *viz.*, earliness (70 days), taller (230 cm), lengthier panicle (16 cm), wider panicle (8.50 cm) and better panicle weight (162.50 g) with bold seeded and high grain yield (149.50 g). These features made M31-2A x MR 14 an ideal restorer on *maldandi* cytoplasm. Similarly y, 104 A x MR 2 was early (70.06 cm), taller (207.50 cm), having panicle weight (132.50 g) with good grain yield (127.50 g), while 401 A x MR 11 was also showing the ideal character to be a good restorer. Hence both the crosses were the best restorer on *milo* cytoplasm. Interestingly, hybrids having MR 1 as a parent have shown top agronomical superiority across all the cytoplasm. Hence, MR 1 was the best restorer and good combiner for yield and yield attributing traits. Further, this line can be used as a potential parent in both *milo* and *maldandi*-based hybrid seed production.

From the performance of F_1 hybrids derived from F_3 MR lines, it was logically evident that only superior F_3 lines should be forwarded to F_4 . Doing this in the early segregating generation will reduce the forwarding of junk material to

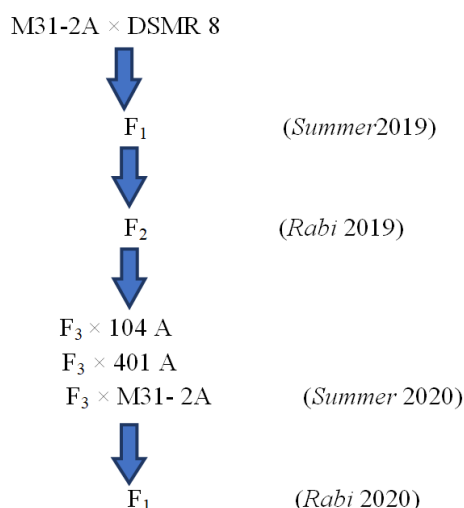


Fig 1: Schematic representation of the experiment conducted across different season



Fig 2: Top five hybrid combinations on *maldandi* cytoplasm for yield and yield attributing traits

Table 1: Top five best hybrids for yield and yield attributing traits on different *ms* sources

S. No.	High yielding hybrids	Grain yield per plant (g)	Days to 50% flowering	Plant height (cm)	Panicle length (cm)	Panicle width (cm)	Panicle weight (g)	100 seed weight (g)
<i>Maldandi</i> (M 31-2A)								
1	M 31-2A × MR 4	149.50	70.00	230.00	16.00	8.50	162.50	5.02
2	M 31-2A × MR 12	138.50	71.73	242.50	21.50	8.65	141.00	5.77
3	M 31-2A × MR 1	112.50	73.50	170.00	15.00	8.60	119.00	6.02
4	M 31-2A × MR 14	107.50	72.03	252.50	20.00	7.75	118.50	5.77
5	M 31-2A × MR 2	107.00	76.00	177.50	16.00	7.50	120.00	5.71
<i>Milo</i> (104 A)								
1	104 A × MR 2	127.50	70.06	207.50	22.00	8.50	132.50	5.23
2	104 A × MR 8	91.00	70.95	202.50	25.50	7.45	111.00	5.56
3	104 A × MR 1	83.00	73.00	180.00	21.00	7.40	92.00	4.85
4	104 A × MR 5	72.00	75.45	200.00	19.85	6.00	64.00	5.78
5	104 A × MR 15	53.50	77.45	184.00	18.00	5.55	72.50	4.65
<i>Milo</i> (401 A)								
1	401A × MR 11	106.00	69.45	212.50	22.00	7.50	116.00	5.55
2	401A × MR 10	90.00	69.95	172.50	21.50	8.50	101.00	4.99
3	401A × MR 5	85.00	71.45	179.00	17.00	7.25	95.00	5.31
4	401A × MR 3	82.00	70.44	210.00	19.00	7.00	101.00	5.04
5	401A × MR 1	80.00	69.45	192.50	17.25	5.45	87.00	4.76

Table 2: Top five best hybrids for grain yield on different *ms* sources

S. No.	M31-2A (<i>Maldandi</i>)	104 A (<i>Milo</i>)	401A (<i>Milo</i>)
1	MR 4	MR 2	MR 11
2	MR12	MR 8	MR 10
3	MR 1	MR 1	MR 5
4	MR 14	MR 5	MR 3
5	MR 2	MR 15	MR 1

the next generation. Though restoration is governed by major and minor genes, screening and selecting over the generation will reduce the environmental influence. Finally, it is possible to isolate potential transgressive segregants for restoration on *maldandi* cytoplasm. These are vital steps to identify promising restorers on *maldandi* cytoplasm because of restoration complexity and environmental influence on minor genes (Verma *et al.*, 2022 and; Elkonin *et al.*, 2005).

Author's Contribution

Conceptualization of research (MCW, BDB, SNC, VSK); designing of the experiment (MCW, BDB, SNC, VSK); contribution of experimental material (BDB).

Conflict of Interest

None.

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RESEARCH ARTICLE

Ensemble Habitat Suitability Studies of *Vetiveria zizanioides* (Linn.): An Important Genetic Resources with Aromatizing, Medicinal and Land Restoration Properties

Manish Mathur¹ and Preet Mathur^{2*}

Abstract

This study evaluated *Vetiveria zizanioides* habitat suitability to its current bio-climatic, root zone characteristics, and land use types to two projected climatic timeframes (2050 and 2070) and four future greenhouse gas scenarios (RCP 2.6, RCP 4.5, RCP 6.0, and RCP 8.5). We compared seven machine learning techniques for habitat suitability modeling of this species utilizing presence records using these predictors. With all RCPs, the random forest and support vector machine algorithms yielded high-quality models. Finally, we used ensemble modeling (averaging of several techniques) to quantify this species' habitat suitability. According to our model, this species' optimal and moderate habitat types are distributed over the southern, south-west, and south-eastern zones, with marginally suitable areas interspersed in between. This species was shown to be unsuited for extreme western, northern, and eastern India in our research. Under both the current and 2070 RCP 8.5 scenarios, isothermality had the biggest effect, but the latter dropped the optimum range from 45–55% to 25–35%. In the RCP 2.6 to 8.5 scenarios for 2050, the precipitation of the wettest quarter (PrWeQ) for this species ranged from 500 to 3000 mm. Precipitation seasonality increases the likelihood of a habitat being favorable for a species. The optimal area, according to this study, is progressively reducing and will be largely in the south and south-west by 2050, with RCPs ranging from 2.6 to 8.5. This research can be used to identify areas that foster vetiver oil quantity and qualities and support higher biomass for land restoration and reclamation.

Keywords: *Vetiveria zizanioides*, Species distribution modeling, Random forest, Ensemble model, Bio-climatic, Root zone, Landcover variables.

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Introduction

Vetiveria zizanioides (Linn.) Nash is a perennial C₄ grass (Figure 1A) that belongs to the Poaceae family, prized for its aromatic and highly valued adventitious roots. *Vetiver* grass has many applications in ecological engineering and environmental restoration, including slope stabilization (Figure 1B and C, Eab *et al.*, 2015), erosion management (Cao *et al.*, 2015), and phytoremediation (Wasino *et al.*, 2019; Abaga *et al.*, 2021). Since the 1980s, this species has been advocated by the World Bank as a way to improve water management (D'Souza *et al.*, 2019). Our current understanding suggests that this grass species can survive in a broad variety of environments, from those with high salt and heavy metal contamination to those with poor fertility and high pH, as well as extended periods of drought and frequent flooding (Itusha *et al.*, 2019; Holanda *et al.*, 2022).

Additionally, its fibrous roots (Figure 1D) contain about 3% of oil on a dry weight basis, making it a particularly useful plant. One liter of oil extract requires about 250 kg of *V. zizanioides* root (Belhassen *et al.*, 2015). Oil from Reunion Island (France) is so superior that it's known as Bourbon oil. Global *vetiver* oil output is projected to reach

300 to 350 tons per year by 2022 (Gupta *et al.*, 2015; Lal *et al.*, 2021a) and the vetiver oil market is forecast to grow to \$ 169.5 million by that same year (Durge and Moon, 2021). The quantity used and interest in it both rise each year. The main exporters of vetiver oil are Indonesia and Haiti, each shipping out between 50 and 100 tons per year. China, meanwhile, imports about 20 tons per year.

Large quantities of vetiver can be found in the Indian states of Uttar Pradesh, the South, and the peninsula. The fragrance, masticatory, attar, and soap sectors (Figure 1E) rely heavily on Indian vetiver oil, and although the country has over 10,000 ha of land dedicated to growing and cultivating the plant (Lal *et al.*, 2018), it only produces 100 tons of oil each year (Lal *et al.*, 2021b). Essential oils from various parts of the world have varied qualities and aromas because of their origins. Numerous studies have been conducted to learn how the agro-climatic conditions of various regions affect the oil content of vetiver through time and space (Ghani, 2003; Pripdeevech *et al.*, 2006; Hammam *et al.*, 2019; Holanda *et al.*, 2022). Northern Indian vetiver oil, in particular, has a reputation for excellence (Gupta *et al.* 2015).

Unfortunately, we still don't know how this species will respond to bio-climatic variables in the context of potential future greenhouse gas scenarios. In light of this, the purpose of the present study was to compare the potential growth of *V. zizanioides* under two future climatic timeframes (2050 and 2070) and four future greenhouse gas scenarios (RCP 2.6, RCP 4.5, RCP 6.0, and RCP 8.5) to its growth under its existing bio-climatic, root zone variable, and land use settings. In this research, we explain and analyze the findings of an evaluation of multiple algorithms for modeling species distributions using presence records of this species, and we do so in terms of an existing criterion for evaluating model quality. The results of such studies on a national scale are useful for evaluating its current and future status and determining whether or not it is essential to preserve.

Materials and Methods

Data Collection

Distributional records for *V. zizanioides* were obtained from data repositories such as the Global Biodiversity Information Facility [9; <https://www.gbif.org/>], the Indian Biodiversity Portal (<https://indiabiodiversity.org/species/show/33318>), published literature Lal *et al.*, 1997; Dudai *et al.*, 2006; Babalola *et al.*, 2007; Edelsterin *et al.*, 2009; Mickovski and Beek, 2009; Lal, 2013; Li *et al.*, 2014; Yaseen *et al.*, 2014; Gupta *et al.*, 2015; Liu *et al.*, 2016; Jaikaew and Nokkaew, 2019; Aziz, 2020; Chintani *et al.*, 2021; Ondo Zue Abaga *et al.*, 2021; Shuai *et al.* 2022) as well as from our field-based inventories (Mathur, 2005; Mathur and Mathur and Sundaramoorthy 2013a b and 2019). Using high-resolution Google Earth satellite image data and GIS ArcMap (Coban *et al.*, 2020), the locations of these sites were determined relative to the WGS84 coordinate datum. In addition, when occurrence data was not available, precise geo-coordinates were determined by utilizing Google Earth (<http://ditu.google.cn/>) to get latitude and longitude values. The distributional locales were compiled into a comma-separated value (.csv) database using the aforementioned sources).

To determine its IUCN conservation category in India, we used a CSV database file to analyze its extent of occurrence and area of occupancy (Sq. km). To accomplish this, we utilized the ConR R-based program.

Bio-Climatic (BC) Variables

Machine learning methods can use the current distribution of species to project their future movements (Sarikaya *et al.*, 2018; Wei *et al.*, 2018). WorldClim version 1.4 (<https://worldclim.org/data/cmip6/cmip6clim30s.html>) provided the observational data (bioclimatic variables: precipitation and temperature accessed on 21st October, 2023) used to predict the present and future distributions of species. We



Figure 1: Pictorial representation of *Vetiveria zizanioides* grass (A), use for slope stabilization (B), prevent sand movement (C), its fibrous roots source of aromatic oil (D), marketed products of *V. zizanioides* (E) and GPS points of natural population of this species (F).

Table 1: Description of predictive bio-climatic variables use in this study (downloaded from WorldClim related to four timeframes: current, 2050 and 2070 Shared Socioeconomic Pathways (RCPs 2.0, 4.5, 6.0 and 8.5))

Code	Environmental variables and their abbreviations	Scaling Factor	Unit
BC-1	Annual mean temperature (AMT)	10	°C
BC-2	Mean diurnal range (MeDR)	10	°C
BC-3	Isothermality (BC2/BC7) (×100) (Iso)	100	Percentage
BC-4	Temperature seasonality (standard deviation ×100) (TempS)	100	°C
BC-5	Max temperature of warmest month (MaTWaM)	10	°C
BC-6	Min temperature of coldest month (MiTCM)	10	°C
BC-7	Temperature annual range (BC 5BC 6) (TAR)	10	°C
BC-8	Mean temperature of wettest quarter (MeTWeQ)	10	°C
BC-9	Mean temperature of driest quarter (MeTDQ)	10	°C
BC-10	Mean temperature of warmest quarter (MeTWaQ)	10	°C
BC-11	Mean temperature of coldest quarter (MeTCQ)	10	°C
BC-12	Annual precipitation (AnPr)	1	mm
BC-13	Precipitation of wettest month (PrWeM)	1	mm
BC-14	Precipitation of driest month (PrDM)	100	mm
BC-15	Precipitation seasonality (Coefficient of Variation) (PrS)	1	Percentage
BC-16	Precipitation of wettest quarter (PrWeQ)	1	mm
BC-17	Precipitation of driest quarter (PrDQ)	1	mm
BC-18	Precipitation of warmest quarter (PrWaQ)	1	mm
BC-19	Precipitation of coldest quarter (PrCQ)	1	mm

used DIVA-GIS version 7.5 (Coban *et al.*, 2020) to convert 19 bioclimatic variables (Hijmans *et al.*, 2005) for the present and two future climate scenarios to ASCII (or ESRI ASCII) at a spatial resolution of 10 arc seconds for India. In 2050 timeframe represents the mean values from 2041 to 2060 and the 2070 timeframe represents the mean values from 2061 to 2080 (Hijmans *et al.*, 2001; Zhang *et al.*, 2021). These are related to four RCPs (RCP 2.6, RCP 4.5, RCP 6.0, and RCP 8.5) and were downloaded. Table 1 lists the various bioclimatic parameters, their respective units, and their respective mathematical expressions and abbreviations.

Non-Bio-climatic variables

Root zone variables

The Global Climate Observation System's (GCOS) Terrestrial Observation Panel for Climate highlighted the 95% rooting depth as a critical variable required to quantify the relationships between climate, soil, and plants. The data on vertical root distribution was provided by the International Land Surface Climatology Project (ISLSCP), and it includes points of data from a wide variety of land coverings (evergreen needle leaf forest, evergreen broadleaf forest, deciduous broadleaf forest, mixed forest, wooded grassland, cropland, urban built-up and open shrub land, closed shrub land, bare ground, Schenk *et al.*, 2009). Data on this parameter was downloaded from <http://daac.ornl.gov>

Rooting zone water storage (mm H₂O) was applied in the current research, which was calculated using the assimilation of NDVI-fPAR (fraction of Absorbed Photosynthetically Active Radiation) and atmospheric forcing data. In this research, estimations of the global distribution of the rooting zone's total plant-available soil water storage capacity ("rooting zone water storage size") on a 1.0-degree global grid (Ramankutty *et al.*, 2010a and b; Kleidon, 2011) were downloaded from [https://daac.ornl.gov/ISLSCP II/guides/root water storage 1deg.html](https://daac.ornl.gov/ISLSCP%20II/guides/root%20water%20storage%201deg.html).

Land-Use-Land Cover Variables

For this study, we downloaded a geographical dataset at a resolution of 5 meters, which included information about five land cover/land use categories: barren and very sparsely vegetated land; irrigated cultivated land; rain-fed cultivated land; forest; and grass/scrub/wood land (Fischer *et al.*, 2008). Details of these variables are available at https://www.fao.org/soils-portal/data-hub/soil-maps-and-databases/harmonized-world-soil-database-v12/en/#jfmulticontent_c284128-4

Issue of multicollinearity

In order to look at cross-correlation, we employed the Pearson Correlation Coefficient (r), and we ran a multicollinearity test to make sure we weren't over-fitting. Also, the Niche Tool Box was used to gradually eliminate variables

Table 2: Model qualities parameters with current bio-climatic and non-bio-climatic (solar and land cover) variables

Current BC, Solar and Landcover	AUC	sensitivity	specificity	Kappa	TSS
GLM	0.78	0.64	0.92	0.3	0.56
GAM	0.83	0.88	0.79	0.2	0.67
MARS	0.78	0.79	0.77	0.16	0.56
CTA	0.81	0.77	0.85	0.62	0.62
RF	0.85	0.82	0.9	0.69	0.7
ANN	0.8	0.85	0.76	0.02	0.61
SVM	0.84	0.92	0.76	0.69	0.68
ESDM	0.83	0.87	0.88	0.61	0.66

with cross-correlation coefficient values greater than or equal to 0.85 (Pradhan, 2016; Osorio-Olivera *et al.*, 2020 <https://github.com/luismurao/ntbox>). In this study, model training and model validation were assigned to 70 and 30% of the data, respectively (Obiakara and Fourcade, 2018).

Projection Assignment and their Transformations

Before extracting data and making predictions using the ensemble model, it is necessary to correct the projections of the Bio-Climatic (BC) and Non-BC variables, which were received from different sources and at different resolutions. In our analysis, we did this by following a set procedure in ArcMap with the help of ArcToolbox. The projection was initially outlined in the “projection and transformation” tab of the Data Management Tools interface. For this, we relied on the World Geodetic System 1984 EASE Geographic Coordinate System (GCS). We transformed the projections of the habitat class raster file to WGS 1984 web Mercator (auxiliary sphere-3857) in order to use the “calculate geometry” window of Arc Map to quantify area under each habitat suitability class (see below). To proceed, let’s say we want to determine the region under a certain class using a measurement system of the user’s choosing (we utilized square kilometers).

Species Distribution Modeling

The following algorithms from the Dismo 1.1-4 package (Hijmans *et al.*, 2017) were used for modeling: Generalized Linear Models using Gaussian distribution (GLM), generalized additive model (GAM) (Wood 2019), support vector machines (SVM), random forest (RF) (Breiman, 2001; Cutler, 2018), multivariate adaptive spline (MARS), and maximum entropy (Maxent v. 3.4.1; Phillips *et al.*, 2017; Layola *et al.*, 2022). Artificial neural network (ANN) (Ripley and Venables, 2020), and classification tree analysis (CTA) (Therneau *et al.*, 2019), the last two being executed through the biomod-2 package (Thuiller *et al.*, 2009). We used the default for all studied algorithms. We split up the SDM analysis into two parts: (a) determining which model or algorithm performed best based on predetermined evaluation criteria (discussed

further down), and (b) doing modeling using an ensemble approach that included both climatic and non-climatic predictors.

For more accurate predictions, models were evaluated using K-fold cross-validation with ten folds and ten replications for each algorithm (Rajamanickam *et al.* 2021). For each replication, the data is randomly split into ten folds, and the model is evaluated using the other nine folds for calibration (Elith *et al.*, 2011). We measured the predictive algorithm’s efficacy with six different metrics: overall accuracy (the percentage of correctly classified data), sensitivity (the probability of predicting the presence of a species), specificity (the probability of predicting the absence of a species), Cohen’s Kappa (a measure of the correctness of predictions), true skill statistic (TSS), and area under the receiver operating curve (AUC). The TSS and KAPPA values typically range from 0.40 to 0.75, with values exceeding 0.75 indicating excellent model performance. The performance of a model is likely to be subpar if the value is less than 0.40. (Beaumont *et al.*, 2016; Ahmad *et al.*, 2019). The AUC was used to evaluate the classifier’s performance on presence/absence datasets. When evaluating the AUC statistic, the following grades are used: exceptional (0.90–1.00), very good (0.8–0.9), good (0.7–0.8), fair (0.6–0.7), and poor (0.5–0.6).

A model’s specificity measures accuracy in detecting absences. The sensitivity of a model is measured by how often it correctly identifies the existence of a target. Using their respective variable importance rankings, the contributions of bio-climatic and non-bio-climatic variables were determined (Irving *et al.*, 2019). To estimate the upper bound of the most influential predictors on the habitat appropriateness of this fungus, we used the `getResponseCurve` function of the `sdm` program in the R language (Naimi and Araujo, 2016).

Post Ensemble Analysis

Habitat suitability

ASCII raster outputs from the ensemble were imported into ArcMap, and cell values (0 to 1) were used to categorize habitats for this species. Based on the continuous point break criteria for each class (Khan *et al.*, 2022), we divide algorithms into four distinct categories based on their level of suitability: optimal, moderate, marginal, low, and absent/inappropriate. We used the raster calculator to determine the total area (in square kilometers) that falls into these categories (spatial Analyst Tool/Map Algebra/Raster Calculator).

Non-Parametric Assessment of Internal Matrix of Habitat Classes

Each of the examined projections was subjected to a regression analysis to determine the link between the number of polygons and the total area covered by the four

Table 3: Model qualities parameters with 2050 bio-climatic times frames and four Representative Concentration Pathways

Algorithms	2050 RCP 2.6					2050 RCP 4.5				
	AUC	Sensitivity	Specificity	Kappa	TSS	AUC	Sensitivity	Specificity	Kappa	TSS
GLM	0.78	0.78	0.78	0.16	0.56	0.78	0.82	0.75	0.15	0.57
GAM	0.78	0.77	0.79	0.16	0.56	0.79	0.82	0.76	0.15	0.58
MARS	0.78	0.8	0.76	0.16	0.56	0.78	0.75	0.81	0.18	0.56
CTA	0.79	0.76	0.82	0.58	0.58	0.81	0.82	0.73	0.55	0.55
RF	0.85	0.93	0.87	0.7	0.7	0.85	0.84	0.9	0.68	0.74
MAXENT	0.79	0.85	0.73	0.02	0.58	0.78	0.82	0.75	0.02	0.57
ANN	0.82	0.87	0.76	0.64	0.64	0.77	0.79	0.76	0.55	0.55
SVM	0.85	0.92	0.77	0.6	0.7	0.84	0.94	0.74	0.61	0.68
ESDM	0.82	0.91	0.84	0.62	0.71	0.81	0.85	0.85	0.56	0.7

Algorithms	2050 RCP 6.0					2050 RCP 8.5				
	AUC	Sensitivity	Specificity	Kappa	TSS	AUC	Sensitivity	Specificity	Kappa	TSS
GLM	0.79	0.82	0.76	0.14	0.58	0.78	0.82	0.75	0.15	0.57
GAM	0.82	0.89	0.76	0.16	0.65	0.80	0.82	0.76	0.15	0.58
MARS	0.78	0.8	0.76	0.15	0.56	0.78	0.75	0.81	0.18	0.56
CTA	0.82	0.91	0.71	0.62	0.62	0.77	0.82	0.73	0.55	0.55
RF	0.85	0.91	0.87	0.67	0.78	0.83	0.89	0.89	0.69	0.78
MAXENT	0.79	0.82	0.76	0.02	0.58	0.78	0.82	0.75	0.02	0.57
ANN	0.81	0.89	0.69	0.58	0.58	0.77	0.79	0.76	0.55	0.55
SVM	0.83	0.91	0.75	0.62	0.66	0.84	0.94	0.74	0.61	0.68
ESDM	0.82	0.86	0.81	0.57	0.67	0.82	0.91	0.84	0.69	0.75

analyzed habitat suitability classes: optimal, moderate, marginal, and low.

Network analysis, a graphical statistical tool, allows for easy visualization and comprehension of relationships between many different variables. In contrast to latent variable modeling, where the structure of the variables is attempted to be reduced to their shared information, we instead directly estimate the relationship between all variables (refers to a diverse group of statistical techniques that employ one or more unobserved variables to explore and explain relationships between a larger set of observed variables, such as regression analysis, principal component analysis and structural equation modeling). Networks are graphical structures comprised of nodes, which are typically circles representing variables. Connections between nodes are represented by lines called edges. Both un-weighted and weighted networks exist. While in unweighted networks, an edge just indicates a connection between nodes, in weighted networks, it also indicates the strength of that connection. A thicker link between two nodes indicates a stronger bond between them.

Both directional and non-directional networks exist. In the former, edges that represent a line of influence or

a route between nodes are marked with arrows. Without arrows, a non-directional network's edges are just straight lines that don't indicate anything about the order in which the variables' interactions should be explored (Epskamp *et al.*, 2018). To better comprehend the connections between the four habitat suitability classes and their respective predictors—the present BC+NBC, 2050, and 2070 with RCPs ranging from 2.6 to 8.5, we used the non-directional weighted network analysis in JASP software (Love *et al.*, 2019).

Results

Based on data gathered from a variety of sources, we were able to pinpoint the exact GPS coordinates of 46 distinct natural populations of this species across India. We used these locations to evaluate the ecological niche of this species under a range of climatic and non-climatic conditions. Figure 1F shows a graphical representation of various locations. The current extent of occurrence (EOO) and area under occupation (AOO), according to our IUCN assessment, were 2123159 and 126 sq. km. These findings indicated a least concern category with EOO greater than 20,000 km², but with AOO it might be considered an

Table 4: Model qualities parameters with 2070 bio-climatic times frames and four Representative Concentration Pathways.

Algorithms	2070 RCP 2.6					2070 RCP 4.5				
	AUC	Sensitivity	Specificity	Kappa	TSS	AUC	Sensitivity	Specificity	Kappa	TSS
GLM	0.79	0.85	0.74	0.14	0.59	0.78	0.86	0.7	0.14	0.56
GAM	0.77	0.78	0.77	0.14	0.55	0.78	0.86	0.7	0.14	0.56
MARS	0.80	0.8	0.78	0.16	0.58	0.80	0.79	0.78	0.19	0.57
CTA	0.78	0.8	0.77	0.57	0.57	0.81	0.73	0.89	0.62	0.62
RF	0.87	0.9	0.88	0.74	0.78	0.82	0.88	0.85	0.68	0.73
MAXENT	0.8	0.84	0.77	0.02	0.61	0.78	0.82	0.73	0.01	0.55
ANN	0.82	0.86	0.77	0.64	0.63	0.82	0.91	0.73	0.64	0.64
SVM	0.83	0.89	0.77	0.67	0.66	0.82	0.92	0.71	0.64	0.63
ESDM	0.81	0.88	0.82	0.58	0.7	0.81	0.84	0.86	0.58	0.7

Algorithms	2070 RCP 6.0					2070 RCP 8.5				
	AUC	Sensitivity	Specificity	Kappa	TSS	AUC	Sensitivity	Specificity	Kappa	TSS
GLM	0.78	0.79	0.78	0.18	0.57	0.75	0.64	0.87	0.2	0.51
GAM	0.81	0.82	0.81	0.25	0.63	0.76	0.73	0.78	0.14	0.51
MARS	0.80	0.73	0.85	0.21	0.58	0.81	0.82	0.73	0.13	0.55
CTA	0.81	0.84	0.78	0.62	0.62	0.82	0.82	0.82	0.64	0.64
RF	0.84	0.88	0.87	0.75	0.75	0.84	0.89	0.87	0.67	0.76
MAXENT	0.79	0.77	0.81	0.03	0.58	0.8	0.82	0.79	0.02	0.61
ANN	0.87	0.81	0.79	0.75	0.6	0.8	0.91	0.7	0.61	0.61
SVM	0.86	0.84	0.85	0.68	0.69	0.81	0.82	0.81	0.6	0.63
ESDM	0.81	0.84	0.83	0.64	0.67	0.82	0.87	0.79	0.58	0.66

endangered species due to its AOO being less than 500 km². Of the 46 sites in the natural population identified by our research, 32 can be classified as subpopulations. We were able to rule out one of the bio-climatic variables that were part of a strongly correlated pair using a multi-collinearity test.

Comparative Assessment of Various Algorithms

We used AUC, Kappa, and TSS to compare the predictive abilities of different machine learning algorithms, including GLM, GAM, MARS, CTA, RF, ANN, SVM, and an ensemble approach (ESDM). For the current set of BC and NBC variables (Table 2), we observed that random forest (RF) performed best. When comparing the aforementioned algorithms, we found that while their AUC values were comparable, their Kappa values varied considerably. Random Forest and SVM both achieved the highest value (0.69). Conversely, ANN yielded the lowest value (0.02). In addition, RF has the highest TSS value. Tables 3 and 4 presented outcomes of model quality parameters for the 2050 and 2070 climatic timeframes using different RCPs. All RCP values greater than 0.80 were observed for the RF and SVM algorithms, indicating excellent model qualities. Both of these methods had TSS values above 0.60, indicating that they produced

satisfactory models. In contrast, the lowest Kappa values were found for GLM, GAM, and MARS, indicating that these machine learning tools are not well suited for making predictions about the habitat suitability of this species. Both the AUC and TSS for the ensemble model, including all of the studied factors, were over 0.80 and 0.65, respectively, showing excellent predictive power.

Assessment of Variables Importance for Habitat Suitability Model

The current bio-climatic, root-zone, and land-cover variables that regulate the different types of habitat suitability for this species are shown in Table 5. Grassland/scrub/forest cover (VIP = 18.86) and isothermality (VIP = 10.6; which is reliant on temperature), were the two most influential bio-climatic indicators. The total plant-available soil water storage capacity in the rooting zone (TPASWSCRZ) was found to have a moderate effect on root zone variables, with a variable importance percentage (VIP) value of 7.7. Other than the above parameters, minimum temperature of the coldest month (BC-6); annual temperature range (BC-7); annual precipitation (BC-12); precipitation seasonality (BC-15); irrigated and rain-fed cultivated lands had VIP values greater than 4.0, implying that both bio-climatic and non-

Table 5: Percentage contribution (or Variable Importance Percentage) of different current Bio-climatic, solar and landcover variables

Current BC, Solar and Landcover Variables		VIP
Bio-Climatic Variables	Isothermality (BC-3)	10.6
	Maximum Temperature of Warmest Month (BC-5)	4.82
	Minimum Temperature of Coldest Month (BC-6)	5.50
	Temperature Annual Range (Bc-7)	5.36
	Mean Temperature of Driest Month (BC-9)	3.40
	Mean Temperature of Warmest Quarter (BC-10)	1.77
	Mean Temperature of Coldest Quarter (BC-11)	2.40
	Annual Precipitation (BC-12)	7.91
	Precipitation Seasonality (BC-15)	5.74
	Ecosystem (95%) Rooting Depth (ERD)	4.27
Root Zone Variables	Total Plant-Available Soil Water Storage Capacity of the Rooting Zone (TPASWSCRZ)	7.7
	Barren and very sparsely vegetated land	3.45
Landcover Variables	Irrigated Cultivated Land	6.89
	Rain-Fed Cultivated Land	6.25
	Forest	4.97
	Grass/Scrub/Woodland	18.86

bioclimatic factors control its current habitat types.

Table 6 displays the VIP values of bio-climatic variables for the years 2050 and 2070 under different RCPs. Precipitation of the wettest quarter (BC-16 PrWeQ) was found to be the most influential variable for this species in 2050 across all four RCPs. Using this information, the results showed that annual precipitation (BC-12; AnPr) and isothermality (BC-3) were the next most influential elements, with RCPs of 2.6 and 4.5, and RCPs of 6.0 and 8.5, respectively (Table 6).

Moreover, under RCP 2.6 and 6.0 for the 2070s, precipitation seasonality (Coefficient of Variation (BC-15 PrS) was found to be the most relevant component, with VIPs of 24.74 and 24.80, respectively. Nonetheless, the most important components for RCP 4.5 and 8.5 were found to be precipitation of the coldest quarter (BC-18; PrCQ) and isothermality (BC-3), with VIPs values of 22.79 and 22.66, respectively (Table 5). It was shown that the driest month precipitation (BC-14; PrDM) had the lowest relative contribution to change (RCPs of 2.6, 6.0, and 8.5). Maximum temperature in warmest month (BC-5; MaTWaM) is an example of an energy variable that RCP4.5 finds to be the least relevant. These findings suggest that water-related factors will ultimately determine whether or not this species' current habitat will continue to be suitable for it in the future. Using response curves, we were able to quantify the

relationship between the presence of *V. zizanioides* and a range of environmental variables, thereby enhancing our understanding of the species' ecological niche. (Figure 2). Although isothermality was found to be an influential factor in both the present and 2070 RCP 8.5 scenarios, the optimal isothermality ranged from 25 to 35% under the current conditions and 45 to 55% under the 2070 RCP 8.5 scenarios. Analyses using RCP 2.6 to 8.5 for the year 2050 showed a greater range (500–3000 mm) of precipitation of the wettest quarter (PrWeQ) for this species (Figure 2). The unpredictability of precipitation is reflected in the seasonality of precipitation (BC-15), with higher percentages indicating greater variability. Our research shows that the species under study is able to thrive in areas with a wider range of precipitation variability and that the suitability of habitat for this species increases rapidly with increasing precipitation seasonality. 2070 RCP 4.5 warmest quarter (BC-18; PrWaQ) precipitation projections showed a significant improvement in habitat for this species, up to 500 mm.

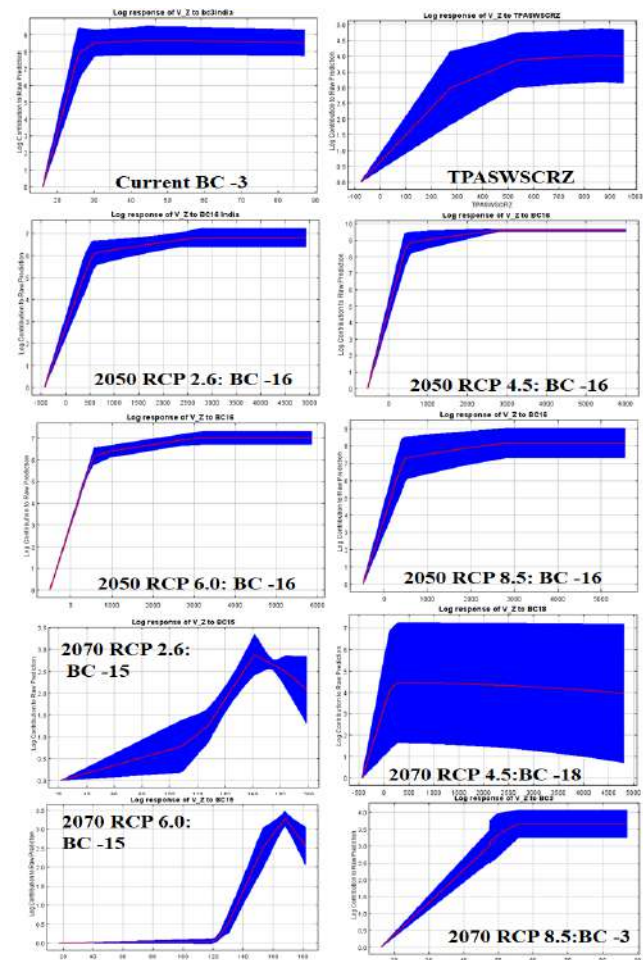


Figure 2: Response curves showing dependence of habitat suitability of *V. zizanioides* with most important variables pertains to different climatic timeframe, RCPs and non-climatic variables

Table 6: Percentage contribution (or Variable Importance Percentage) of Bio-climatic variables of two-time frames with four RCPs. Bioclimatic variables with higher VIP values were used to make the link between species and their environment

Variables	2050				2070			
	RCP 2.6	RCP 4.5	RCP 6.0	RCP 8.5	RCP 2.6	RCP 4.5	RCP 6.0	RCP 8.5
BC-1	7.21	x	x	x	x	x	x	x
BC-3	x	6.90	14.96	13.86	13.81	12.83	11.32	22.66
BC-4	8.20	x	x	x	x	x	x	x
BC-5	x	3.78	6.08	5.10	7.78	6.10	x	12.88
BC-6	x	4.37	10.40	7.79	x	9.45	4.49	x
BC-7	3.75	x	x	x	x	7.06	x	x
BC-8	3.44	6.0	6.84	6.19	7.69	9.5	x	12.86
BC-9	x	11.32	9.17	7.63	10.57	x	14.72	x
BC-10	5.24	3.47	7.09	5.57	x	x	5.0	x
BC-11	x	x	x	x	x	x	x	x
BC-12	17.67	16.13	x	x	x	x	x	x
BC-13	x	x	x	x	x	22.15	19.54	x
BC-14	6.99	12.57	5.13	4.69	5.73	x	6.65	4.20
BC-15	14.4	x	x	10.85	24.74	10.0	24.80	12.19
BC-16	18.50	21.09	20.79	19.29	13.78	x	x	19.50
BC-17	6.77	5.65	6.59	5.83	6.91	x	x	4.52
BC-18	7.74	8.78	12.91	13.19	8.96	22.79	13.43	11.16

x =eliminated variables due to their significant co-linearity with other variables

Table 7: Area under different habitat suitability classes of --- with studied bio-climatic and non-bioclimatic variables

Projections	Habitat Suitability Classes (Sq. Km.)			
	Optimum	Moderate	Marginal	Low
Current BC+NBC*	137595.41	1196523.70	1122624.31	500677.47
2050 RCP 2.6	91910.45	1380420.14	1206374.25	330904.42
2050 RCP 4.5	51140.08	865688.25	1154459.85	867698.00
2050 RCP 6.0	116271.13	1255448.17	1217124.55	531528.35
2050 RCP 8.5	51049.55	658782.93	1701987.07	505182.51
2070 RCP 2.6	73212.83	826888.05	1608108.96	358589.61
2070 RCP 4.5	123293.74	1251106.89	1286825.39	198589.61
2070 RCP 6.0	106214.45	718015.16	1704007.06	535545.43
2070 RCP 8.5	133593.34	1069453.86	1402216.93	511527.66

NBC* root zone and Landcover variables

Spatial Extent (Square Kilometres) of different Habitat Classes under various Projections

Table 7 shows the area (square kilometers) that falls into each of the four analyzed habitat suitability classes (optimum, moderate, marginal, and low). Their spatial range suggests that the optimum area is largest with current BC, root zone, and land cover variables (13,7595.41 km²), and the smallest with 2050 RCP 8.5 (51,094.95). The marginal habitat

class showed the opposite pattern, with the largest area (1701987.07) reported for 2050 RCP 8.5 and the smallest area (1122624.31) recorded under current BC, root zone, and land cover variables. The largest area covered by the moderate category (1380420.14) was found for 2050 RCP 2.6, while the smallest area (65872.93) covered by this category was found for 2050 RCP 8.5.

Figure 3 (current BC and 2050) and Figure 4 (2070) depict

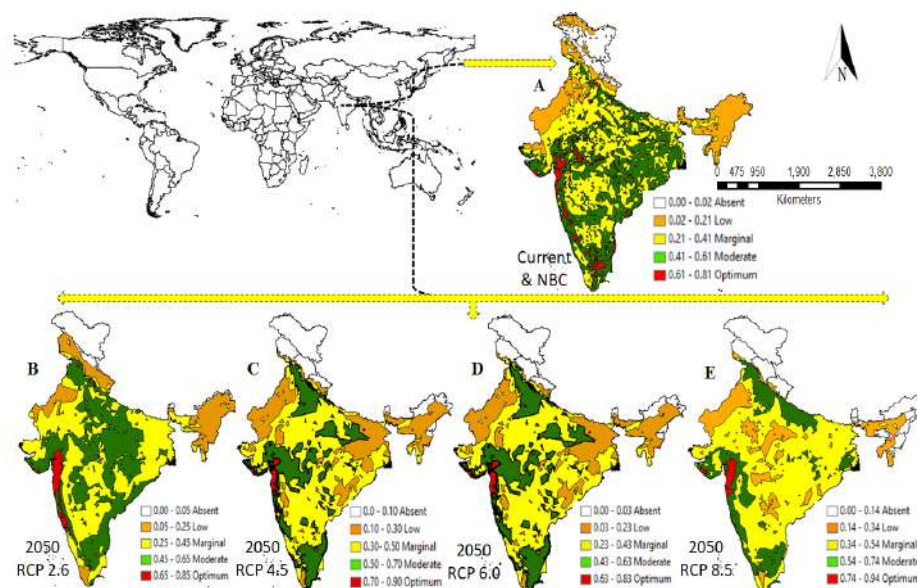


Figure 3: Habitat suitability of *V. zizanioides* current bioclimatic and non-bioclimatic (A) as well as with 2050 bio-climatic predictors (B) RCP 2.6; (C) RCP 4.5; (D) RCP 6.0 and (E) RCP 8.5.

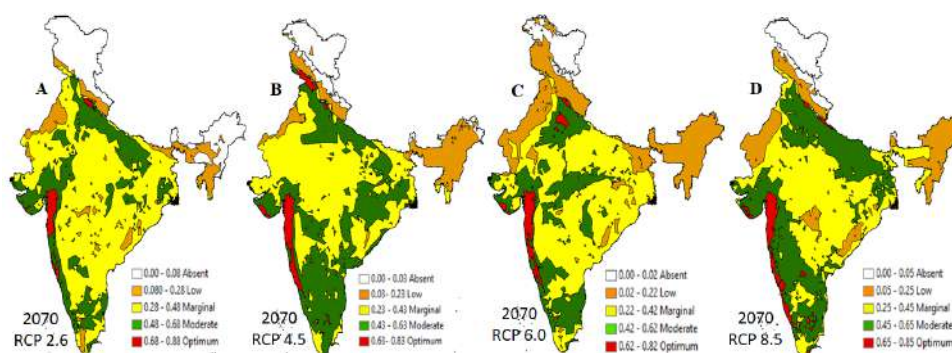


Figure 4: Habitat suitability of *V. zizanioides* with 2070 bio-climatic predictors (A) RCP 2.6; (B) RCP 4.5; (C) RCP 6.0 and (D) RCP 8.5.

the spatial extents of these classes. Using current bioclimatic, root zone, and land cover variables (Figure 3A), this analysis concluded that parts of Gujarat (Somnath, Anand, Vadodara, Surat), Maharashtra (Vapi, Silvassa, Nasik, Mumbai, Kolhapur, Chandoli National Park, Malkapur), and Karnataka (Topinkatti, Khnapur, Nandgad) are the best places for this species. Similarly, southern locations such as Thuraiyur, Mayanur, Pallapatii, and Chennimalai in Tamil Nadu, as well as some northern places such as Ayodhya, Kanpur, Pryagraj, and Etowah in Uttar Pradesh, have been identified as optimal areas. While the majority of eastern Indian states, such as West Bengal, Odisha, Chhattisgarh, and Jharkhand, as well as parts of Bihar, provide a moderate habitat for this species (Figure 3). Tamil Nadu, Karnataka, western territories such as Gujarat, and sections of Madhya Pradesh (Central India) have also been recognized as moderate sites. The

majority of Madhya Pradesh, Haryana, and Uttar Pradesh are marginal areas for this species, whereas Nagaland, Arunachal Pradesh, Assam, Manipur, Mizoram, and western dry areas of Rajasthan have been recognized as low suitable habitats for this species under present climatic circumstances.

With a 2050 estimate and RCPs from 2.6 to 8.5 (Figure 3 B to E), the analysis shows that the optimum area is shrinking and becoming more concentrated to the south-west. Specific tendencies for modest area contraction and marginal area expansion were also observed. We found optimum habitats in Uttar Pradesh, Bihar, Uttarakhand, Madhya Pradesh, Punjab, Haryana, and the Southern Ghats using 2050 RCP2.6. From RCP 4.5 to RCP 8.5, such optimum zones will erode and be replaced by marginal areas. This analysis demonstrates the emergence of low-appropriate habitat types in various parts of central India with 2050 RCP 8.5 as

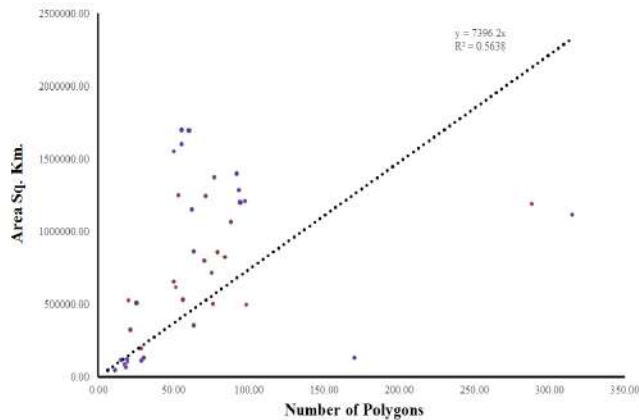


Figure 5: Regression analysis between number of polygons and areas under different suitability class with studied climatic and non-climatic projections (Includes all RCPs)

compared to the current condition (Figure 3). The south-western regions of a country are predicted to be optimum under all RCP scenarios from 2.6 to 8.5 in 2070 (Figure 4 A to D). Intriguingly, RCP2.6 and 6.0 showed higher moderate-area loss than RCP4.5 and 8.5. (Figure 4).

The optimal habitat for this species is just 4.19% of India's total land area, while the lowest value for this category (1.55%) was recorded with 2050 RCP 8.5. (Table 8). From the current BC, root zone, and land cover variables to other climatic epochs and the RCPs projection, it has been documented that the area occupied by this optimum category is steadily decreasing. Although this group has seen minimal land area expansion since 2050, this trend does not appear to have reversed by 2070. Moderate (41.99%) and low (26.40%) habitat types both had significant increases in

Table 8: Per cent of total land area of India

Projections	Habitat suitability classes			
	Optimum	Moderate	Marginal	Low
Current BC+NBC	4.19	36.40	34.15	15.23
2050 RCP 2.6	2.80	41.99	36.70	10.07
2050 RCP 4.5	1.56	26.33	35.12	26.40
2050 RCP 6.0	3.54	38.19	37.03	16.17
2050 RCP 8.5	1.55	20.04	51.78	15.37
2070 RCP 2.6	2.23	25.15	48.92	10.91
2070 RCP 4.5	3.75	38.06	39.15	6.04
2070 RCP 6.0	3.23	21.84	51.84	16.29
2070 RCP 8.5	4.06	32.53	42.66	15.56

area with a 2050 climate time frame but with RCP 2.6 and RCP 4.5, respectively (Table 8). Across all variables tested, we found a linear relationship between the number of polygons and their total area (square kilometers) and it can be equated as area (Sq. Km.) = 7396.20 x number of polygons ($R^2 = 0.56$ $p > 0.01$; Figure 5).

Figure 6 depicts the results of the network analysis. This non-parametric test for assessing the internal matrix of different habitat classes identified with different bio-climatic projections and RCPs. It revealed a significant negative relationship between current habitat classes (optimum, moderate, marginal, and low) and all RCPs of optimum and moderate habitat types in 2050 and 2070. However, such connections were only documented for the marginal class with 2070 and RCPs. For all habitat classes, we found substantial intra-RCP connections of bio-climatic time frame with distinct RCPs.

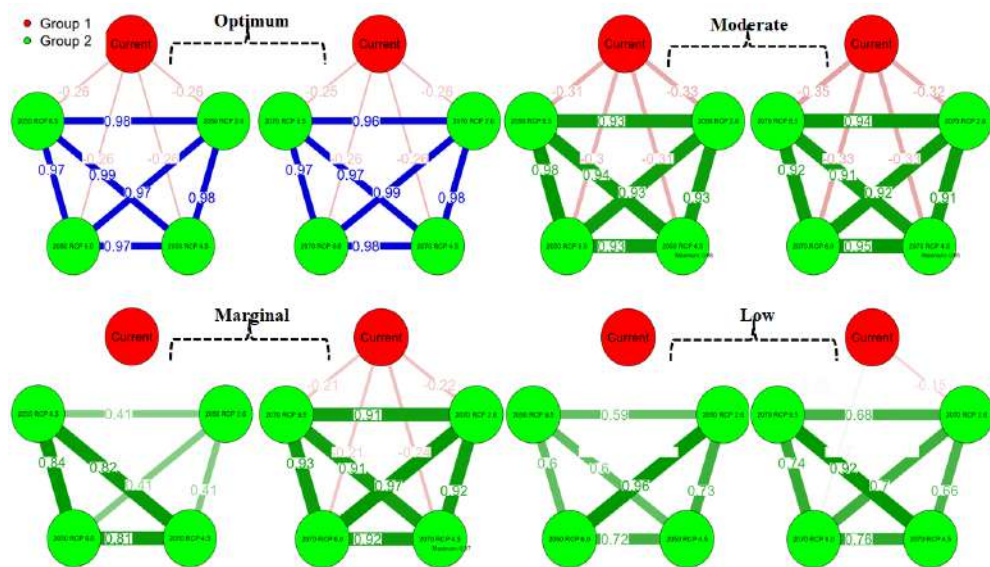


Figure 6: Network comparison between the areas (Sq. km.) classified under different suitability classes with different projections. Circle represent the projection; line or edge showed relationship with different nodes (edge width also corresponds to the strength of their

Discussion

Given the rapid pace of climate change, SDM has emerged as the most important method for predicting species' distributions and informing management decisions. In addition, the IUCN has started to formally incorporate SDMs for determining the frequency of occurrence as a proxy for extinction risk and for studying the impacts of climate change on species distribution (Cassini, 2011). Researchers in the Indian subcontinent have mostly relied on a single model approach, such as Maxent, when simulating the patterns of plant species' distributions. This has the potential to lower prediction accuracy and consequently lower confidence in projected species distributions (Bhandari *et al.*, 2020; Boral and Moktan, 2021; Purohit and Rawat, 2021; Shekhar *et al.*, 2022). In contrast to the standard practice, which relies solely on one algorithm (Maxent), we use a suite of seven algorithms (including Maxent) to predict the distribution dynamics of *V. zizanioides* and conduct a risk assessment across the entirety of India. When compared to single-model algorithms, the ensemble modeling approach provides several benefits (Ahmad *et al.*, 2019; Hamid *et al.*, 2019). (Mathur and Mathur, 2023). This study adds to the findings of Kumari *et al.* (2022), showing that using an ensemble approach to predict species distributions improves both the accuracy and confidence with which those predictions can be made. AUC, Kappa, and TSS were used to compare GLM, GAM, MARS, CTA, RF, ANN, SVM, and ESDM. SVM algorithms had good model quality, but RF performed best with BC and NBC variables. AUC and TSS of ensemble models with all predictors > 0.80 and > 0.65 indicate very good model predictive power.

From the perspective of protecting biodiversity, models of species distribution are crucial. They make maps by drawing on relationships between environmental factors and occurrence data on species' distributions. These maps are useful for gaining ecological understanding and making predictions about the distribution of species in various habitats. The evolution of SDM has aided biodiversity conservation by bridging the gap between research and policymaking. With the help of SDM, interested groups can determine with precision where to direct their efforts. Other uses include understanding the effects of climate change, cataloging species diversity, and pinpointing prime locations for preservation and reintroduction efforts. We found no previous research that attempted to predict whether or not the entire range of *V. zizanioides* in India would contain a variety of habitat types that were conducive to the survival of this species. Our findings have direct implications for identifying areas that support higher above- and below-ground biomass, which is significant because vetiver has the potential to be a candidate for land restoration and reclamation, as well as for selecting appropriate habitats that foster vetiver oil quantity and quality.

By using data from 46 natural population sites, we determined the EOO and AOO according to IUCN criteria, and our analysis places this species in the least concern and endangered categories, respectively and providing vital information to conservationists about the species' distribution and how to best ensure its survival. Khan *et al.* (2022) found analogous things for *Pinus geradiana* in the Himalayas.

In present, our model predicts that this species' optimal and moderate habitat types are dispersed across the southern, south-west, and south-eastern zones, with marginally suitable areas presented here and there in between the aforementioned two classes. However, as we progressed through the climatic time frame and RCPs, we noticed two distinct patches of optimum and moderate areas located in opposite directions (Figures 3 and 4), roughly northern and southern. In contrast, marginal habitat types occupy the majority of central India. Based on our research, it appears that the western, northern, and eastern parts of the country are not good places to grow this species. SDM modeling of such patchy behavior for various plant species has been reported by researchers from different regions of the country, including Song *et al.* (2004), Xiaodan *et al.* (2011), Rashid *et al.* (2015), Manish *et al.* (2016), and Hamid *et al.* (2018). Such studies, however, reported the likelihood of a shift in vegetation and a rearrangement of plant assemblages to the north, both of which can be linked with future warming as predicted. Based on the predators we've studied, we know that this particular species prefers to head in a south-westerly direction.

There are two different kinds of vetiver. Unlike its southern Indian counterpart, the northern Indian variety is typically smaller and is gathered from the wild rather than farms. Even in wet conditions, it will reliably bloom and produce seed. The other variety is a staple in agriculture and is thought to have originated in southern India, where it was domesticated. It produces almost no usable seeds and has a low flowering rate, so it must be grown from cuttings (Lal *et al.*, 2021b). The use of vetiver as a permanent field boundary and in contour strips in southern India has been shown to be effective in reducing soil erosion. Due to low night-to-day temperature swings and an early start to the monsoon season, February and April are ideal for planting in South India (Lal *et al.*, 2018).

In North Indian varieties, self-sown seeds lead to abundant seeding and natural regeneration. The typical seed yield per hectare is between 400 and 650 kilograms. The newly harvested spikelets are underdeveloped and will need to sit for an additional three months. Taking the caryopsis out of its shell helps the seed germinate. Most spikelets on south Indian varieties are sterile, and the thin, short-germinating seeds that are occasionally produced are not very hardy. The rhizome is not disturbed when

propagating these non-seeding varieties; instead, the 15 to 20 cm long aerial portion is used to produce slips for further growth. North Indian varieties take only 35 to 40 hours to distill due to their high boiling point and low volatile oil content, while south Indian varieties take 72 to 96 hours. Two different oil fractions can be extracted from vetivers, one of which is lighter than water and the other of which is heavier. Oil's quality rises as its density rises. Finding out what traits this species possesses that will allow it to thrive in the central Indian plains and other moderately favorable sites in 2050 and 2070 under RCP 2.6, 4.5, and 6.0 would be a top priority for researchers based on the results of this study (Figures 3 and 4). In addition to assessing the influence of climatic predictors, we also evaluated the effect of landcover and root zone variables in our study, which is something that hasn't been done before for grass or Poaceae genera. Grassland/scrubland/woodland land cover type is the strongest predictor for this species, followed by isothermality and then barren, irrigated, rain-fed, and forest. In agreement with Lu *et al.* (2019) this finding suggests this species as a possible candidate for grassland restoration and management. To the best of our knowledge, this study was the first to apply Total Plant-Available Soil Water Storage Capacity of the Rooting Zone (TPASWSCRZ) to the modeling of the distribution of any indigenous Indian plant species. When precipitation is spotty and intermittent, as is typical in tropical countries like India, TPASWSCRZ values have a significant impact on grain crop yields.

Crop response to nutrient applications is influenced by a number of factors, including the efficiency with which nutrients are taken up by the plant and used by the plant (Leenaars *et al.*, 2015). According to the TPASWSCRZ response curves (Figure 2), the species' habitat becomes more and more suitable at an exponential rate. It rises to a maximum of 600 mm and then levels off at 900 mm. Therefore, one must guarantee this minimum level of soil water storage to boost its biomass. Dudai *et al.* (2006), who found that it thrives in regions with a moderately humid climate and annual rainfall of 1000 to 2000 mm, reached the same conclusion. Conventional wisdom holds that climate is the single most important factor in determining where species can be found, as it reveals crucial details about the kinds of environments in which they can thrive (Marino *et al.*, 2011). Furthermore, three precipitation-related variables (Precipitation of Driest Month, Precipitation Seasonality, and Precipitation of Warmest Quarter) were found to significantly influence the distribution of *Betula utilis* by Hamid *et al.* (2019).

Among temperature-related factors, the diurnal temperature range and isothermality were the most important. *Vetiveria* can grow in temperatures as low as -15°C and as high as +55°C. At 25°C, soil is at its most conducive to root development. Temperatures below 5°C trigger root

dormancy. The shoots may turn purple and wither or even die in cold weather, but the underground growing points remain alive and will quickly sprout again once the weather warms up.

Visualizing the responses of key bio-climatic variables to *V. zizanioides* suitability, as indicated by the Variable Importance (VI) score and their response curves, can shed light on this species' ecological niche. Under both the current and 2070 RCP 8.5 scenarios, isothermality was found to have the greatest effect; however, under the latter, the optimal range for this climatic parameter is reduced from 45–55% to 25–55%. The precipitation of the wettest quarter (PrWeQ) for this species varied more widely, from 500 to 3000 mm, across the range of representative concentration pathways (RCP) 2.6 to 8.5 for the year 2050. (Figure 2). The BC-15 seasonality of precipitation metric is a reflection of the rainfall variability, with larger percentages indicating more pronounced swings. Our findings demonstrated that this species does well in regions with a greater degree of precipitation variability and that the suitability of habitat for this species raises precipitation seasonally. With 2070 RCP 4.5 warmest quarter (BC-18; PrWaQ) precipitation, up to 500 mm, the suitability of this species significantly increased.

The northwest Himalayan perennial herb *Rheum webbianum* was found to be most sensitive to the amount and timing of precipitation during the driest month and the seasonality of precipitation (Wani *et al.*, 2021). Global habitat suitability for the annual grass *Cenchrus spinifex* was predicted by Cao *et al.* (2021) in light of present and future climate change scenarios. Under the RCP 2.6 and RCP 8.5 scenarios, they found that *C. spinifex* would have less suitable habitats than it does now in 2050 and 2070. The seasonality of precipitation (bio15), the mean temperature of the warmest quarter (bio10), the mean temperature of the coldest quarter (bio11), and the precipitation of the driest quarter (bio17) together accounted for 96.8% of the variance, indicated that optimal conditions for *C. spinifex* growth appear to be determined by temperature and precipitation. Previous research (Liang *et al.*, 2016 and 2018; Wang *et al.*, 2018 and 2021) and our own results agree that the annual total precipitation and its seasonal variation pattern, as well as temperature variations and their seasonality, are the most influential variables for its habitat suitability. Consistent with our findings, Chen *et al.* (1991) determined that the optimal temperature range for vetiver grass growth in the current bioclimatic conditions is between 25 and 30°C. According to Verma (2020) findings, the optimal range of isothermality would rise from 25 to 35% under current conditions to 45 to 55% by 2070 under RCP 8.5 scenarios.

This is in line with the well-known trait and biogeographical differences between the distributions of C_3 and C_4 species (Pau *et al.*, 2013), which show that C_4 species, on average, have a higher optimal temperature

and water-use efficiency and thus prefer drier habitats at lower latitudes. In their modeling of C_3 and C_4 grass species' distributions, Havrilla *et al.* (2022) found that these species' distributions are extremely sensitive to seasonality in precipitation. These variations may be explained, at least in part, by the fact that the various functional groups experience the seasons differently. C_3 grasses typically benefit more from cool-season and stored soil moisture than C_4 grasses, which are more reliant on summer rainfall due to their later seasonal phenology.

To ensure the survival of these valuable species, habitats must be conserved and regularly monitored, as future climate change will have significant effects on such species across all GHG projections (Khan *et al.*, 2022; Mathur and Mathur, 2023). Multiple studies, consistent with one another, attribute this species' limited distribution to the far north and east because of the extensive and prolonged snow-covered landscape in temperate climates (Sofi *et al.*, 2022; Rather *et al.*, 2022). The northern and eastern Himalayas have cooler temperatures and more precipitation over a longer period of time than the hot, arid and semi-arid western areas of India, which both pose threats to the species' survival (Rana *et al.*, 2020).

Comparing the spatial extent of the four habitat suitability classes—optimum, moderate, marginal, and low under different projections reveals that the optimum area is largest under the current BC, root zone, and land cover variables, while the lowest area under this class was recorded with the 2050 RCP 8.5. The marginal habitat class, on the other hand, recorded the highest area with 2050 RCP 8.5 and the least with current BC, root zone, and land cover variables. The largest region falling within the moderate category was found for 2050 RCP 2.6, while the smallest was found for 2050 RCP 8.5.

This research shows that the optimal area is consistently decreasing and is concentrated in the south and west based on projections to 2050 and RCPs 2.6 to 8.5. There were also discernible patterns noted for the decline of intermediate-sized regions and the growth of small- and very-small-sized regions. We found the best conditions in the states of Uttar Pradesh, Bihar, Uttarakhand, Madhya Pradesh, Punjab, Haryana, and the Southern Ghats using the 2050 RCP2.6 scenario. Such optimal regions will deteriorate between RCP 4.5 and RCP 6.0, and be replaced by marginal regions. Based on the current trends and the 2050 RCP 8.5 scenario, this study predicts the emergence of low-suitable habitat types in a portion of central India (Figure 3). The south-western regions of a country are predicted to be the best places to live under all RCP scenarios from 2.6 to 8.5 by 2070. Interestingly, RCP2.6 and 6.0 showed more moderate-area shrinkage than RCP4.5 and 8.5 (Figure 4).

The optimal habitat for this species is only 4.19 percent of India's total land area, and the lowest value for this

category (1.55 percent) was recorded with 2050 RCP 8.5. (Table 8). From the present-day BC, root zone, and land cover variables to other climatic epochs and RCPs projection, the area occupied by this category has been steadily decreasing. There has been minimal growth in land area for this category between 2050 and 2070. Under the 2050 climate scenario, however, RCP 2.6 and 4.5 showed the greatest increase in moderate (41.99%) and low (26.40%) habitat types, respectively (Table 8).

Currently, *V. zizanioides* is more common in the south (including the western and eastern ghats) and in the center of India, but it is less common in the west and east, where it faces stiffer competition. Our ensemble model predicted that the climatically suitable area for *V. zizanioides* would shrink or become unsuitable by the end of the century under both the 2050 and 2070 climate change scenarios. Part of Gujarat, Maharashtra, Karnataka, Ayodhya, Kanpur, Pryagraj, and Etawah are ideal sites for future research based on existing bio-climatic, root zone, and land cover variables. Eastern areas, such as Nagaland, Arunachal Pradesh, Assam, Manipur, and Mizoram, and western arid areas of Rajasthan are low suitable habitats.

Using a variety of projections, our network analysis shows how the space utilized by this species is distributed internally across size classes. The presence of intra-RCP correlation denotes the extent to which a certain set of predictors influences a particular class of habitat; identifying such regions and taking measures to preserve or restore them would have an undeniable impact on future time periods and RCPs.

Conclusion

To our knowledge, this is the first study to assess the risk posed by *V. zizanioides*, map its current distribution, and speculate on its possible future distribution in the Indian subcontinent. This study used ensemble modeling to predict the distribution dynamics of *V. zizanioides* in the Indian subcontinent. Results showed that the optimum places for this species are located in the south, the south-west, and the south-east, with marginal spots strewn about in between. The root zone's total plant-available soil water storage capacity (TPASWSCRZ) was found to be strongly related with its current habitats. In both the current and 2070 RCP 8.5 scenarios, isothermality was found to have the greatest impact, but under the latter, the optimal range for this climatic parameter is reduced from 45–55% to 25–35%. *V. zizanioides* is a species that does well in regions with a wider range of precipitation variability, and the suitability of habitat for this species is determined by the annual total precipitation and, its seasonal variation pattern and the seasonality of temperature variations. Studies show that the optimal area is steadily shrinking and is concentrated in the south and some portions of the north, with intermediate-

sized regions declining and small and very small regions expanding according to a discernible pattern. Low suitable habitats can be found in the eastern regions of Nagaland, Arunachal Pradesh, Assam, Rajasthan and Gujarat. The traits (aromatic oil quality and quantity, root biomass) now existing in northern and southern India vetiver varieties would be used to set research targets for future regionally unique types with varying climate projections.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The data sets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

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Author Contribution

Senior author conceptualized the chapter theme and interpretation of output of various machine learning techniques. Co-Author prepared various types of language codes in python, Java and in R scripts and convert the various file format from ASCII to KMZ, Raster, dbf, CSV etc. for software's like QGIS 3.10.0; Wallace; DIVA-GIS version 7.5; MaxEnt 3.4.1 software; SDM toolbox; Map Comparison Kit; ENMTools and Ntbox; SSDM R packages.

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RESEARCH ARTICLE

Genetic Diversity Assessment of *Cordia gharaf* (Forsk.) Germplasm for Morpho-Physiological and Fodder Traits in Hot-arid Kachchh Region of India

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Abstract

About 81% of India's arid areas fall in western Rajasthan and Gujarat. In arid regions, *Cordia gharaf* (Forsk.) is one of the important under-utilized multipurpose shrubs that is well adapted to drought, salt, dry winds, and extreme temperatures. This investigation aimed to unveil potential genotypes of species for fruit and fodder use. Sixteen accessions of *C. gharaf* were collected from the hot-arid Kachchh region of Gujarat, India. Collected germplasm was characterized for 14 morpho-physiological and 13 fodder quality traits during the year 2017 – 19, indicating wide variation. Morpho-physiological traits viz. plant height ranged from (155–244.67 cm), canopy spread (140–254 cm²), number of branches per plant (6.67–11.67) leaf area (5.88–11.3 cm²), relative water content (62.79–96.0%), relative stress injury (8.6–17.46%) and canopy temperature depression (2.17–5.83°C). Fodder quality traits like above-ground biomass (AGB) varied from (2.22–19.63 kg/ plant), calcium (0.56–3.70%), crude protein (9.07–17.31%), neutral detergent fiber (NDF, 35.9–68.9%) and acid detergent fiber (ADF, 35.62–58.7%). A high coefficient of variation was recorded for phosphorus (78.85%), AGB/plant (57.79%), silica (36.68%), and calcium (29.55%). The genotypes CG-11, CG-12, CG-13, and CG-15 were reported promising for morphological and above-ground biomass traits, whereas genotypes CG-5, CG-6, and CG-8 reported promising for fodder quality traits.

Keywords: *Cordia gharaf*, Characterization, Genetic diversity, Germplasm, Kachchh.

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Introduction

The hot-arid region of India covers around 31.7 million ha, mainly spread in the states of Rajasthan, Gujarat, Punjab and Haryana, Maharashtra, Karnataka, and Andhra Pradesh. About 81% of this area falls in India's western Rajasthan and Gujarat states (Sharma and Tewari, 2005). These regions are characterized by low and erratic rainfall with a high coefficient of variation and harsh climatic conditions. This hot-arid region is endowed with rich plant diversity and excellent adaptations to survive harsh climatic conditions. Many of these plants are a boon for this region, and the genus *Cordia* has an important role among them. The *Cordia* belongs to the Boraginaceae family and is a large genus comprised of about 300 species of shrubs; small to medium-sized trees have widely been grown in dry and hot regions of the world (Bouby *et al.*, 2011). Around 21 species of this genus have been reported in India (BSI, 2022). Out of these, four species, namely, *Cordia gharaf* (Forsk.), *C. myxa* L., *C. macleodii* (Griff.) Hook. f. et Thomson and *C. sebestena* L. were reported in hot-arid regions of India (Bhandari, 1990). Among them, *C. gharaf* (2n=72), (syn. *C. sinensis* Lam., *C. rothii* Roem. & Schult.), locally known as "Gondi", is one of the important under-utilized multipurpose shrub or small sized tree of this region. Worldwide, it is distributed in drier areas of tropical Africa, Saudi Arabia, Abyssinia, Ceylon, and India (Bhandari, 1990).

In India, it is reported in the states of Rajasthan, Gujarat, Andhra Pradesh, Goa, Haryana, Jammu & Kashmir, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Punjab, Tamil Nadu, Uttarakhand, and Uttar Pradesh (BSI, 2022). In the hot-arid Kachchh region of Gujarat, it most occurred on leveled topography on field boundaries and scrub forests with a soil pH of 8 to 8.5 (Dev *et al.*, 2020). It adapted to drought, salt, dry winds, and extreme temperatures due to morphological and physiological adaptability. It is a multi-stemmed shrub or small tree, growing up to 12 m high. Leaves are opposite or sub-opposite, ovate to obovate, and light green with pale hairs on both surfaces. Flowers are small, born in terminal and axillary cymes, white or cream, and are sweetly scented. Flowering and fruiting occurred from March to June. The fruit is 9 to 12 mm long, ovoid, drupe and bright red or yellow-orange color. One fruit contains 1 to 4 hard and rough seeds (Bhandari, 1990; Warfa, 1990; Orwa *et al.*, 2009). This multipurpose species is valued for its edible berries, fuel wood, and leaf fodder, which is available for a long season and has great medicinal importance. The fruits are eaten and sold in local markets. The children chew the bark to redden their lips as a substitute for *Pan*. The wood is used for fuel and in the manufacture of agricultural implements. The decoction of the bark is used for gargles. It has great potential to withstand abiotic pressure and reduce soil erosion because of its strong tap root system, providing livelihood security against drought and famine in the hot-arid Kachchh region of India. Leaves are mainly used as fodder by browsing animals such as goats, sheep, and camels in hot-arid regions. Its leaves are a good source of crude protein (13.8%) and dry matter (41.2%), neutral detergent fiber (NDF, 56.6%), acid detergent fiber (ADF, 43.3%), and acid detergent lignin (ADL, 18.9%) with high amount of minerals like calcium, zinc, potassium, phosphorus, and iron. Its fruits are also a rich source of energy (18.9 kcal/100 g dry wt.), crude protein (12.6%) and dietary fiber (11.6%) (Kuria *et al.*, 2005).

C. gharaf is an important fodder shrub for small ruminants, and hot-arid regions have a wide genetic diversity for the traits. As a multipurpose and under-utilized fodder and fruit shrub species in the hot-arid region of India, it is very important to collect available genetic diversity from natural habitats and characterize them for different traits for genetic improvement and further exploitation. But more scientific information is needed on germplasm collection, characterization and utilization of this species. Further, on-farm conservation and studying the genetic diversity of this under-explored shrub is very important for the identification of superior genotypes for its exploitation and utilization in alternate land use systems. Thus, the present study was carried out to collect available germplasm from the hot-arid Kachchh region of India and characterize them for further genetic improvement and utilization.

Materials and Methods

Field survey and Germplasm Collection

Field surveys were carried out in five talukas, namely Bhuj, Mandvi, Nakhatarana, Bhachau, and Rapar of arid Kachchh, Gujarat, during summer (April - May) and monsoon (July - September) seasons of two consecutive years, 2015 and 2016 for collection of germplasm of *C. gharaf*. The passport information was also collected during germplasm collection.

Evaluation of Morpho-physiological and Fodder Quality Traits

Collected seeds were sown in polythene bags, and seedlings were raised in the nursery. A total of sixteen survived germplasm lines were transplanted in the experimental field at 6×6 m spacing during August - September 2016 using RBD with five replications at the age of eight months at ICAR-Central Arid Zone Research Institute, Regional Center, Kukma-Bhuj, Gujarat for evaluation. The field evaluation, flowering, fruiting and seed photograph are presented in Fig. 1. Growth pattern traits like plant height, canopy spread, number of leaves/plant, and stem thickness were recorded at regular one-year intervals up to three years during the years 2017, 2018 & 2019 after the monsoon season. Observations were recorded for 14 morpho-physiological and 13 fodder quality traits after three years of transplanting in the field after the monsoon season (Mahajan *et al.*, 2002). The leaf area of individual genotypes was measured by taking third newer leaves from the top of five different branches with the help of a leaf area meter (CI-203 laser area meter, CID, Inc. USA).

Relative water content (RWC)

The RWC expresses the water content (%) at a given time as related to the water content at full turgor. RWC was estimated as follows (González and González-Vilar, 2001).

$$\text{RWC (\%)} = \frac{\text{Fresh weight} - \text{dry weight}}{\text{Turgid weight} - \text{dry weight}} \times 100$$

Similarly, leaves' dry weight and moisture content were calculated by weighing the fresh leaf weight and oven-dry weight (samples oven-dried for 72 hours at a constant temperature of 72°C) as per the procedure advocated by van de Sande-Bakhuyzen (1928).

Canopy Temperature Depression (CTD)

CTD is calculated as the difference between air temperature and canopy temperature using an infrared thermometer (Balota *et al.*, 2008).

$$\text{CTD} = T_{\text{air}} - T_{\text{canopy}}$$

Relative Stress Injury (RSI)

RSI is estimated as per the procedure of Dionisio and Tobita (1998). The leaf sample (0.1 g) from three randomly selected plants was cut into uniform pieces. The samples were set in 10 ml of double distilled water in two sets. One set was kept at 40°C for 30 minutes, and its conductivity (ECa) was measured. The subsequent set was held in a boiling water bath (100°C) for 10 minutes and its conductivity (ECb) was noted. RSI was calculated as follows;

$$\text{RSI (\%)} = \frac{\text{ECa}}{\text{ECa} + \text{ECb}} \times 100$$

Fodder Nutrient Analysis

Total ash was determined according to the AOAC (1990), nitrogen was estimated using the Kjeldahl method (AOAC, 2019) and crude protein was determined by increasing the nitrogen content with a factor of 6.25. The NDF and ADF were estimated as suggested by Van Soest *et al.* (1991). All the samples were analyzed thrice for nutrient analysis in *C. gharaf* leaves.

Statistical Analysis

The experiment was planned with a complete randomized block design in five replications. The mean values were used for the statistical analysis of recorded trial data for 14 morpho-physiological and 13 fodder quality traits tested at the hot-arid region of Kachchh. The plant growth and fodder quality characteristics were taken in five and three repetitions, respectively. The descriptive statistics of studied traits were derived using IBM SPSS statistics software version 20.0 (IBM SPSS, 2011). Pearson's correlation coefficient, cluster, and biplot analysis used R software version 3.6.1 (R Core Team, 2019). The "PAST4" program was used to perform principal component analysis (Hammer *et al.*, 2001).

Results and Discussion

Field survey, collection and distribution pattern

During the field survey, 20 distinct germplasm in the form of ripened fruits were collected from different habitats. Its distribution was found in various natural habitats like wild areas, field boundaries, backyards, roadsides, and near human settlements in Bhuj, Rapar, and Mandvi mandals of the Kachchh district. The passport information of collection sites was also collected and is presented in Table 1.

Growth pattern of *C. gharaf* from the year 2017 to 2019

The growth pattern of *C. gharaf* germplasm showed good plant growth concerning plant height (cm), canopy spread (cm²), number of leaves per plant and stem thickness (mm) during the years 2017 to 2019 in the field (Table 2). The plant height increased up to 206.3 cm after the third year

of planting in 2019 and varied from 41 to 120 cm, 121.7 to 212 cm, and 155 to 244.7 cm during 2017, 2018, and 2019, respectively. The canopy spread ranged from 27.5 – 73.0 cm², 118 to 223 cm², and 140 to 254 cm², with mean values of 48.8 cm², 177.5 cm² and 203.3 cm² during 2017, 2018 and 2019, respectively. The number of leaves per plant increased considerably up to the third year in 2019, reaching a value of 4201 leaves per plant. The stem thickness also substantially increased to 32.8 mm after the third year of planting in 2019. The abundant plant growth of this species in adverse climatic conditions of hot-arid regions makes it suitable for introduction in the rangeland improvement programs.

Genetic variability for morpho-physiological traits

Descriptive statistics carried out for 14 morpho-physiological traits of sixteen *C. gharaf* accessions showed a wide range of variability (Table 3 and Supplementary Table 1). The plant height and canopy spread ranged from 155 to 244.67 cm and 140 to 254 cm², respectively, with a mean value of 206.33 cm and 203.3 cm², respectively. The number of branches and leaves per plant range from 6.67 to 11.67 and 2430 to 6047.33, respectively, with a mean value of 8.81 and 4201.33. Similarly, leaf length and width ranged from 5.21 to 6.63 cm and 7.57 to 9.57 cm, respectively, with a mean of 5.78 cm and 8.75 cm, respectively. The trait stem thickness was recorded between 21.59 and 46.0 mm, with a mean value of 32.8 mm. Petiole length was measured between 1.97 and 2.93 cm with a mean of 2.5 cm, whereas petiole width was recorded in the 3.22 and 4.18 mm range with a mean of 3.67 mm. The important trait contributing mainly to photosynthesis and biomass production, i.e., leaf area, varied between 5.88 and 11.30 cm² with an average value of 9.35 cm².

A considerable level of variation was found among the physiological traits that were evaluated in the third year. These characteristics showed how resilient and adaptable a genotype is under stress conditions. The RWC, CTD, and RSI values range from 62.79 to 96%, 2.17 to 5.83°C, and 8.6 to 17.46%, with a mean value of 71.18%, 3.72°C, and 13.33%, respectively.

Among the tested morpho-physiological traits, the highest coefficient of variation (CV) was recorded for CTD (23.99%) followed by the number of leaves per plant (23.28%). The lowest CV was observed for leaf width (6.01%), followed by leaf length (6.95%) and petiole width (7.53%).

Among the studied genotypes, the genotype CG-13 collected from the roadside of Mandvi showed maximum plant height (244.67 cm) followed by CG-14 (243.33 cm) and minimum CG-02 (155.0 cm). Similarly, the maximum canopy spread was noted for genotype CG-11 (254.0 cm²), followed by CG-13 (249.33 cm²) and minimum in CG-02 (140.0 cm²) in the third year of evaluation. The genotype with the maximum leaf area was CG-12 (11.30 cm²), followed by CG-16 (10.80 cm²), and the minimum was CG-01 (5.88 cm²). The highest RWC was recorded for CG-16 (96.0%), followed by

Table 1: Passport information of 16 *C. gharaf* (CG) genotypes collected from Kachchh region of Gujarat

Genotypes	Mandal	District	Latitude	Longitude	Habitat
CG-01	Bhuj	Kachchh	23.12330	69.78845	Wild*
CG-02	Bhuj	Kachchh	23.23361	69.73668	Field boundary**
CG-03	Bhuj	Kachchh	23.23561	69.73169	Field boundary
CG-04	Rapar	Kachchh	23.54710	70.73110	Backyard
CG-05	Rapar	Kachchh	23.56288	70.70563	Road side
CG-06	Bhuj	Kachchh	23.18147	69.66737	Wild
CG-07	Bhuj	Kachchh	23.18124	69.66693	Wild
CG-08	Bhuj	Kachchh	23.18139	69.66775	Wild
CG-09	Bhuj	Kachchh	23.21644	69.72455	Field boundary
CG-10	Bhuj	Kachchh	23.21576	69.72282	Field boundary
CG-11	Bhuj	Kachchh	23.19508	69.85644	Forest nursery
CG-12	Mandvi	Kachchh	23.08289	69.52191	Road side
CG-13	Mandvi	Kachchh	22.89927	69.39358	Road side
CG-14	Mandvi	Kachchh	22.89152	69.40769	Road side
CG-15	Mandvi	Kachchh	22.84624	69.37469	Near human settlement
CG-16	Mandvi	Kachchh	22.85733	69.29186	Wild

*'Wild' is used about an area of land in its natural state, not changed by people.

**'Field boundaries' are the geometric borders of agricultural fields. A field, in this case, is an area of land, enclosed or otherwise, used for agricultural purposes such as cultivating crops.

Table 2: Morphological growth pattern of *C. gharaf* during year 2017, 2018 & 2019

Traits	Years	Range		Mean $\pm$ SEM	SD	CV (%)
		Min.	Max.			
Plant height (cm)	2017	41.0	120.0	83.3 $\pm$ 6.1	24.4	29.2
	2018	121.7	212.0	162.6 $\pm$ 6.9	27.4	16.9
	2019	155.0	244.7	206.3 $\pm$ 7.3	29.4	14.2
Canopy spread (cm ²)	2017	27.5	73.0	48.8 $\pm$ 4.2	16.8	34.4
	2018	118.0	223.0	177.5 $\pm$ 7.8	31.0	17.5
	2019	140.0	254.0	203.3 $\pm$ 8.7	34.9	17.2
No. of leaves/plant	2017	91.7	620.0	251.6 $\pm$ 41.0	164.0	65.2
	2018	322.3	2991.3	1271.6 $\pm$ 211.7	846.9	66.6
	2019	2430.0	6047.3	4201.3 $\pm$ 244.6	978.2	23.3
Stem thickness (mm)	2017	6.4	14.4	10.3 $\pm$ 0.6	2.3	22.6
	2018	17.5	38.7	26.4 $\pm$ 1.5	6.2	23.3
	2019	21.6	46.0	32.8 $\pm$ 1.8	7.1	21.5

CG-13 (90.0%), and the minimum was for CG-14 and CG-12 (62.79% each). Likewise, the highest CTD was recorded for genotype CG-03 (5.83°C), followed by CG-02 (4.77°C). The genotype CG-12 showed the highest (17.46%) RSI, followed by CG-01 (16.72%) and minimum in genotype CG-07 (8.6%). By looking at the growth performance of *C. gharaf* in the adverse conditions of hot-arid Kachchh of Gujarat, it might be used in silvipasture systems or rangeland improvement programs for sustainable development in hot-arid regions of India.

Meghwal *et al.* (2014) reported the presence of wide variability in important fruit characters viz., bunch weight, number of fruits per bunch, fruit weight and pulp: stone ratio of 14 *C. myxa* L. germplasm collected from Rajasthan and Gujarat. Earlier studies also revealed the wide genetic diversity of *C. myxa* in natural populations for its morphological traits (Pareek and Sharma, 1993; Saini *et al.*, 2002; Samadia, 2005; Malik *et al.*, 2010; Sivalingam *et al.*, 2012). The morphological and molecular (RAPD marker) diversity study in 22 *Cordia* accessions of the Indian hot-arid

Table 3: Descriptive statistics of 14 morpho-physiological and 13 fodder quality traits recorded in 16 *C. gharaf* accessions

S No.	Traits	Range		Mean $\pm$ SEM	SD	CV (%)
		Mini.	Maxi.			
	Morpho-physiological traits					
1.	Plant height (cm)	155.00	244.67	206.33 $\pm$ 7.34	29.36	14.23
2.	Canopy spread (cm²)	140.00	254.00	203.3 $\pm$ 8.7	34.9	17.2
3.	No. of branches/plant	6.67	11.67	8.81 $\pm$ 0.37	1.50	16.97
4.	No. of leaves/plant	2430.00	6047.33	4201.33 $\pm$ 244.55	978.21	23.28
5.	Stem thickness (mm)	21.59	46.0	32.8 $\pm$ 1.8	7.1	21.5
6.	Leaf length (cm)	5.21	6.63	5.78 $\pm$ 0.10	0.40	6.95
7.	Leaf width (cm)	7.57	9.57	8.75 $\pm$ 0.13	0.53	6.01
8.	Petiole length (cm)	1.97	2.93	2.50 $\pm$ 0.07	0.27	10.94
9.	Petiole width (mm)	3.22	4.18	3.67 $\pm$ 0.07	0.28	7.53
10.	Fresh leaf weight (10 leaves)	1.85	3.15	2.44 $\pm$ 0.09	0.34	14.10
11.	Leaf area (cm²)	5.88	11.30	9.35 $\pm$ 0.34	1.37	14.63
12.	RWC (%)	62.79	96.00	71.18 $\pm$ 2.39	9.55	13.42
13.	CTD (°C)	2.17	5.83	3.72 $\pm$ 0.22	0.89	23.99
14.	RSI (%)	8.60	17.46	13.33 $\pm$ 0.70	2.79	20.92
	Fodder quality traits after three years of transplanting					
1.	AGB/plant (kg)	2.22	19.63	8.08 $\pm$ 1.17	4.67	57.79
2.	Moisture content (%)	55.41	72.51	65.93 $\pm$ 1.17	4.69	7.11
3.	Dry weight (%)	27.49	44.59	34.07 $\pm$ 1.17	4.69	13.77
4.	Total ash (%)	6.65	18.20	13.55 $\pm$ 0.80	3.18	23.50
5.	Organic matter (%)	81.65	93.10	86.32 $\pm$ 0.78	3.11	3.60
6.	Carbon (%)	41.10	46.70	43.55 $\pm$ 0.39	1.57	3.62
7.	Silica (%)	0.65	3.35	1.78 $\pm$ 0.16	0.65	36.68
8.	Calcium (%)	0.56	3.70	2.73 $\pm$ 0.20	0.81	29.55
9.	Phosphorus (%)	0.07	0.68	0.18 $\pm$ 0.03	0.14	78.85
10.	N ₂ (%)	1.45	2.77	1.72 $\pm$ 0.09	0.36	21.04
11.	CP (%)	9.07	17.31	10.75 $\pm$ 0.57	2.26	21.04
12.	NDF (%)	35.90	68.90	50.51 $\pm$ 2.05	8.20	16.24
13.	ADF (%)	35.62	58.70	48.87 $\pm$ 1.60	6.38	13.06

RWC-Relative water content, CTD-Canopy temperature depression, RSI-Relative stress injury, AGB-Above ground biomass, CP-Crude protein, NDF-Neutral detergent fiber, ADF-Acid detergent fiber

region revealed considerable genetic diversity in studied accessions (Sivalingam *et al.*, 2012). Kumar *et al.* (2017) studied the morphometric variability in *C. gharaf* collection, especially for seed, fruit and seedling traits from the Jaisalmer district. They found that the Badabagh collection (CAZJCG-1) performed better concerning seed germination and seedling growth parameters. The study of other under-utilized hot-arid shrub species like *Grewia tenax* was also carried out by Patidar *et al.* (2022) using morphological and phytochemical traits under hot-arid condition of Jaisalmer and observed the highest CV for number of branches per plant (31.82%) followed by plant height (23.25%). Similarly, Dev *et al.* (2017) also studied the diversity of *G. tenax* using morphological and fruit traits after one year of planting

Table 4: Distribution of 16 *C. gharaf* (CG) accessions into different clusters

Clusters	Number of genotypes	Genotypes
I	2	CG-01, CG-02
II	7	CG-03, CG-06, CG-09, CG-10, CG-11, CG-12, CG-15
III	3	CG-04, CG-05, CG-08
IV	4	CG-07, CG-13, CG-14, CG-16

in hot-arid climatic conditions in Kachchh, Gujarat. They reported diverse plant heights from 20.8 to 78.5 cm among the different genotypes.



Fig. 1: Field evaluation of *Cordia gharaf* at ICAR-Central Arid Zone Research Institute, Regional Center, Kukma-Bhuj, Gujarat (a), Flowering twig (b), Fruiting branches (c), Seeds of *C. gharaf* (d)

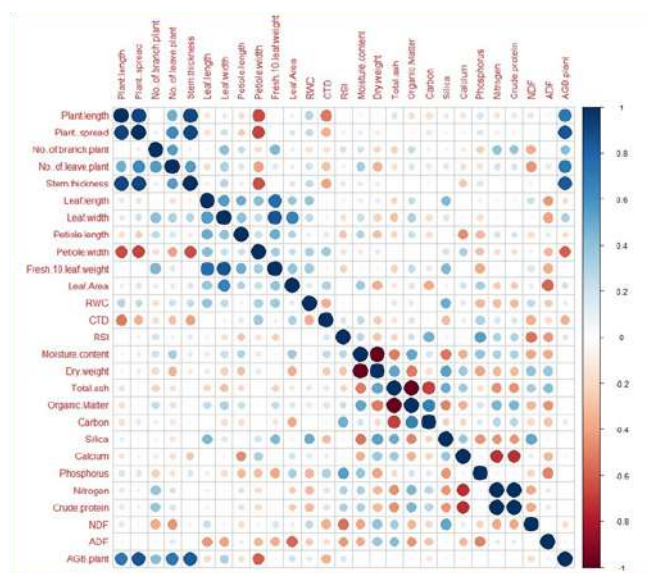


Fig. 2: Pearson's correlation coefficient plot of 16 *Cordia gharaf* accessions for 27 morpho-physiological and fodder quality traits

Genetic variability for fodder quality traits

Descriptive statistics for 13 fodder quality traits in sixteen *C. gharaf* accessions showed a wide range of variability among them (Table 3 and Supplementary Table 2). Above-ground biomass (AGB) per plant ranged from 2.22 to 19.63 kg/plant with a mean value of 8.08 kg/plant. Similarly, leaf moisture content varied between 55.41 to 72.51% and dry matter in the leaves ranged from 27.49 to 44.59%. The maximum organic matter and carbon content were estimated in genotype CG-08 (93.1 and 46.7%) and minimum in CG-01 (81.65) and CG-02 (41.1%), respectively. The silica content in the leaves ranges between 0.65 and 3.35%. In comparison, calcium content in leaves ranged from 0.56 to 3.7%. Nitrogen and crude protein are two important parameters of quality fodder of any plant species. In this study, these two contents varied from 1.45 to 2.77% and 9.07 to 17.31%, respectively,

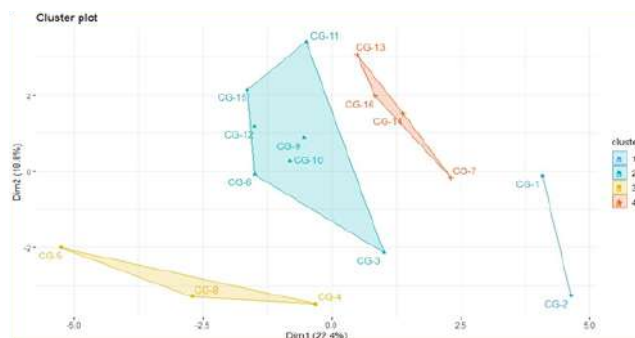


Fig. 3: Cluster plot of 16 *Cordia gharaf* accessions using 27 morpho-physiological and fodder quality traits

and found minimum in CG-11 and CG-01 (1.45 and 9.07%) genotypes and maximum in CG-05 (2.77 and 17.31%) followed by CG-15 (2.23 and 13.93%), respectively. The NDF and ADF content ranges between 35.9 to 68.9% and 35.62 to 58.7%, respectively.

Among the fodder quality traits, the highest CV was recorded for phosphorous content (78.85%), followed by AGB per plant (57.79%) and silica (36.68%). At the same time, the lowest CV was observed for organic matter (3.60%) followed by carbon content (3.62%) and moisture content (7.11%). Kuria *et al.* (2005) also studied the nutritive value of different range forage species. They reported that the leaves of *C. sinensis* are a good source of crude protein (13.8%), dry matter (41.2%), NDF (56.6%), ADF (43.3%) and ADL (18.9%) with high amounts of minerals like calcium, zinc, potassium, phosphorus, and iron. Jain and Patel (2016) estimates of descriptive statistics for nine quantitative parameters revealed wide variation among sorghum genotypes. Similarly, Dev *et al.* (2023) found significant variety in *Grewia tenax* germplasm for fodder and related quality parameters. Above-ground biomass (AGB) ranged from 0.4 to 2.9 kg/plant, with significant variance in crude protein (2.2–15.2%), NDF (37.264.2%), and ADF (12.0–47.6%) levels. The fodder quality value can indicate its suitability as a feed source for browsing animals and in silvipasture systems within India's hot-arid regions.

Pearson's correlation of morpho-physiological and fodder quality traits

Pearson's correlation coefficients were estimated among 27 morpho-physiological and fodder quality traits in sixteen *C. gharaf* genotypes (Fig. 2). Among the various characteristics studied, plant height was positively correlated with canopy spread, stem thickness, above-ground biomass per plant, and a number of leaves per plant. Likewise, canopy spread was also positively correlated with stem thickness, above-ground biomass per plant, and number of leaves per plant. The fodder-yielding traits like above-ground biomass were positively associated with morphological traits like plant height, canopy spread, number of branches per plant, number of leaves per plant, and stem thickness; all these

traits contribute to higher plant biomass. Within fodder quality traits, a strong positive correlation was observed between nitrogen content and crude protein. Conversely, significant negative correlations were identified between dry weight and moisture content, as well as between total ash and organic matter (Fig. 2). The correlation studies conducted have revealed a positive correlation between fodder-yielding traits, such as above-ground biomass, and the associated morphological traits. These findings suggest that selecting and emphasizing these specific traits could be highly beneficial in developing cultivars with high fodder yield potential. Guleria *et al.* (2020) tested *Grewia optiva* clones for fodder yield and other features and discovered a favorable relationship between collar diameter, fresh fodder weight, and fuel wood weight. Sivalingam *et al.* (2016) found a positive association between leaf, fruit, stone characteristics, and pulp: stone ratio but a negative or negligible correlation with flower count per cyme in *C. myxa* germplasm. According to the correlation study by Jain and Patel (2016), the fodder yield of sorghum was positively connected with the number of leaves per plant, leaf breadth, leaf length, plant height, and days to maturity. The good correlation among these parameters indicated that they are crucial for the direct selection of high forage-producing genotypes.

Cluster analysis

The cluster plot was generated using 27 morpho-physiological and fodder quality traits of sixteen *C. gharaf* genotypes based on Ward's approach and grouped all the studied genotypes into four major clusters (Table 4 and Fig. 3). The first cluster consists of only two genotypes and contributed only 12.5% of the total genotypes. On the other hand, the second cluster included seven genotypes with 43.75% of the total genotypes. The clusters third and fourth consisted of 3 and 4 genotypes, respectively. The genotypes included in the same cluster are similar and different to the genotypes of other clusters. Sivalingam *et al.* (2012) discovered that the clustering pattern is mostly determined by leaf size and pulp:stone ratio, rather than the region from where seeds were collected. They may have evolved from various beginnings by diverging from a common ancestor and establishing their own niche. This difference might be attributed to seed-grown plants, cross-pollination, and seed dispersal by birds, animals, and humans from one location to another. Cluster analysis divided the *G. tenax* genotypes into two primary clusters, each with two sub-clusters, confirming the presence of a substantial degree of genetic variability among the tested genotypes (Dev *et al.*, 2023).

Biplot analysis

PCA biplot visually represents the distribution and diversity of both studied traits and the *C. gharaf* germplasm (Fig. 4). The biplot provides insights into the patterns and

relationships between these variables, allowing for a better understanding of their interplay. This also aids in selecting genotypes or groups of genotypes with superior traits, facilitating their selection and utilization for species improvement. In contrast, traits heading in the same direction show a positive correlation, while traits in opposite directions represent a contrast or negative correlation between variables. This analysis provides valuable insights into trait relationships and their impact on species enhancement. The high variance was observed for plant height, plant spread, stem thickness, above-ground biomass (AGB) per plant, number of leaves per plant, moisture content, nitrogen content, crude protein, organic matter, petiole length, dry weight, and total ash. The leaf area, leaf length, leaf width, fresh ten leaves weight, petiole length, phosphorus, and RSI exhibited comparatively lower variance in the observed data. Fig. 4 shows that traits like above-ground biomass, petiole width, total ash and organic carbon are in opposite directions and form an independent group. The traits like AGB per plant, plant spread, plant height, stem thickness and number of leaves per plant were positively correlated and grouped in the same AGB group. Similarly, the total ash content of the genotypes is positively associated with fodder quality traits like silica content, ADF, NDF and dry weight. The Eigenvalue and principal component (PC) number were graphed to generate the scree plot (Fig. 5). It explains the variance associated with each PC. The first four

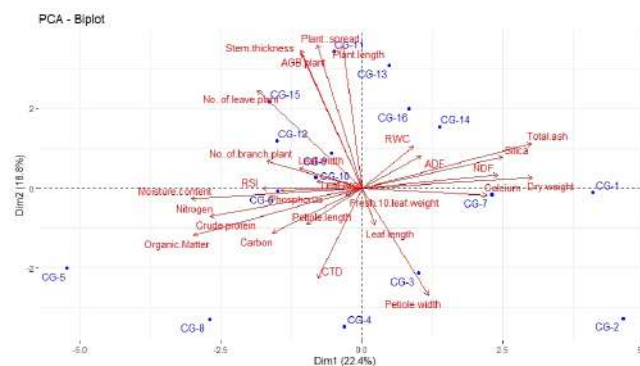


Fig. 4: Biplot

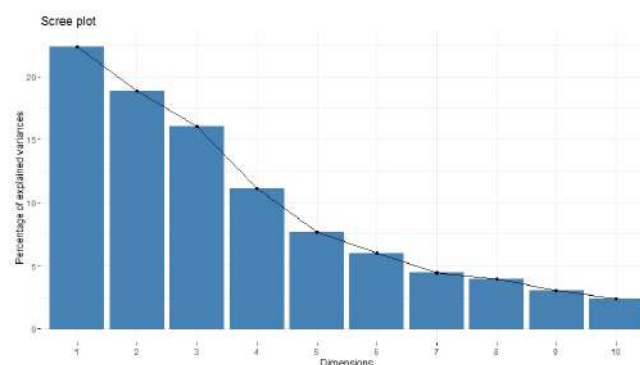


Fig. 5: Scree plot

PCs contributed 68.25% of the total variation revealed in 16 *C. gharaf* germplasm. The initial four principal components (PCs) contain the maximum variation in the data. Therefore, selecting accessions from these PCs would be beneficial for further studies and analyses. The plotting of variables against genotypes and correlation study suggests that the genotypes CG-11, CG-12, CG-13, and CG-15 exhibit favorable characteristics for morphological traits and above-ground biomass per plant. Genotypes CG-05, CG-06, and CG-08 also show good characteristics for fodder quality. These genotypes can be selected as promising candidates for future improvement programs. PCA biplot was used to order the genotypes and to visualize the genetic relationships among accessions. Jain and Patel (2016) discovered that biplot between PCs 1 and 2, distributed genotypes in all four quadrants, indicating high genetic variability in quantitative features in sorghum germplasm. Dev *et al.* (2023) found distinct genotypes (GT-15, GT-1, GT-3, GT-11, GT-13, GT-29, GT-32, GT-33, and GT-36) in *Grewia tenax* through biplot analysis using PC1 and PC2 information.

Conclusion

C. gharaf, a versatile and underexplored/underutilized shrub/small tree species, thrives well in harsh climatic conditions characterized by frequent droughts and crop failures. It is valued for its edible berries, fuel wood and leaf fodder, which is available for a long season and has great medicinal importance. The species' leaves are essential fodder for browsing animals like goats, sheep, and camels in hot-arid regions. Given its importance in challenging environments, it is crucial to characterize the existing variability of heterogeneous germplasm to tap the species' potential. The present study demonstrated a wide range of diversity for morpho-physiological and fodder quality traits in studied germplasm and *ex-situ* on-farm conservation for further improvement for exploitation of its untapped potential value. The promising genotypes identified for morphological, above-ground biomass traits and fodder quality traits can be used in silvipasture systems in hot-arid regions of India.

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Supplementary Table 1: Morpho-physiological traits along with descriptive statistics of 16 *C. gharaf* accessions

Acc.	Plant height (cm)	Canopy spread (cm ²)	No. of branches/plant	No. of leaves/plant	Stem thickness (mm)	Leaf length (cm)	Leaf width (cm)	Petiole length (cm)	Petiole width (mm)	fresh leaf weight (10 leaves)	Leaf Area (cm ²)	RWC (%)	CTD (°C)	RSI (%)
CG-01	202.33	196.8	6.67	2763.33	30.82	5.34	7.57	1.97	3.67	1.85	5.88	76.46	3.00	16.72
CG-02	155.00	140.00	8.00	2430.00	21.59	6.25	8.53	2.60	4.12	2.46	10.05	68.29	4.77	11.55
CG-03	174.67	181.33	7.67	3955.00	25.40	5.49	8.90	2.57	3.94	2.42	9.82	66.07	5.83	10.80
CG-04	168.33	165.33	8.67	3255.00	23.05	5.81	9.03	2.23	4.18	2.67	9.91	75.44	3.90	12.50
CG-05	195.33	186.33	8.67	4206.67	33.09	5.69	8.60	2.70	3.73	2.19	9.01	69.35	4.60	16.70
CG-06	196.00	177.67	11.00	5107.67	33.01	5.64	9.30	2.57	3.76	2.65	9.55	63.64	3.27	14.08
CG-07	202.00	181.33	8.33	3881.67	27.47	5.92	8.40	2.57	3.64	2.42	8.75	71.70	2.17	8.60
CG-08	167.00	166.67	9.00	4156.67	27.15	6.46	8.90	2.93	3.75	2.68	9.22	66.67	4.43	15.14
CG-09	219.33	215.33	9.33	5318.00	29.72	5.21	7.80	2.50	3.32	2.04	7.00	64.58	3.30	12.77
CG-10	205.67	191.33	10.67	4175.00	32.22	5.57	8.77	2.90	3.56	2.47	10.56	69.57	3.57	13.69
CG-11	226.33	254.00	10.00	6047.33	45.99	5.25	8.70	2.23	3.51	2.05	10.16	71.43	3.53	14.72
CG-12	241.33	240.33	7.00	3629.00	39.87	5.66	8.47	2.10	3.32	2.00	11.30	62.79	4.37	17.46
CG-13	244.67	249.33	7.00	5023.33	43.06	5.88	9.10	2.60	3.56	2.53	9.77	90.00	2.80	9.74
CG-14	243.33	228.00	7.67	3492.67	38.37	5.85	9.10	2.60	3.54	2.55	9.36	62.79	3.47	8.90
CG-15	218.00	241.00	11.67	4610.00	37.61	5.88	9.23	2.27	3.22	2.85	8.50	64.10	3.37	14.63
CG-16	242.00	237.33	9.67	5170.00	36.84	6.63	9.57	2.73	3.95	3.15	10.80	96.00	3.20	15.23
Mini.	155.00	140.00	6.67	2430.00	21.59	5.21	7.57	1.97	3.22	1.85	5.88	62.79	2.17	8.60
Maxi.	244.67	254.00	11.67	6047.33	46.02	6.63	9.57	2.93	4.18	3.15	11.30	96.00	5.83	17.46
Range	89.67	114.00	5.00	3617.33	21.47	1.42	2.00	0.97	0.96	1.30	5.42	33.21	3.67	8.86
Mean	206.33	203.32	8.81	4201.33	32.80	5.78	8.75	2.50	3.67	2.44	9.35	71.18	3.72	13.33
SEM	7.34	8.71	0.37	244.55	1.8	0.10	0.13	0.07	0.07	0.09	0.34	2.39	0.22	0.70
SD	29.36	34.90	1.50	978.21	7.1	0.40	0.53	0.27	0.28	0.34	1.37	9.55	0.89	2.79
CV (%)	14.23	17.2	16.97	23.28	21.5	6.95	6.01	10.94	7.53	14.10	14.63	13.42	23.99	20.92

RWC-Relative water content, CTD-Canopy temperature depression, RSI-Relative stress injury

Supplementary Table 2: Fodder quality traits along with descriptive statistics of 16 *C. gharaf* accessions

Acc.	AGB/plant (kg)	Moisture content (%)	Dry weight (%)	Total ash (%)	Organic matter (%)	Carbon (%)	Silica (%)	Calcium (%)	Phosphorus (%)	N ₂ (%)	CP (%)	NDF (%)	ADF (%)
CG-01	4.82	58.93	41.07	18.10	81.65	45.95	2.65	3.34	0.14	1.45	9.07	55.10	53.10
CG-02	2.22	55.41	44.59	18.20	82.15	41.10	2.25	3.66	0.12	1.55	9.69	53.20	51.70
CG-03	3.29	70.16	29.84	16.15	83.60	41.68	1.65	3.18	0.14	1.59	9.92	51.35	52.10
CG-04	2.97	67.91	32.09	8.35	91.45	45.68	1.45	3.70	0.15	1.59	9.95	55.10	35.62
CG-05	6.95	72.51	27.49	9.10	90.65	45.45	0.65	0.56	0.27	2.77	17.31	42.25	48.75
CG-06	8.78	66.44	33.56	14.10	85.75	42.95	1.35	2.68	0.16	1.97	12.29	35.90	50.50
CG-07	4.77	64.70	35.30	15.05	84.95	42.73	1.95	2.31	0.13	1.55	9.68	68.90	52.10
CG-08	4.19	68.43	31.57	6.65	93.10	46.70	1.55	2.50	0.20	1.77	11.07	40.50	45.90
CG-09	6.75	65.92	34.08	14.05	85.75	42.95	1.25	2.53	0.13	2.02	12.61	45.30	58.70
CG-10	8.54	72.28	27.72	14.95	85.10	42.55	1.95	2.42	0.15	1.54	9.60	47.30	48.75
CG-11	19.63	64.02	35.98	13.30	86.45	43.35	1.55	3.56	0.15	1.45	9.07	42.30	51.25
CG-12	9.12	70.58	29.42	13.55	86.35	43.38	1.05	3.42	0.68	1.48	9.26	48.75	37.80
CG-13	11.65	66.53	33.47	14.45	85.40	42.98	1.50	2.22	0.07	1.46	9.11	52.20	53.20
CG-14	10.18	60.56	39.44	13.35	86.35	43.28	2.05	2.07	0.11	1.58	9.90	59.80	54.70
CG-15	14.80	64.39	35.61	12.55	87.30	43.60	2.25	2.32	0.14	2.23	13.93	55.05	48.90
CG-16	10.64	66.06	33.94	14.90	85.10	42.55	3.35	3.28	0.11	1.53	9.53	55.10	38.90
Mini.	2.22	55.41	27.49	6.65	81.65	41.10	0.65	0.56	0.07	1.45	9.07	35.90	35.62
Maxi.	19.63	72.51	44.59	18.20	93.10	46.70	3.35	3.70	0.68	2.77	17.31	68.90	58.70
Range	17.41	17.10	17.10	11.55	11.45	5.60	2.70	3.14	0.60	1.32	8.24	33.00	23.08
Mean	8.08	65.93	34.07	13.55	86.32	43.55	1.78	2.73	0.18	1.72	10.75	50.51	48.87
SEM	1.17	1.17	1.17	0.80	0.78	0.39	0.16	0.20	0.03	0.09	0.57	2.05	1.60
SD	4.67	4.69	4.69	3.18	3.11	1.57	0.65	0.81	0.14	0.36	2.26	8.20	6.38
CV (%)	57.79	7.11	13.77	23.50	3.60	3.62	36.68	29.55	78.85	21.04	21.04	16.24	13.06

AGB-Above ground biomass, CP-Crude protein, NDF-Neutral detergent fiber, ADF-Acid detergent fiber

RESEARCH ARTICLE

Stem of Woody Pepper (*Piper pendulispicum* C. DC) as a Source of Phenolic Acids and Piperine

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Abstract

Woody pepper (*Piper pendulispicum* C. DC) is a medicinal spice recently reported from Andaman Islands apart from its natural distribution in Vietnam, Thailand etc. The species is unique as the thickened stem is used as a spice. As very limited information is available about the species, the present study was undertaken to understand the biochemical composition of its edible portion. Results revealed that stem thickness did not influence the moisture content and recovery of scraped stem. Piperine and phenolic acid profiling suggested that piperine content increased with an increase in stem thickness. Stem samples dried using vacuum drying had higher piperine content (7.665 mg/g) than those dried using oven drying (7.423 mg/g). Profiling of phenolic compounds revealed the presence of 18 phenolic compounds, ferulic acid being the dominant one. The concentration of ferulic acid was higher in thicker stems (867.945 µg/g) than in the thin stems (699.043 µg/g).

Keywords: Ferulic acid, Piperaceae, woody climber, tropical species, vacuum drying.

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Introduction

Members of the family Piperaceae are known to be rich sources of secondary metabolites (Tamuly *et al.*, 2015) and hence several *Piper* species have been commercially used for various food, pharmaceutical and aroma industries (Salehi *et al.*, 2019). However, of more than 1,000 reported *Piper* species so far (Parthasarathy *et al.*, 2006), a large number of species have remained under-studied and thus less understood. Woody pepper (*Piper pendulispicum* C. DC) is an underutilized species of the Piperaceae family, which has recently been identified as a new addition to the Indian Flora (Waman *et al.*, 2024). Further, this species has been found to be a potential crop for commercial cultivation in the warm and humid tropical Andaman and Nicobar Islands, India (Waman *et al.*, 2019). It is also found distributed naturally in the Northern, North-eastern Thailand and Vietnam up to an altitude of 200-1200 m above mean sea level (Chaveerach *et al.*, 2008).

Unlike the berries of black pepper, stem pieces of this vine species are used for imparting spiciness to the traditional food in the Andaman Islands (Waman *et al.*, 2019; 2024). In Thailand, the species is one of the eight popular *Piper* species (Chaveerach *et al.*, 2006), wherein stems of this species are often used in the preparation of local food- *kaeng khae* (Chaveerach *et al.*, 2017) apart from its use as an ingredient in herbal tea (Adekoya *et al.*, 2021). It is cultivated in home gardens of northern Thailand, while it is collected from wild stands in Loei province of North-eastern Thailand (Arisa *et al.*, 2014). Ethanolic extract of *P. pendulispicum*

exhibited cytotoxic activity against cancerous cell lines (Mahavorasirikul *et al.*, 2010). Systematic studies have not been conducted in woody pepper so far to understand its potential. Unfortunately, the natural population is dwindling due to destructive and unsustainable harvesting (Waman *et al.*, 2019). In order to promote cultivation of such potential spice, knowledge about the biochemical aspects is important.

Piperine, an alkaloid with IUPAC name- (2E, 4E)-5-(benzo[d][1,3] dioxol-5-yl)-1-(piperidin-1-yl) penta-2,4-dien-1-one, has been regarded as one of the important bioactive compounds responsible for the pungent taste in *Piper* species (Takahashi *et al.*, 2018; Weil *et al.*, 2017). Piperine is known for its versatile properties including antioxidant, anti-tumor, hepato-protective, antidepressant, antimicrobial, immune-modulatory, anti-diarrheal, analgesic *etc.* (Chopra *et al.*, 2016; Salehi *et al.*, 2019; Shityakov *et al.*, 2019; Tiwari *et al.*, 2020). Further, piperine has proven to serve as a bio-enhancer, which enhances the efficiency and activity of other bioactive molecules and antibiotics (Shityakov *et al.*, 2019). Several formulations including those based on Ayurveda and Homeopathy are being sold in the markets across the globe (Chopra *et al.*, 2016), thereby suggesting the acceptability of this compound.

Phenolic compounds are among the most abundant and broad group of secondary metabolites, which are equally functional when present in the plant system or the products obtained after harvest. Several phenolic compounds are known to exhibit anti-inflammatory, antioxidant, cardio-protective, anti-allergic, vaso-dilatory, and antimicrobial properties (Giada and Mancini-Filho, 2006). In the case of the *Piper* species, piperine and phenolic compounds have been identified as the prime constituents contributing to the antioxidant activity (Takahashi *et al.*, 2018). Drying methods and plant maturity have been considered important factors in determining the content of secondary metabolites in various plant species (Takahashi *et al.*, 2018; Thamkaew *et al.*, 2021). Some report also suggests use of dry powdered formulation in Thai medicines (Adekoya *et al.*, 2021); however, no biochemical studies are available in this species (Arisa *et al.*, 2014). Fresh produce of woody pepper is sold in the markets of Andaman Islands based on the stem thickness and hence, understanding the biochemical differences at different stem thickness (harvest stages) is required. The present study thus aimed at studying the effect of two drying methods on piperine content and phenolic compounds in the stems of woody pepper of different maturity.

Materials and Methods

Collection and primary processing of samples

Harvested stem pieces of woody pepper vines were

collected from farmer's field in North and Middle Andaman District, India. The material was transported to the authors' laboratory at the Division of Horticulture and Crop Improvement, Port Blair, South Andaman Island, India for further processing. Stems were graded into two categories viz. thin (mean thickness 19.1 ± 0.52 mm) and thick (mean thickness 30.8 ± 0.74 mm), based on thickness. These pieces were then cut into small segments of equal length, and each segment was weighed using a digital weighing balance (Citizen India, CY-120). Outer layer of the vine comprising of dead tissues and adventitious roots was scraped off and weight of each segment was recorded to know the usable stem recovery percentage. Observations were recorded using ten replications. Moisture content was determined in triplicate and expressed as percentage.

Dehydration of samples

Samples were cut into small pieces using stainless steel secateurs (Falcon, India) and used for dehydration studies using two methods. In the first method, samples were dried in a hot air oven (50 °C), while in the second method, samples were dried in a vacuum tray dryer (RE 11142, Ruchi Enterprises, Delhi, India) at 50 °C temperature and -570 mm Hg vacuum. After drying, the samples were powdered using an electric grinder, sieved using 212-micron sieve and packed in air-tight glass containers with screw caps.

Phenolic acid profiling

Phenolic acid profiling in the samples was done using Waters Acquity UPLC H Class coupled with TQD MS/MS with methanol (80%) following the procedure described earlier (Weidner *et al.*, 2000) with a slight alteration. Known quantity (500 mg) of the sample was homogenized in methanol (80%) followed by centrifugation (6,000 rpm for 10 min at 15°C) and the volume of the extract was made up to 20 mL. The extract was then evaporated at 45°C under vacuum to get near dried product. It was then diluted to 5 mL with water and was extracted thrice with ethyl acetate using a separating funnel. The aqueous layer was discarded and ethyl acetate extract was evaporated to dryness under vacuum. In the resultant dried extract, 4 mL of 2N NaOH was added and allowed for hydrolyzing overnight. It was then acidified to pH 2 using 2N HCl (5 mL) and again re-extracted with ethyl acetate (10 mL). The ethyl acetate layer carrying the phenolic acids was evaporated to complete dryness under vacuum and the residue was dissolved in MS grade methanol (2 mL). It was then filtered through 0.2µm nylon filter prior to injection in LC-MS/MS.

Phenolic acids were resolved on BEH-C18 (2.1 × 50 mm, 1.7 µm) analytical column (Waters India Ltd.) that was protected by Vanguard BEH C-18 (Waters, USA) guard column. The gradient flow of organic and aqueous phases with the flow rate of 0.3 mL/min was adopted. In the mobile phase, solvent A consisted of 0.1% formic acid in

water, while solvent B was 0.2% formic acid in methanol. The column was maintained at 25°C temperature during analysis. The injection volume used for analysis was 2 µL. The eluted phenolic acids were pumped directly without any split into TQD-MS/MS system (Waters, USA) optimized for analysis of phenolic acids. The obtained values of phenolic acids were expressed in µg/g.

Estimation of Piperine

Known quantity (100 mg) of powdered sample was mixed with ethanol (80%), followed by sonication and centrifugation. The supernatant was collected and extracted once again with ethanol (80%) before making up the volume to 20 ml. From this, 0.1 ml volume was drawn; volume was made up to 2 ml with mobile phase and filtered through a nylon filter (0.2 µm) before injecting 2 µl in LC-MS/MS for estimation.

For estimation of piperine, an analytical column BEH C18 (2.1 × 50 mm, 1.7 µm from Waters India Ltd.) with Vanguard BEH C18 (Waters, USA) guard column was used. Isocratic mode with a flow rate of 0.15 mL/min was used for analysis with a sample injection volume of 2 µL. Mobile phase consisted of solvent A (Acetonitrile: water: Acetic acid:: 65:34.5:0.5) and solvent B (Methanol: Acetonitrile: Water :: 10:50:40). The column temperature was maintained at 25°C during analysis. Run time employed was 15 min. The eluted piperine by UPLC column effluent was pumped directly without any split into TQD-MS/MS (Waters, USA) system optimized for piperine analysis. Values of piperine were expressed in mg/g.

Results and Discussion

Recovery of stems and moisture content

Many *Piper* species are grown in tropical regions and are valued for their use as spices and medicines. Woody pepper is a unique species of the Piperaceae family in which stem is economically important. It is amongst the most important species in Thailand for food and medicinal purposes (Arisa *et al.*, 2014). It is known to have creamish soft stems, which are commonly employed in the preparation of soup called 'kaeng khae' in Thailand. During the present investigation, the thickness of thick samples ranged from 25.2 to 36.3 mm with a mean thickness of 30.8 mm (Table 1). The stems of thin group, on the other hand, had a mean thickness of 19.1 mm and a range of 11.9 to 24.6 mm. With the advancement of maturity, the thickness of the stem increases, and thicker stems fetch better prices in the local markets of Andaman Islands. Branches of primary and secondary origin generally have less thickness than the main stem. In the present case, most of the produce from the thick category was from main stem, while that from the thin category was from primary branches.

Table 1: Recovery (%) and moisture content (%) in woody pepper samples of different stem thickness

Sample	Thickness (mm)		Recovery (%)	Moisture content (%)
	Range	Mean ± Sem		
Thick	25.2 to 36.3	30.8 ± 0.74	75.9 ± 0.61	70.6 ± 0.19
Thin	11.9 to 24.6	19.1 ± 0.52	76.0 ± 1.22	70.4 ± 0.60

The outer bark of the species is rough and it produces heavy tufts of roots, especially on the nodes (Chaveerach *et al.*, 2008). These roots facilitate clinging of vines, while growing on a support (Waman *et al.*, 2019). However, while using the spice, such root masses are completely trimmed off and the outer layer of bark is scraped off using a stainless steel knife. To quantify the recovery of usable part from the produce, samples were weighed after scraping of the outer layer and percentage recovery was calculated. Interestingly, analysis of data suggested that the recovery of edible produce was not influenced by its stem thickness. Recovery of 75.9% was observed from stems of the thick category, while 76.0% recovery was observed in thin stems. Additionally, the moisture content in the edible part of the stem also remained on par in both samples. The moisture content of 70.4 to 70.6% was recorded in the peeled produce (Table 1).

Piperine content as influenced by stem thickness and drying methods

Fig. 1 depicts variations in the piperine content among the woody pepper samples dried using two different methods. Piperine content in the vacuum-dried sample was significantly higher (7.665 mg/g) than that observed in the oven-dried sample (7.423 mg/g). Many drying methods have commonly been applied for the dehydration of various spices and each of those methods is known to have its advantages and disadvantages (Thamkaew *et al.*, 2021). In general, the suitability of the drying method varies with species. Experiment with *Piper nigrum* suggested significant role of the drying method on piperine content as oven drying improved piperine content, when compared with freeze-drying (Namjoyan *et al.*, 2012). On the other hand, drying methods did not influence piperine content in *P. borbonense* (Weil *et al.*, 2017). Maturity of plant part is one of the factors reported to influence the accumulation of piperine. In the present study, piperine content in the stem pieces of woody pepper was also influenced by the stem thickness used for estimation. Piperine content of 6.230 mg/g was observed in the sample of the thin category (Fig. 2), while it was significantly higher (6.654 mg/g) in the thick samples. Various biochemical and physiological changes in a plant system are known to vary with the species, plant part studied, position of the part on the plant, *etc.* (Salehi *et al.*, 2019).

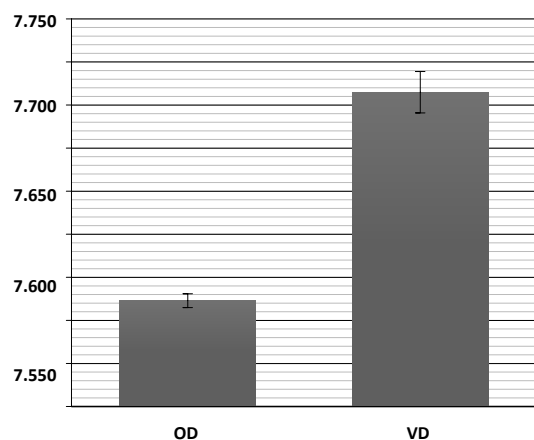


Fig. 1: Piperine content (mg/g) as influenced by drying methods

Piperine has been reported to occur in various plant parts such as fruits of *P. nigrum* (Kotte *et al.*, 2014), *P. borbonense* (Weil *et al.*, 2017), *P. retrofractum* (Takahashi *et al.*, 2018), *P. longum* (Khound *et al.*, 2017), the stem of *P. retrofractum* (Roy *et al.*, 2018) and multiple parts of *P. nigrum*, *P. guineense* and *P. longum* (Adosraku *et al.*, 2013; Prasad *et al.*, 2018; Semler and Gross, 1988). Also, the concentration of secondary metabolites varies among different parts of a plant e.g. piperine was detected in the leaves of a female plant in *P. longum* while it was absent in the leaves of a male plant (Prasad *et al.*, 2018). Accumulation of various bioactive molecules in the plant tissues has been reported to vary with the stage of tissue/ plant part's growth. In *P. retrofractum* fruits (Takahashi *et al.*, 2018), piperine content decreased from the green mature stage (23.59 mg/g-FW) to the red mature stage (12.97 mg/g-FW). These reports support the variations observed for piperine content in the present study.

Higher piperine content in the thick stem could be taken as an indicator of its spiciness as used for other species (Takahashi *et al.*, 2018; Weil *et al.*, 2017). Several bioactive constituents are lost during processing (Cascais *et al.*, 2021); hence, such higher levels of bioactive molecules could be of advantage during the preparation of processed products. Such additional quantities of piperine could help in the maintenance of the optimum level of spiciness in the processed products as well. Hence, use of thicker stems could be advocated for the preparation of products requiring higher levels of piperine.

Phenolic acid profiling as influenced by stem size

Phenolic acid composition of woody pepper was influenced by the maturation of the stem i.e. size of the stem used for analysis. In general, eighteen compounds were present in woody pepper of both classes (Table 2). Significantly higher quantities (1142.860 mcg/g) of phenolic acids were found in thin stem than its thicker counterpart (1113.099 mcg/g). Study on ingredients of a Thai medicine (Adekoya *et al.*, 2021) was conducted which revealed low content of phenols in

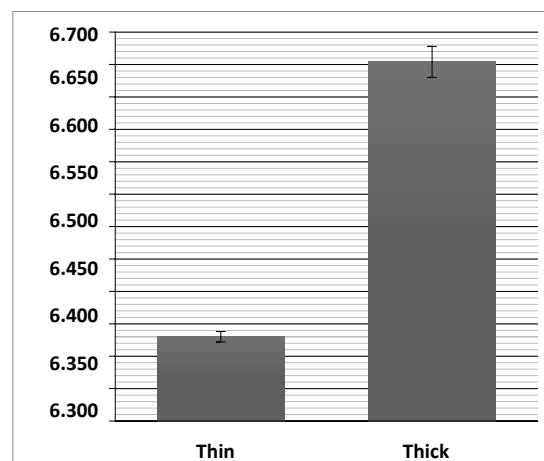


Fig. 2: Piperine content (mg/g) as influenced by stem thickness

the stem of *P. pendulispicum* collected from Thailand (0.06 mg/g total phenolic content, GAE) when compared with the present study. Geographical region, genetic variations and other unknown factors could be considered responsible for this variation. Reduction in phenolic content was evident in present study with increase in stem thickness. An earlier study in the fruits of *P. retrofractum* suggested reduction in total phenolic content with maturity from green mature to red mature stage (Takahashi *et al.*, 2018), while the antioxidant activity of leaves of *P. sarmentosum* increased with maturity from young to middle age followed by drop at full maturity (Ibrahim and Sayidah, 2019). This suggests that the maturity of plant part has a role in the accumulation of phenolic compounds, though the contents might vary with the species and plant part. Of the 18 compounds, 14 compounds were found in significantly higher quantities in thin stems, two compounds (Ferulic acid and Salicylic acid) were relatively higher in thick stems, while the concentration of Gentisic acid and Chlorogenic acid remained statistically similar. In both the stem sizes, Ferulic acid was found to be the dominant phenolic acid, followed by *p*-Coumaric acid in woody pepper. The concentration of Ferulic acid was significantly higher (867.945 mcg/g) in thick stems than in thin stems (699.043 mcg/g) studied. It means Ferulic acid constituted about 61.17% of the total phenolics in thin stems, while its share was 77.98% in thick stems. The second dominant compound i.e. *p*-Coumaric acid was comparatively higher (188.510 mcg/g) in thin samples than its thick counterpart (99.620 mcg/g).

In terms of relative concentrations, thin samples had *o*-Coumaric acid (68.400 mcg/g), *t*-Cinnamic acid (41.310 mcg/g), Caffeic acid (39.570 mcg/g), Gallic acid (35.569 mcg/g), Sinapic acid (25.148 mcg/g) and Vanillic acid (19.944 mcg/g); while thick samples showed the presence of *t*-Cinnamic acid (35.923 mcg/g), Caffeic acid (29.229 mcg/g), *o*-Coumaric acid (25.100 mcg/g) and Gallic acid (23.026 mcg/g) as other constituents. Minor constituents were also present in relatively smaller quantities in both

Table 2: Phenolic acid content ($\mu\text{g/g}$) in woody pepper as influenced by stem thickness

Phenolic acids	Thin	Thick
Benzoic acid	5.418 ± 0.0272	2.932 ± 0.0161
p-hydroxy benzoic acid	0.689 ± 0.0026	0.349 ± 0.0022
Salicylic acid	1.917 ± 0.1062	2.639 ± 0.0058
3-Hydroxy benzoic acid	2.070 ± 0.0220	0.876 ± 0.0168
t-Cinnamic acid	41.310 ± 0.6008	35.923 ± 0.1601
2,4-dihydroxybenzoic acid	7.278 ± 0.1648	1.300 ± 0.0140
Gentisic acid	2.893 ± 0.1722	2.988 ± 0.0643
Protocatechuic acid	3.381 ± 0.0727	2.018 ± 0.1187
p-Coumaric acid	188.510 ± 0.6655	99.620 ± 0.8803
o-Coumaric acid	68.400 ± 0.7558	25.100 ± 0.0944
Vanillic acid	19.944 ± 0.1179	11.394 ± 0.0367
Gallic acid	35.569 ± 0.1983	23.026 ± 0.0822
Caffeic acid	39.570 ± 0.2219	29.229 ± 0.5244
Ferulic acid	699.043 ± 1.1475	867.945 ± 1.7603
Syringic acid	1.657 ± 0.0067	0.965 ± 0.0062
Sinapic acid	25.148 ± 0.0820	6.743 ± 0.1316
Ellagic acid	0.061 ± 0.0012	0.051 ± 0.0045
Chlorogenic acid	0.001 ± 0.0001	0.001 ± 0.0001
Total	1142.860 ± 1.3978	1113.099 ± 0.1767

samples. The results obtained here are in agreement with the earlier study in which eighteen phenolic compounds were reported from the leaves of *Ficus carica* (Nadeem and Zeb, 2018). They have reported variations in the composition of phenolic compounds with the advancement of maturity. However, the dominant compound (Caffeic acid) remained the same during all stages of development, which was also the case in woody pepper.

Phenolic acid profiling as influenced by drying methods

Use of an appropriate drying method has been emphasized for drying herbs to obtain products with better phenolics and antioxidant activities (Orphanides *et al.*, 2013). Hence, the effect of two drying methods was studied which suggested a significant role of the drying method on the concentration of phenolic acids in woody pepper. In general, oven-dried samples had higher quantities of phenolics (1400.317 mcg/g) than vacuum-dried samples (1141.873 mcg/g) (Table 3). This result is in line with a report on green tea in which higher total phenolic content was observed in oven-dried samples (Roshanak *et al.*, 2016). Also, relative concentrations of 11 compounds were higher in oven-dried produce, while the concentration of five compounds (Caffeic acid, Gallic acid, 2,4-dihydroxybenzoic acid, Salicylic acid, and 3-hydroxyl benzoic acid) was higher in vacuum dried produce. The varied composition of phenolic compounds in response to different processing methods has been reported earlier (Brandão *et al.*, 2021).

Table 3: Phenolic acid content ($\mu\text{g/g}$) in woody pepper as influenced by drying methods

Phenolic acids	Oven drying	Vacuum drying
Benzoic acid	5.254 ± 0.0636	4.076 ± 0.0374
p-hydroxy benzoic acid	1.159 ± 0.0356	0.701 ± 0.0076
Salicylic acid	3.877 ± 0.0791	4.321 ± 0.0380
3-Hydroxy benzoic acid	0.601 ± 0.0018	1.510 ± 0.0103
t-Cinnamic acid	98.235 ± 0.9480	63.798 ± 0.5229
2,4-dihydroxybenzoic acid	6.889 ± 0.0925	13.161 ± 0.0826
Gentisic acid	12.538 ± 1.1917	3.777 ± 0.1799
Protocatechuic acid	5.882 ± 0.2835	4.699 ± 0.1108
p-Coumaric acid	161.626 ± 2.1589	101.016 ± 2.4899
o-Coumaric acid	31.743 ± 0.5920	25.146 ± 0.0529
Vanillic acid	14.677 ± 0.1078	14.657 ± 0.2774
Gallic acid	32.338 ± 0.1611	41.502 ± 0.1439
Caffeic acid	45.374 ± 0.1298	64.110 ± 1.5065
Ferulic acid	938.796 ± 1.8019	771.576 ± 4.3171
Syringic acid	1.419 ± 0.0152	0.743 ± 0.0016
Sinapic acid	39.845 ± 0.1151	27.039 ± 0.8282
Ellagic acid	0.063 ± 0.0047	0.040 ± 0.0021
Chlorogenic acid	0.001 ± 0.0001	0.001 ± 0.0000
Total	1400.317 ± 3.7595	1141.873 ± 6.4196

Ferulic acid, the dominant constituent of woody pepper stem, was in higher quantities (938.796 mcg/g) in oven-dried (OD) produce than in vacuum dried (VD) (771.576 mcg/g). Similarly, *p*-Coumaric acid was present in a significantly higher amount (161.626 mcg/g) in OD than in VD samples. In OD, compounds present in their order of relative abundance were *t*-Cinnamic acid (98.235 mcg/g), Caffeic acid (45.374 mcg/g), Sinapic acid (39.845 mcg/g), Gallic acid (32.338 mcg/g), *o*-Coumaric acid (31.743 mcg/g) and Gentisic acid (12.538 mcg/g) apart from other minor constituents. On the other hand, compounds such as Caffeic acid (64.110 mcg/g), *t*-Cinnamic acid (63.798 mcg/g), Gallic acid (41.502 mcg/g), Sinapic acid (27.039 mcg/g), *o*-Coumaric acid (25.146 mcg/g), Vanillic acid (14.657 mcg/g) and 2,4-dihydroxy benzoic acid (13.161 mcg/g) were found in the vacuum dried samples. These findings are in line with the earlier report in which the significant influence of drying methods on the concentration of phenolic compounds (caffeic acid and gallic acid) and antioxidants was reported in spearmint (Orphanides *et al.*, 2013).

Ferulic acid (4-hydroxy-3-methoxycinnamic acid) is one of the potent bioactive molecules reported to occur in a number of plants (Zhao and Moghadasin, 2008). The compound has been considered a ubiquitous phenolic derivative of cinnamic acid (Rosazza *et al.*, 1995). Owing to its proven benefits, it has been considered an important constituent in the food, nutraceutical, and pharma industries (Kumar and Pruthi, 2014).

Conclusion

The present study could generate valuable information to understand the biochemical composition of this lesser-known plant genetic resource. It was observed that the maturity of the stem, as judged by its thickness in the market, did not influence moisture content and scraping percentage; however, piperine content increased with an increase in stem thickness. Ferulic acid was identified as the most abundant phenolic acid in the stems of both sizes. The use of vacuum drying resulted in high piperine recovery, while oven drying showed higher recovery of phenolic compounds. These studies would form the basis for utilizing this medicinally important and antioxidant-rich spice.

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Competing Interests

None declared.

Authors' Contributions

AAW: Conceptualization, exploration and collection of material, draft preparation; PB: processing of samples and data analysis; RKD: processing of samples; ANL: Biochemical analysis; KSS: Biochemical analysis. All the authors read and approved the manuscript.

Availability of Data and Material

All the relevant data has been included in the manuscript.

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RESEARCH ARTICLE

Collecting Plant Genetic Resources from Remote and Isolated Marwah-Warwan Valley of Jammu and Kashmir, India

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Abstract

Marwah-Warwan is an isolated remote valley falling in the Kishtwar district of Jammu Kashmir, India. Considered one of the great wildernesses in the inner Himalayas, the valley is located in the south-east of Jammu & Kashmir sandwiched between the Anantnag district of Kashmir in the west and Suru Valley of Kargil district of Ladakh in the east separated by lofty mountains. There are a total of 27 villages in the valley and several of these villages are still not connected by road. An exploration and germplasm collection programme was undertaken by ICAR-NBPGR in this region and 82 germplasm accessions of diverse agri-horticultural crops belonging to 27 genera and 30 (15 cultivated and 15 wild) species were collected from 24 sites ranging between 33°36' to 33°56' N latitude, 75°26' to 75°48' E longitude with an altitudinal range of 2159 - 3529 m MSL. The germplasm collected included that of cereals, pseudocereals, minor millets, grain legumes, minor fruits/nuts and medicinal plants. The collected germplasm has been submitted for conservation in the National Gene Bank (NGB) which has negligible representation of crop genetic resources from this region. Besides, these collections assume importance especially when the area is increasingly being exposed to the outside world with the construction of roads and other developmental activities, posing a challenge to local agro-biodiversity. Cultivation of paddy, barley and several minor crops has already started vanishing from the system. This article highlights information on the germplasm collected, cultivation practices, genetic erosion and future exploration potential in the region.

Keywords: Exploration, Germplasm collection, Inner Himalayas, Marwah-Warwan Valley.

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Introduction

Marwah-Warwan situated in the Inner Himalayas is an isolated remote valley falling in Kishtwar district of Jammu and Kashmir, India. Located in the south-east of Jammu and Kashmir, Kishtwar district is flanked by lofty Himalayan mountains from all sides and surrounded by districts of Anantnag and Doda and Kargil, Ladakh and touching the northern boundaries of Himachal Pradesh state. Kishtwar proper is the main town of the district connected by a 110 km road with the Srinagar-Jammu national highway at Batote hill station. Kishtwar is also connected with Kashmir valley by Daksum Anantnag - Synthan top (33°34', 75°30', 3793 m MSL) - Chatroo road. The main town of Kishtwar branches off into three main valleys, namely Paddar Valley, Chatroo Valley and Marwah-Warwan Valley. Warwan and Marwah are two separate sub-districts or tehsils among 11 in the district, each one is constituted of about a dozen thinly populated villages. Inshan village is the tehsil HQ of Warwan while Nowapachi village is the tehsil HQ of Marwah. Marwah-Warwan (local "Madeaw-Wardwan") regarded as one of the remotest places in Jammu and Kashmir is a long narrow valley more than 50 km long stretching from village Sukhnai Warwan

in the north to village Hanzal Marwah in the south and on an average less than half a km wide sandwiched between Kashmir valley and Chatroo valley in the west and Suru valley of Ladakh in the east separated by lofty mountains. The valley is drained by Warwan River flowing from Warwan in the north to Marwah in the south with several streams/nallahs flowing into it from both sides of the valley forming the Maru or Marusudhar River as it enters Marwah tehsil. It is considered the largest tributary of the river Chenab meeting it at Bhanderkoot just above Kishtwar town. Renai *nallah* is one of the several prominent *nallahs* flowing southwest of the valley bordering the north side of Kishtwar National Park meeting the Maru River at village Qaderna. Mineral springs of hot water (local "Tatapani") and cold water (local "Shifapani") are located on the banks of this *nallah*. In the higher altitudes of the Kishtwar National Park famous for snow leopards, extensive alpine scrub and meadows, open rocks and glaciers are present. These meadows present a picturesque serene view during the peak blooming period of June-July with colorful flowering plants such as Himalayan cinquefoil (*Potentilla astrosanguinea* Lodd.), *Myosotis alpestris* F.W. Schmidt. etc. On the banks of the *nallahs*/streams in lower catchment areas, one can see lush green forests of fir [*Abies pindrow* (Royle ex D.Don) Royle], deodar [*Cedrus deodara* (Roxb.) G.Don], kail/blue pine (*Pinus wallichiana* A.B. Jacks.), spruce [*Picea smithiana* (Wall.) Boiss.] and Himalayan yew (*Taxus wallichiana* Zucc.).

Marwah-Warwan is biodiversity rich fertile valley with agriculture as the mainstay of almost entire population. The people are simple and hospitable and majority of them speak *Kashmiri* with a localized accent. Interestingly, although Marwah-Warwan is the part of district Kishtwar of Jammu Province, the only motorable road to this area is from district Anantnag of Kashmir Province through Marghan Top. Alternatively, people have to cross arduous hilly tracts through villages of Tellar, Hanzal and then Dachhan tehsil areas by foot to reach main town of Kishtwar. The area remains cut off for 4-5 months in a year from rest of the country due to heavy winter snowfall. Earlier during the years 2013, 2015 and 2018, systematic expeditions were conducted by ICAR-NBPGR in Chatroo Valley, Padder Valley and Kishtwar main including some areas of Dachhan, Surror, Sarthal, Thakraie, Bhonjwa and Drabshala and valuable germplasm of cereals, pseudocereals, millets, pulses, vegetables etc. has been collected and conserved in the National Gene Bank (Sultan *et al.*, 2019b). Since Marwah-Warwan part of Kishtwar district was not yet explored by ICAR-NBPGR and there is almost negligible representation of crop genetic diversity in the National Gene Bank from this region, the present exploration and germplasm collection programme was undertaken systematically to collect the available genetic diversity from this unexplored place, especially at a time when the area is being increasingly opened up to outside

world for tourism and other developmental activities, posing a challenge to local agro-biodiversity.

Materials and Methods

Exploration and germplasm collection programme approved under National Exploration Plan 2022-23 was undertaken in Marwah-Warwan Valley of Jammu and Kashmir, India during September 2023. The villages Mulwarwan and Inshan of Warwan tehsil were the entry points into the valley through the Marghan mountain pass (33°44'N, 75°29'E, 3702 m MSL). Before exploration, information on the region's flora, agriculture etc. was gathered from available literature and other sources, for example, Govt. agencies, NGOs etc. and analyzed with the specific aim of better planning of the exploration. Scientists of Rice Research Station, SKUAST-K, Khudwani, Anantnag (J&K), High Altitude Maize Research Station, SKUAST-K, Sagam, Kokernag, Anantnag (J&K) and High Altitude Research Station, SKUAST-K, Larnoo, Kokernag, Anantnag (J&K) were also consulted in this regard. Standard procedures of germplasm exploration and collection developed by ICAR-NBPGR, New Delhi (Tyagi *et al.*, 2016) were followed. The main sources for collection of germplasm samples of cultivated crops were farmer's fields or the threshing yards/farm stores in case the crop had already been harvested. In general, random sampling was followed for field collection and farm stored material, whereas small samples were bulked in case of wild species. The germplasm of wild species was collected from roadsides, alongside farmer's fields, grasslands, hill slopes and from rock crevices. The samples were collected in the form of seeds, fruits, bulbs, corms, rhizomes, tubers, etc. based on their mode of propagation. At each collection site a passport data sheet was filled as per standard format (Moss and Guarino, 1995) including data from hand-held GPS system on latitude, longitude and altitude of the place of collection. For recording ethno-botanical information, discussions were held with local knowledgeable farmers and elderly people. Each collection was assigned a unique collector number. One set of the collected materials was deposited in National Gene Bank, ICAR-NBPGR, New Delhi for conservation, while another set of majority of the collections were retained for characterization and multiplication.

Results and Discussion

Marwah-Warwan Valley is an isolated remote place with almost entire population directly or indirectly involved with the agriculture and allied sectors (Fig. 1). It is mostly a mono cropped zone with low production and productivity and the people are largely practising subsistence farming. Maize, wheat, potato and common beans are the main crops. "Ambri" apple plants are also grown at places in the area. These crops are essentially cultivated using organic and traditional eco-friendly farming methods with very

limited use of modern day agricultural inputs and still based on local cultivars, human labour, animal power and indigenous implements. Potatoes and other such vegetables are traditionally stored in deep pits covered with soil for winter use. Marwah tehsil is comparatively bigger in area, more populated and richer in vegetation than Warwan tehsil. The two tehsils are about 30 kms apart and Hajika nallah near Sarkund village separate the two. While moving from Warwan to Marwah, countless wild walnut trees can be seen growing on both sides of the road. Wherever sizable population of these walnut trees is present, local people refer to the place as "Doonkhal" ("Doon" is local name for walnut). Similarly, the place with larger population of *Sambucus wightiana* Wall. (local "Gonji") is referred to as "Gonjikhall". The entire valley is guarded by lofty mountains on all sides, and it is this isolation that has probably protected and preserved local agro-biodiversity for a long time. However, according to locals, during last decade there has been tremendous loss of biodiversity especially of useful medicinal herbs in the valley. Earlier medicinal plants grew more commonly and livestock used to graze these herbs. The consumption of different products from such livestock led to healthier lifestyle free from modern day ailments in local population. Several important medicinal herbs still grow in the higher reaches of the valley which are overharvested now for commercial purposes. During present exploration in the area, the authors have observed alien invasive weeds such as *Xanthium spinosum* L. growing abundantly along tracks. Such invasive alien species pose a significant threat to the local biodiversity in general. The authors have earlier reported this weed growing in several other places of Kishtwar district as well (Sultan *et al.*, 2019b). In fact, species which are listed to be of immediate concern in the Kishtwar district include *Parthenium hysterophorus* L., *Xanthium spinosum* L., *Cirsium arvense* (L.) Scop. and *Anthemis cotula* L. (Bhutyal *et al.*, 2014). Since Marwah-Warwan valley is now being increasingly opened up for the outside world and developmental activities including construction of roads etc. have been initiated, the erosion of the local

biodiversity is inevitable. First time concerted efforts have been made by ICAR-NBPGR in an organized way to survey and explore almost all the villages of the region falling in the altitudinal range of 2159 - 3529 MSL, 33°36' - 33°56' N latitude and 75°26' - 75°48' E longitude for collection of crop genetic resources. The exploration has yielded a total of 82 germplasm accessions (Table 1) from 24 different collection sites (Table 2) of following crops:

Cereals

Maize and wheat are the main cereal crops grown in the region. According to locals barley and paddy were also commonly cultivated earlier but these crops especially paddy has now been almost abandoned. During the present exploration programme seven (07) accessions of maize (*Zea mays* L.), six (06) accessions of wheat (*Triticum aestivum* L.), four (04) accessions of naked barley (*Hordeum vulgare* L.) and two (02) accessions of paddy (*Oryza sativa* L.) were collected. Maize landraces collected were short with small cobs having yellow or white grains. Wheat also has small plants with short spikes (Fig. 2A). One of the accessions collected from Warwan area (SHEIKH-1251) has small reddish grains in contrast to the straw-colored grains of other accessions. Naked barley is cultivated as in neighboring Ladakh. This is quite interesting as in other parts of Kishtwar district hulled barley is cultivated instead and authors have collected several accessions of hulled barley (local "Jau") earlier (Sultan *et al.*, 2019b). According to elderly farmers of Warwan area, their forefathers have originally brought barley and wheat seed from Ladakh which is being cultivated up to this day. The two paddy accessions collected were awned with dull white and bold grains (Fig. 2C). Earlier, paddy was commonly cultivated, largely in the fields located on the banks of nallahs/streams especially in Marwah area, but now as the water level in the river/nallahs has significantly fallen below, badly affecting paddy field irrigation, the farmers have discontinued paddy cultivation. Paddy cultivation still has the potential to be revived once electricity supply reaches the area and lift irrigation pumps get installed.

Pseudocereals

Nine (09) accessions of Tartary buckwheat [*Fagopyrum tataricum* (L.) Gaertn.], two (02) accessions of grain amaranth (*Amaranthus caudatus* L.) and two accessions of grain chenopod (*Chenopodium album* L.) were collected during present exploration. Earlier, these crops were more commonly cultivated but now farmers are increasingly losing interest in these valuable and climate resilient crops. Interestingly, in the entire Warwan-Marwah region only bitter buckwheat [*Fagopyrum tataricum* (L.) Gaertn.] is cultivated and we have not seen sweet buckwheat (*Fagopyrum esculentum* Moench) being cultivated anywhere. It seems due to harsh climate and isolated conditions, the farmers of this region have preferred and selected bitter buckwheat for



Fig. 1: Map of UT of Jammu & Kashmir. Shaded portion within Kishtwar district is the study area of Marwah-Warwan (arrow mark in red color denotes the Marghan Top entry point into the area)

Table 1: Plant Genetic Resources (PGR) collected from Marwah-Warwan valley in Kishtwar district of Jammu & Kashmir, India

S No.	Crop	Species	Local name	No. of accessions	Traits
<i>Cereals</i>					
1	Maize	<i>Zea mays</i> L.	Maki	07	Shorter plants and cobs
2	Wheat	<i>Triticum aestivum</i> L.	Kanak	06	Shorter plants and spikes
3	Barley	<i>Hordeum vulgare</i> L.	Grim/Vishki	04	Naked greenish
4	Rice	<i>Oryza sativa</i> L.	Madeaw Dhani	02	Awed, bold dull white grains
5	Wild rye	<i>Elymus semicostatus</i> (Steud.) Melderis		02	
6	Wild rye	<i>Elymus caninus</i> L.		01	
7	Wild rye	<i>Elymus nutans</i> Griseb.		01	
<i>Pseudocereals</i>					
8	Buckwheat	<i>Fagopyrum tataricum</i> (L.) Gaertn.	Trumba/Dhruv	09	Lesser seed shattering
9	Grain amaranth	<i>Amaranthus caudatus</i> L.	Seoul	02	
10	Grain chenopod	<i>Chenopodium album</i> L.	Kankudroo	02	
<i>Millets</i>					
11	Foxtail millet	<i>Setaria italica</i> (L.) P. Beauvois	Shol	04	Primitive features, seed shedding
12	Finger millet	<i>Eleusine coracana</i> Gaertn.	Kudroo	03	
13	Proso millet	<i>Panicum miliaceum</i> L.	Anne	02	
<i>Grain legumes</i>					
14	Common bean	<i>Phaseolus vulgaris</i> L.	Madeaw-Wardwan rajma	12	Medium sized, reddish grains common
15	Soybean	<i>Glycine max</i> (L.) Merr.	Muthi	03	One acc. with smaller brownish grains
<i>Fruits and nuts</i>					
16	Sea buckthorn	<i>Hippophae rhamnoides</i> subsp. <i>turkestanica</i> Rousi	Kandi phall	01	Plants less thorny
17	Hawthorn	<i>Crataegus songarica</i> K. Koch	Reng/Khring	02	One acc. thornless
18	Himalayan viburnum	<i>Viburnum grandiflorum</i> Wall. ex DC.	Kulma	02	
19	Hip rose	<i>Rosa canina</i> L.	Gulab	02	
20	Hip rose	<i>Rosa macrophylla</i> Lindl.	Gulab	01	
21	Stone bramble	<i>Rubus saxatilis</i> L.	Chanch	01	Fruits shiny red and tasty
22	Himalayan bird cherry	<i>Prunus cornuta</i> (Wall. ex Royle) Steud.	Zanbi dach	01	
23	Hazelnut	<i>Corylus jacquemontii</i> Decne.	Jangli badam/Virvoin	01	
<i>Medicinal & aromatic plants</i>					
24	Yams	<i>Dioscorea deltoidea</i> Wall. ex Griseb.	Kinns/Discorea	03	
25	Henbane	<i>Hyoscyamus niger</i> L.	Bajarbangh	03	
26	Indian costus	<i>Saussurea costus</i> (Falc.) Lipsch.	Kuth	01	
27	Himalayan yew	<i>Taxus wallichiana</i> Zucc.	Postul phall	01	The aril of the berries is edible and has medicinal value

Other crops					
28	Tomato	<i>Solanum lycopersicum</i> L.	Tamatar	01	Smaller fruits
29	Mountain spinach	<i>Atriplex hortensis</i> L.	Wust-e- haakh	01	
30	Sunflower	<i>Helianthus annuus</i> L.	Gul-e-aftab	01	

Table 2: Geographical coordinates of collection sites of crops species/CWR collected in Marwah-Warwan valley in Kishtwar district of Jammu & Kashmir, India

S. No.	Collection site/Area/ Village	Latitude	Longitude	Altitude (m)	Crop/CWR collected
1	Dhobpathar, 5 kms ahead of Gawran	33°43'	75°26'	2729	<i>Rosa canina</i>
2	Upper Batinallah, 3 kms before Marghan Top	33°46'	75°31'	3529	<i>Elymus nutans</i>
3	Nadibalan Warwan	33°46'	75°32'	3366	<i>Rubus saxatilis</i>
4	Lower Nadibalan Warwan	33°46'	75°32'	3285	<i>Elymus caninus</i>
5	Uuriwan Warwan	33°48'	75°33'	2503	<i>Triticum aestivum</i>
6	Afti Warwan	33°51'	75°32'	2586	<i>Hippophae rhamnoides</i> ssp. <i>turkistanica</i> , <i>Rosa canina</i> , <i>Saussurea costus</i>
7	ChoiDraman Warwan	33°51'	75°32'	2559	<i>Triticum aestivum</i> (2 acc.), <i>Phaseolus vulgaris</i> , <i>Fagopyrum tataricum</i>
8	Basmana Warwan	33°50'	75°29'	2689	<i>Phaseolus vulgaris</i> , <i>Triticum aestivum</i> , <i>Fagopyrum tataricum</i> , <i>Rosa macrophylla</i> , <i>Zea mays</i>
9	Upper Basmana Warwan	33°50'	75°29'	2704	<i>Solanum lycopersicum</i> , <i>Atriplex hortensis</i> , <i>Triticum aestivum</i>
10	Gumri Warwan	33°55'	75°31'	2768	<i>Hyoscyamus niger</i> (2 acc.), <i>Fagopyrum tataricum</i>
11	Rikenwas Warwan	33°56'	75°31'	2775	<i>Fagopyrum tataricum</i>
12	Barayan Warwan	33°50'	75°32'	2571	<i>Phaseolus vulgaris</i> , <i>Hordeum vulgare</i> , <i>Triticum aestivum</i> , <i>Fagopyrum tataricum</i>
13	Sarkund Marwah	33°42'	75°38'	2327	<i>Dioscorea deltoidea</i> , <i>Viburnum grandiflorum</i> , <i>Zea mays</i> , <i>Prunus cornuta</i>
14	Qaderna Marwah	33°38'	75°43'	2221	<i>Elymus semicostatus</i> , <i>Crataegus songarica</i> , <i>Setaria italica</i> , <i>Hordeum vulgare</i> , <i>Fagopyrum tataricum</i> , <i>Oryza sativa</i> , <i>Eleusine coracana</i> (2 acc.), <i>Glycine max</i> (2 acc.), <i>Panicum miliaceum</i> , <i>Phaseolus vulgaris</i> , <i>Zea mays</i>
15	AstanGam Marwah	33°40'	75°42'	2159	<i>Setaria italica</i> , <i>Phaseolus vulgaris</i> , <i>Zea mays</i> , <i>Amaranthus caudatus</i>
16	Satarwagen Yuordo Marwah	33°40'	75°42'	2346	<i>Chenopodium album</i> , <i>Fagopyrum tataricum</i> , <i>Amaranthus caudatus</i> , <i>Hordeum vulgare</i> , <i>Phaseolus vulgaris</i>
17	Chanjier Marwah	33°37'	75°42'	2184	<i>Zea mays</i> , <i>Glycine max</i> , <i>Phaseolus vulgaris</i> , <i>Hordeum vulgare</i> , <i>Chenopodium album</i> , <i>Oryza sativa</i> , <i>Setaria italica</i> , <i>Eleusine coracana</i> , <i>Fagopyrum tataricum</i>
18	One km before Teller Marwah	33°36'	75°43'	2198	<i>Crataegus songarica</i>
19	Teller Marwah	33°36'	75°43'	2202	<i>Zea mays</i> , <i>Phaseolus vulgaris</i> , <i>Viburnum grandiflorum</i> , <i>Dioscorea deltoidea</i> , <i>Corylus jacquemontii</i> , <i>Taxus wallichiana</i>
20	Hynan Marwah	33°37'	75°42'	2182	<i>Zea mays</i> , <i>Phaseolus vulgaris</i> , <i>Dioscorea deltoidea</i>
21	Yurodo Marwah	33°39'	75°43'	2174	<i>Helianthus annuus</i>

22	Three kms ahead of Yurodo Marwah	33°39'	75°44'	2318	<i>Elymus semicostatus</i>
23	Anyar Marwah	33°39'	75°47'	2337	<i>Hyoscyamus niger</i> , <i>Phaseolus vulgaris</i>
24	Zaban Renaie Marwah	33°39'	75°48'	2378	<i>Setaria italica</i> , <i>Panicum miliaceum</i> , <i>Fagopyrum tataricum</i> , <i>Phaseolus vulgaris</i> (2 acc.)



Fig 2: A characteristic wheat field in Warwan area (A), *Elymus nutans* collected at the entrance of Warwan valley near Marghan Top (B), Paddy (Local "Madeaw Dhani") collected from Marwah area (C), Buckwheat (*Fagopyrum tataricum*) collection SHEIKH-1242, in the inset buckwheat pancake (Local "Dhull") prepared on a stone pan (D), Some variable common bean and soybean collections (E and F), Seabuckthorn (*Hippophae rhamnoides* subsp. *turkestanica*) population growing at Afti Warwan (G) and Himalayan yew (*Taxus wallichiana*) collected near Teller Marwah (H)

cultivation; it is considered to be healthier containing greater amount of rutin and having higher antioxidant activity than common or sweet buckwheat. It has been reported that rutin content in *Fagopyrum tataricum* is almost 100 times higher than that of *Fagopyrum esculentum* (Kitabayashi *et al.*, 1995). In other areas of Kishtwar *Fagopyrum esculentum* is cultivated besides *Fagopyrum tataricum*. Interestingly, lesser seed shattering was observed by us during buckwheat harvesting in farmer fields in Warwan areas. The buckwheat flour dough is fermented overnight before being made into pancakes on a stone pan. The resultant sourness in the dough according to the locals neutralizes the bitterness in

pancakes to some extent. These pancakes are locally called as "Dhull" and are eaten with honey or sugar (Fig 2D).

Millets

Four (04) accessions of foxtail millet [*Setaria italica* (L.) P. Beauvois], three (03) accessions of finger millet (*Eleusine coracana* Gaertn.) and two (02) accessions of proso millet (*Panicum miliaceum* L.) have been collected mostly from Marwah areas. Earlier these crops were cultivated commonly and consumed in the form of porridge but now younger generation has lost interest in these crops which are now unfortunately vanishing from the system. Elderly people fondly mention these crops and their nutritional worth. Primitive form of *Setaria italica* is being cultivated more commonly than other two minor millets and is used as animal feed and fodder (Fig. 3 B and C). Its grains are boiled in water and then fed to the cattle and as per locals it then increases milk yield. The millet is characteristically threshed by trampling with bullocks. According to locals, in earlier times it was cooked and eaten like rice. This primitive type of foxtail millet has earlier been collected by us from Padder area of Kishtwar where it is locally known as "Sallan"; its roasted grains are ground and used as satu there (Sultan *et al.*, 2019b). Seed shedding is a problem with it, besides morphologically it is closer to *Setaria viridis* (L.) P. Beauv., the wild relative of foxtail millet.

Grain legumes

Of all the crops collected during our present exploration programme, maximum number of accessions (12) have been collected in common beans indicating their common cultivation in the region. The common bean landraces in Jammu and Kashmir are frequently named according to the area of production and every region claims to produce unique and tasty common beans. Similarly, common beans produced in Marwah-Warwan are known as Marwah-Warwan rajma (Madeaw-Wardwan rajma) and are believed to be superior in quality and taste. The common beans produced here are often reddish colored with medium sized grains (Fig. 2E) in contrast to other regions of Kishtwar and in Gurez valley of Kashmir where good variability in size and color has been collected (Sultan *et al.*, 2019a). One accession of short duration elongated yellow grained common bean has been collected from Marwah region (SHEIKH-1309). Three (03) accessions of soybean [*Glycine max* (L.) Merr.] have also been collected during present exploration including one unique small, brown-seeded collection from Qaderna Marwah (SHEIKH-1277) (Fig. 2F).



Fig. 3: A man working in a buckwheat field in Warwan village (A), A man carrying harvested foxtail millet landrace on his back in Marwah village (B), Harvested foxtail millet landrace stacked in front of a typical house before being threshed by trampling with bullocks in remote Zeban Renai village of Marwah (C) and a traditional watermill in Teller Village of Marwah (D)

Fruits and nuts

In Marwah area “Ambri” apple plants with fragrant tasty fruits were found growing in front of several homes. Small populations of sea buckthorn (*Hippophae rhamnoides* subsp. *turkestanica* Rousi) were found growing on sandy stream banks near village Afti of Warwan (Fig. 2G) and village Qaderna of Marwah. Strangely locals are quite ignorant about this important plant genetic resource; its local name “Kandi phall” apparently indicates the uselessness of the plant. Unlike the plants growing in Ladakh and Telail Gurez, sea buckthorn plants growing in Marwah-Warwan valley are less thorny; one accession of this plant has been collected from Afti Warwan. The value and importance of sea buckthorn and need for its conservation was highlighted during an interaction meeting with elderly people of Warwan area during the present exploration programme. The locals were informed about the nutritional importance of this minor fruit which in neighboring Ladakh is known by the name of “Leh berry” and is a source of livelihood of many people there. Two accessions each of hawthorn (*Crataegus songarica* K. Koch), Himalayan viburnum (*Viburnum grandiflorum* Wall. ex DC.), hip rose (*Rosa canina* L.), one accession each of *Rosa macrophylla* Lindl., *Rubus saxatilis* L. and *Prunus cornuta* (Wall. ex Royle) Steud. were also collected. One accession of *Crataegus songarica* (SHEIKH-1296) is significantly thorn less with tasty fruits. While moving from Warwan to Marwah, countless plants of wild walnuts (*Juglans regia* L.) were seen growing in the forests at several places. These plants are a source of livelihood for local people who collect their comparatively smaller, harder and difficult to crack nuts and sell them to the contractors

at a meagre price. One accession of hazelnut (*Corylus jacquemontii* Decne.) has been collected near village Teller of Marwah. Small population of dispersed plants can be seen growing here. *Sorbus* L. spp. (local “Muilli”) was also found growing at this place and locals fondly eat its soft tasty fruits. It could not be collected during the present exploration as it was not at suitable stage of collection.

Medicinal and aromatic plants

The area abounds in wealth of medicinal plants mostly growing in higher reaches. These include *Angelica glauca* Edgew., *Viola odorata* L., *Podophyllum hexandrum* Royle, *Atropa belladonna* L., *Rheum emodi* D. Don, *Arnebia benthamii* (Wall. ex G. Don) LM. Johnston, *Picrorhiza kurroo* Royle ex Benth., *Inula royleana* DC., *Delphinium roylei* Munz, *Jurinea dolomiaea* Boiss. etc. Three (03) accessions each of yams (*Dioscorea deltoidea* Wall. ex Griseb.) and Henbane (*Hyoscyamus niger* L.) and one (01) accession of Indian costus [*Saussurea costus* (Falc.) Lipsch.] have been collected during the present exploration. One accession of *Taxus wallichiana* Zucc. has also been collected some 2 kms ahead of Teller village. Its berries are eaten by locals and are believed to be general tonic, effective against cough. The berries are about 0.9cm long and 0.7cm broad; reddish berry flesh or aril is the edible part while the single stone/seed enclosed within this aril is highly toxic (Fig 2H).

Other crops

Besides common beans, maize and wheat, potato is the fourth common crop grown in both Warwan and Marwah areas. Potatoes cultivated in the area are tasty and both white and red skinned types are grown. One accession

each of tomato (*Solanum lycopersicum* L.) with small attractive fruits and mountain spinach (*Atriplex hortensis* L.) has been collected in Warwan area. Apart from potatoes, vegetable cultivation in the entire area seems to be limited. Interestingly, one accession of sunflower (*Helianthus annuus* L.) has also been collected from Marwah region and according to the farmer, it is being grown from the time of his forefathers but its cultivation has now been stopped.

Besides, these crops wild rye (*Elymus nutans* Griseb.) accession (SHEIKH-1315) has been collected near Margan Top at an altitude of 3529 mMSL (Fig. 2B). Two (02) accessions of *Elymus semicostatus* (Steud.) Melderis and one (01) accession of *Elymus caninus* L. have also been collected during the course of present exploration.

To sum-up, eightytwo (82) germplasm accessions of diverse agri-horticultural crops thus collected belong to 27 (15 cultivated and 12 wild) genera and 30 (15 cultivated and 15 wild) species (Table 1). Maximum number of accessions have been collected in common beans (12), followed by *Fagopyrum tataricum* (09), *Zea mays* (07) and wheat (06) indicating diversity of these crops in the region. Maximum number of accessions of various crops (13) have been collected from Qaderna village area. This crop diversity is of primary importance in the nutritional and livelihood security of the local population. One set of all the 82 collected germplasm accessions has been submitted for conservation in National Gene Bank at ICAR-NBPGR, New Delhi while another set of majority of these accessions has been retained for characterization/maintenance. Genetic diversity of crops, gathered and protected from extinction in the gene banks worldwide is no doubt essential for dealing with changing and more demanding environments. However, it would always be wiser to mobilize and support local communities to preserve crop genetic resources *in situ* (on farm), through the sharing of knowledge, publicity and cooperation with scientific researchers and governmental organizations.

The remote, isolated and picturesque area of Marwah Warwan is surrounded by a difficult terrain which has made development of transportation infrastructure and logistic services difficult. This has badly hampered access to electricity, sanitation and communications services. Entire belt is still devoid of electricity supply with virtually no telecommunication facilities. In almost every household, solar panels have been installed for lighting purposes. Since the area has so far been devoid of electricity supply, traditional watermills still operate in almost every village (Fig. 3D). There are several villages which are still inaccessible and one has to travel several kilometers on foot to reach there. Sukhnai in the north, Teller, Deharna and Hanzal in the south west and Sudhar Renai, Zaban Renai and lastly Metwan on the banks of Renai nallah in the south east, for example, are far from being connected by roads. The geographical isolation has however protected and

preserved local biodiversity. Crops grown here especially common beans and potatoes are in great demand in other parts of Jammu and Kashmir. Now various developmental activities including construction of roads have been initiated in the entire region. Improved transportation will result in economic benefits through tourism and resource extraction, opening up new livelihood opportunities for local people. Local agricultural produce including rajmash, potatoes etc. besides forest produce including valuable herbs and medicinal plants will have easy access to the market. With market demand economic activity will improve; consequently, the cost of living will ease. Careful consideration should, however be given to ensure the sustainable management of these resources. According to locals, during last decade there has been tremendous loss of biodiversity especially of useful medicinal herbs which are highly endangered now. Engagement with stakeholders, particularly those who are most likely to bear the costs of negative external pressures, is also critical in ensuring social, environmental and agricultural sustainability.

Conclusion

The present exploration has yielded 82 germplasm accessions, maximum number of accessions are of common bean (12), followed by *Fagopyrum tataricum* (09), *Zea mays* (07) and wheat (06). As the region has remained largely isolated without modern day facilities like telecommunication, electricity supply, road connectivity, the agriculture system has been truly eco-friendly and sustainable with local crops being superior in taste and nutritional value. This valuable agro-biodiversity is now threatened as the valley is being increasingly opened up for tourism and other developmental related activities. Cultivation of paddy, barley and several minor crops has already started vanishing from the system. Medicinal and aromatic plants now occur only in higher reaches, while just a decade ago these grew within village premises as well. Community support initiatives are required to preserve crop genetic resources *in situ* (on farm), through the sharing of knowledge, publicity and cooperation with scientific researchers and governmental organizations. Rich species and genetic diversity in medicinal and aromatic plants, minor fruits, nuts and wild ornamentals indicate the need for urgent continued collecting missions in the region.

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RESEARCH ARTICLE

Characterization of Gola *Ber* Genotypes under Arid Conditions of Western Rajasthan for Qualitative and Quantitative Characters

DK Sarolia*, Kamlesh Kumar, Lokesh Kumar, D Singh and MK Choudhary

Abstract

About 350 genotypes of *ber* are being maintained at the National Active Germplasm Site (NAGs) of ICAR-CIAH, Bikaner, Rajasthan. These *ber* accessions were characterized for two consecutive years to assess variability among the accessions for qualitative and quantitative characters. In *ber*, ambiguity exists in local nomenclature as a result of different names in different localities for the same cultivar and the same name for different cultivars. For instance, Gola collected from different locations (CAZRI, HAU, IARI, MPKV, Bahadurgarh, Jodhpur) with another name (Delhi Gola, Gola Gurgaon, Kakrola Gola, and Popular Gola) may be the same name for different genotypes, i.e., Sasni Gola entirely different than original Gola. In this context, sixteen different Gola genotypes were collected earlier from different locations and characterized to assess similarity or dissimilarity among the genotypes on morphometrically and fruit quality traits basis. These 16 *ber* genotypes showed a range of variability with respect to plant growth, yield attributes and fruit quality parameters. The canopy volumes ranged from 12.21 to 19.84 m³, leaf size (length 7.2–11.5 cm and width 4.5–6.7 cm), and fruit yield 50.4 to 75.2 kg/plant. Further, fruit quality in terms of fruit weight, TSS, TSS acidity, total sugars and ascorbic acid content ranged from 18.5 to 28.8 g, 25.2 to 30.6° Brix, 60.71 to 89.53, 13.23 to 16.40% and 76.8 to 82.2 mg/100 g of edible pulp, respectively. Among sixteen genotypes, three have been found superior and showed marked variability with respect to fruit shape, size and color etc. viz., Gola CIAH (Diggi No.1) genotype recorded with the biggest fruit size, round fruit shape and golden yellowish color followed by Gola CIAH (N-1) and Gola Gurgaon (S3-5). Quality-wise, genotypes Gola CAZRI (S1-1) and Gola MPKV (S6-13) were found to be better than rest of the genotypes. Moreover, these genotypes were marked for further exploitation as commercial multiplication.

Keywords: Gola *ber* genotypes, Growth and Quality traits.

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Introduction

Ber (*Ziziphus mauritiana* Lamk.) belongs to the family Rhamnaceae and is said to be an indigenous fruit crop. According to De Condolle (1886), the center of origin of *ber* is Central Asia, where it is found under varying climatic conditions. It is grown in India traditionally from ancient times, where it has been in use for almost 4000 years (Mittre, 1961). It is one of the most suitable fruit trees for arid and semi-arid regions. It is also known as Chinese date, Chinese fig or poor man's fruit. It is distributed worldwide, including the Indian sub-continent, Southeast Asia, Australia, China, Africa, the Mediterranean region and the American center but its cultivation is confined over drier parts of the globe and commercial cultivation occurs in India. It is an example of an extremely drought-hardy species and is a dominant component of the natural vegetation of the Indo-Pak deserts. Both leaves and fruits of *ber* are very important from a nutritional point of view for being richer in mineral contents (Ivanova, 1982), vitamin C (Wang *et al.*, 1992) and other important nutritional contents. In both China and India, *Ziziphus* varieties have a long tradition of selection and cultivation, which

resulted in better-known and more widely released varieties than in other regions (Cherry, 1985). The important Indian states for jujube cultivation are Punjab (Sangrur, Bathinda, Ferozpur, Ludhiana, Patiala, and Mukatsar), Haryana (Hisar, Rohtak, Jind, Panipat, Mahendergarh and Gurgaon), Uttar Pradesh (Varanasi, Aligarh, Ayodhya and Agra), Rajasthan (Bharatpur, Jaipur and Jodhpur), Gujarat (Banaskantha, Sabarmati, Bhavnagar, Surendranagar, Patna, Ahmedabad, Bharuch, Vadodara, Sabarkantha and Mehsana), Tamil Nadu (Tirunelveli, Ramanathapuram, Dharmapuri and Salem), Karnataka (Bijapur, Bellary, Gulbarga, Belgaum, Raichur and Bidar), West Bengal (Murshidabad, Malda, Bankura and Birbhum), etc (Fig. 1). Presently, in India area under *ber* cultivation is around 54,000 ha with a production of 596,000 MT annually (Sawant *et al.*, 2023). More than 300 varieties of *ber* are available in the various parts of the country due to the suitability of the edapho-climatic conditions that prevailed in the region, but only a few are commercially important. They are Gola, Seb, Mundia; suitable for extremely dry areas, whereas Banarasi Kadaka, Umran and Meharun are suited for comparatively humid regions (Chadha, 2019). Various scientists have reported genetic and morphological variation among *ber* cultivars due to one or other reasons.

In northern India, Gola is the earliest to ripen, the ruling variety and covers about 60% area of *ber* cultivation (Anonymous, 2018). Gola has spreading tree habit, leaf cordate, broad (L/W: 1.1); apex obtuse; old leaves curved towards the ventral surface; broad-based. The petiole is light green in color on both surfaces, having an average length of 1.2 cm. Flowering starts late in August, with a peak in early September and ends late in September. Fruit set starts early in September with a peak late in September and ends early in October. Fruits mature earlier (January 7-25) than other commercial varieties. Fruit round; style end flat; stem end round; skin bright yellow, smooth and glossy; the cavity in pericarp at stem end; pulp white, soft; size 3.8 x 3.5 cm; pulp/stone ratio 14.3; average weight 21.4 g; TSS 21°Brix. Chovatia *et al.* (1992) have reported 160 mg/100 g vitamin C content in fruit pulp of this variety.

At farmer's fields a lot of complaints arise in the Gola genotype regarding poor flowering, pollination, fertilization, fruit set and fruit shape, size, yield, etc. The possible solution might be related to genuine of planting material, edapho-climatic factors and, crop bounded agro techniques, etc. Present climatic variables influencing a lot to the genotypic performance which needs reassessments in such conditions. In the case of *ber*, considerable confusion has arisen as a result of different names in different localities for the same cultivar and the same name for different cultivars. For instance, Gola collected from different locations (CAZRI, HAU, IARI, MPKV, Bhadurgarh, Jodhpur) with another name (Delhi Gola, Gola Gurgaon, Kakrola Gola, and Popular Gola) may be the same name for different genotypes, i.e., Sasni

Gola entirely different than original Gola. Therefore, the present study was conducted at ICAR- Central Institute for Arid Horticulture, Bikaner on existing Gola genotypes which were earlier collected from different localities such as CAZRI, HAU, IARI, MPKV, Bhadurgarh, Jodhpur with the nomenclature as Delhi Gola, Gola Gurgaon, Kakrola Gola, Laddu Gola, Popular Gola etc. with prefix or suffixed as Gola to know the similarity or dissimilarity morphometrically and fruit quality wise. The evaluation and improvement of *ber* for Rajasthan need to be intensified with genuine saplings of superior fruit quality. Keeping in view the above facts present study was conducted to evaluate and utilize the existing variability of the *ber* for crop improvement.

Materials and Methods

A field observation trial was conducted on *ber* cv. Gola genotypes were collected from different sources and planted at varied locations at ICAR - Central Institute for Arid Horticulture, Bikaner, situated in the middle of Thar desert at N 28.12°N latitude, 73.34° E longitude and at an altitude of 234.84 m above sea level. This is a national active germplasm site and maintains the largest field repository of *ber* crop in the country. In the present experiment, full-grown trees were selected having almost uniform age groups (15 years); three trees of each genotype planted at a spacing of 6 x 6 m that were collected earlier from different locations and being maintained in a field gene bank. A selective sampling method was adopted for the selection of genotypes of Gola *ber* and tagged with recording geographical position with the help of hand-held GPS device (Garmin Ltd.) and numbered as 1. Gola CAZRI (S1-1), 2. Gola, Jodhpur (S1-21), 3. Gola, Gudgaon, (S3-5), 4. Gola Kakrola (S4-6), 5. Gola CIAH (N-1) in nursery, 6. Gola by cuttings (N-2) in nursery, 7. Gola CIAH (N-3) in nursery, 8. Gola CIAH (Diggi No.1), 9. Gola HAU (S1-25), 10. Gola Laddu (S2-28), 11. Gola CIAH (R-1), 12. Gola Kakrola (Rahuri S4-7), 13. Gola Popular-Hisar (S6-9), 14. Gola MPKV (S6-13), 15. Gola Kakrola (Bahadurgarh S8-17) and 16. Gola IARI (S9-17). The study in each genotype was conducted during two consecutive years *i.e.*, 2018-19 and 2019-20. Observations with respect to plant growth (plant type, lead size, canopy volume, fruit load, shape) and fruit quality (fruit weight, fruit size, stone weight, pulp weight, pulp: stone, shape index, TSS, acidity, sugars, ascorbic acid) attributes were recorded at right stage and following standard methodology mentioned in descriptor of *ber* crop (Anonymous, 2017), especially canopy volume (m³) calculated by formula = $r^3 \times (2/3 X + X^3/3)$ whereas r = canopy radius and x = canopy height (Reddy *et al.*, 2008). Further, the fruit sample comprising 15 mature fruits of each accession was used for the physicochemical analysis. Fruit weight, stone and pulp weight were determined on electronic balance and fruit diameter was measured by use of Vernier caliper and shape index by dividing equatorial to polar diameter. Total

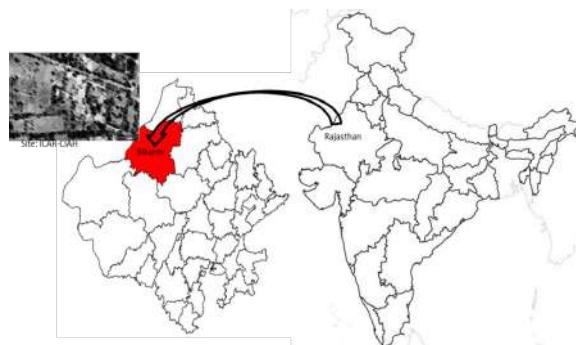


Fig. 1: National active germplasm site (NAGs) of Indian jujube

soluble sugars (TSS) content was recorded with the help of a hand refractometer and readings were corrected at 20°C with the help of a temperature correction chart. However, titratable acidity, total sugar, reducing sugars and ascorbic acid contents were determined as per standard methods described by AOAC (1990). All data were statistically analyzed by one way of analysis of variance using statistical online OPSTAT software developed by Sheoran *et al.* (1998).

Results and Discussion

Plant Growth and Yield

It is evident from the data presented in Tables 1, 2 and 3 that variability exists with respect to plant growth characteristics, fruit yield and quality attributes in the analyzed genotypes of Gola *ber*. From the observations, it was noticed that all genotypes have plant type tall or bushy, spreading type canopy with medium to dense foliage density. The canopy volume of Gola genotypes varied from 12.21 to 19.84 m³, leaf length 7.5 to 11.5 cm and width 4.5 to 6.7 cm. Variation in fruit yield was also noticed and fruit yield ranged from 50.4 to 75.2 kg plant per plant. Gola CIAH (N-3) genotype recorded maximum canopy volume (19.84 m³) followed by (19.02 m³) Gola HAU (S1-25) and minimum in Gola IARI (12.21 m³). Likewise, the highest yield (75.10 kg/plant) also noted in genotype Gola CIAH (N-3) and the lowest (50.40 kg/plant) in Gola IARI. This wide difference in canopy and yield among Gola genotypes probably might be due to differences in the genetic makeup of the each genotype (Table 1), which need to be study for clear conformity as also supported by Abdel-Sattar *et al.* (2021) who observed similar disparities in growth and yield attributes among eleven Indian *ber* cultivars. They suggested that variations among genotypes may arise from genotype-specific traits and their edapho-climatic suitability in a specific region, a notion previously supported by Mahajan and Dhillon (2000) and Kumar *et al.* (2014).

Physical Fruit Quality Parameters

Fruiting load from all genotypes was observed as medium to heavy. Fruits of all 16 Gola genotypes were round in shape (shape index value 0.95–1.02) and yellowish in color

at maturity with average fruit weight, pulp weight and pulp: stone varied from 18.5 to 28.8 g, 17.29 to 26.90 g and 13.04 to 14.50, respectively. Maximum fruit weight (28.80 g) and diameter (3.68 cm polar and 3.50 cm equatorial) was registered in Gola CIAH (D-1) followed by Gola CIAH (N-1), i.e., 22.80 g and minimum in Gola Kakrola (18.50 g). Similarly, pulp weight was also noted as a maximum (26.90 g) in Gola CIAH (D-1) and a minimum (17.29 g) in Gola Kakrola. Further, the highest (1.90 g) stone weight was recorded in Gola CIAH (D-1) and the lowest (1.20 g) in Gola Popular genotype, which was at par with Gola Kakrola (S4-7). Whereas pulp:stone ratio was found to be a maximum (14.50) in Gola Popular and a minimum (13.04) in Gola CIAH(N-2) (Table 2). The fruit weight, pulp weight, and pulp: stone ratio displayed variability among the Gola *ber* genotypes examined. Various researchers have documented similar observations in their studies on *ber* varieties, including investigations by Razi *et al.* (2013), Islam *et al.* (2015) and Abdel-Sattar *et al.* (2021).

Biochemical Quality Attributes of Gola Genotypes

As far as biochemical fruit quality traits are concerned, the TSS content among 16 Gola genotypes ranged from 25.2 to 30.6 °B. The highest TSS content (30.6 °B) was noticed in the Gola CAZRI genotype planted at N28° 6' 3 E 73° 20' 4 site, followed by Gola MPKV (30.1 °B) and minimum in Gola Laddu (25.2 °B). The TSS: acidity ratio and ascorbic acid content in these genotypes also varied from 60.71 to 89.53 and 76.8 to 82.2 mg/100 g pulp, respectively. Further, total sugar, reducing sugars and non-reducing sugars ranged from 13.23 to 16.40%, 6.02 to 7.55% and 7.74 to 9.71%, respectively. Maximum sugars (16.40%) were found in the Gola CAZRI genotype, closely followed by (16.14%) Gola MPKV (N28° 6' 4 E 73° 20' 5) and Gola Kakrola (15.47%), Rahuri (N28° 6' 4 E 73° 20' 5). The minimum respective fruit quality traits viz., TSS (25.2 °Brix), total sugar (13.23%), and reducing sugars (6.02%) were registered in Gola Laddu (N28° 6' 4 E 73° 20' 4). All these Gola genotypes did not register any significant difference with respect to acidity and ascorbic acid content.

The fruit quality attributes, such as total soluble solids (TSS) and the TSS: acidity ratio, exhibited noticeable variation among the examined *ber* genotypes. However, no significant differences were observed among these Gola genotypes in terms of acidity and ascorbic acid content. These findings closely align with those reported by Chen *et al.* (2019). Variations in TSS values may be attributed to climatic differences in the region and the genetic makeup of the cultivar, as noted by Shukla *et al.* (2007). The accumulation of ascorbic acid in fruits is primarily influenced by genotype, cultivation conditions and management practices (Chen *et al.*, 2019). The findings of the present study regarding titratable acidity and ascorbic acid are negatively correlated with the results of Abdel-Sattar *et al.* (2021). They found varied titratable acidity (0.38–1.27%) and ascorbic acid contents (55.27–164.47 mg/100 g) in eleven studied Indian

Table 1: Characterization of *Ber* cv. Gola accessions for growth and yield attributes

S. No	Genotype Gola and site of plantation	GPS position of plant	Plant type and foliage density	Canopy volume (m ³)	Fruit shape and color	Leaf length and width (cm)	Fruiting load	Yield (kg/plant)
1	Gola CAZRI (S1-1)	N28° 6' 3" E 73° 20' 4" Elevation 194.8 m	Bushy spreading plant with heavy foliage density	13.01	Big round and yellow color	11.4 x 6.6	Heavy	74.2
2	Gola Jodhpur (S1-21)	N28° 6' 4" E 73° 20' 4" Elevation 211.7m	Bushy spreading plant with medium foliage density	17.05	Big round and yellow color	7.2 x 4.5	Medium	68.4
3	Gola Gudgaon, (S3-5)	N28° 6' 4" E 73° 20' 5" Elevation 201.9m	Bushy compact plant with heavy foliage density	18.79	Big round and yellow color	8.8 x 5.1	Medium	70.6
4	Gola Kakrola (S4-6)	N28° 6' 4" E 73° 20' 5" Elevation 206.1m	Tall spreading plant with medium foliage density	12.91	Medium round and yellow color	10.2x5.1	Medium	60.7
5	Gola CIAH(N-1)	N28° 7' 2" E 73° 21' Elevation 197.3 m	Tall spreading plant with heavy foliage density	18.76	Medium round and yellow color	9.6 x 5.8	Heavy	75.1
6	Gola by cuttings CIAH(N-2)	N28° 6' 4" E 73° 21' Elevation 203.6 m	Bushy spreading plant with dense foliage density	18.87	Medium round and yellow color	11.5 x 6.2	Medium to heavy	70.4
7	Gola CIAH (N-3)	N28° 6' 1" E 73° 20' Elevation 196.3 m	Bushy spreading plant with dense foliage density	19.84	Medium round and yellow color	9.0 x 5.1	Medium to heavy	75.5
8	Gola CIAH (Diggi No.1)	N28° 6' 9" E 73° 21' Elevation 217.3 m	Tall spreading with medium foliage density	17.96	Big round and yellowish	10.4 x 5.6	Medium	70.3
9	Gola HAU (S1-25)	N28° 6' 1" E 73° 20' 3" Elevation 198.6 m	Tall spreading plant with medium foliage density	19.02	Medium round and yellow	9.4 x 5.5	Medium	71.4
10	Gola Laddu (S2-28)	N28° 6' 4" E 73° 20' 4" Elevation 190.5m	Bushy spreading compact plant with medium foliage density	15.78	Big large, round and yellowish	10.6 x 6.7	Heavy	72.1
11	Gola CIAH(R-1)	N28° 6' 1" E 73° 20' 3" Elevation 199.6 m	Bushy spreading and medium foliage density	17.54	Medium round and yellow	9.7 x 5.5	Heavy	72.1
12	Gola Kakrola (Rahuri S4-7)	N28° 6' 4" E 73° 20' 5" Elevation 204.7 m	Bushy spreading and medium foliage density	13.96	Medium round and yellowish	9.6 x 5.3	Medium	64.2
13	Gola popular-Hisar (S6-9)	N28° 6' 4" E 73° 20' 5" Elevation 206 m	Bushy spreading and medium foliage density	12.50	Medium round and yellowish dull appearance	9.1 x 5.0	Medium	62.3
14	GolaMPKV (S6-13)	N28° 6' 4" E 73° 20' 5" Elevation 197.7m	Tall spreading medium foliage density	17.66	Medium round and yellowish	8.4 x 4.5	Medium	67.4
15	Gola Kakrola (Bhadargarh S8-17)	N28° 6' 4" E 73° 20' 5" Elevation 204.4m	Bushy compact medium foliage density	14.00	Medium round and yellowish	8.7 x 5.1	Medium	64.3
16	Gola IARI (S9-17)	N28° 6' 4" E 73° 20' 5" Elevation 203.4m	Tall spreading medium foliage density	12.21	Medium round and yellowish	9.7 x 5.5	Low to medium	50.4
SEm±		-	-	1.60	-	0.27x 0.14	-	1.92
CD (p=0.05)		-	-	4.80	-	0.80x0.41	-	5.78

ber cultivars and reported that these differences are due to the unique genetic makeup of each cultivar.

Thus, *ber* genotype 'Gola CIAH (D-1)' was recorded big size (28.80 g) round fruit and golden yellowish color followed

by (22.80 g) Gola CIAH (N-1), which was at par (22.70 g) with Gola Gudgaon (S3-5). However, yield potential was found to be maximum (75.50 kg/plant) in genotype Gola CIAH(N-3), closely followed by (75.10 kg/plant) Gola CIAH (N-1) and Gola

Table 2: Evaluation of *Ber* cv. Gola for physical fruit quality traits

S. No	Genotype Gola and site of plantation	Fruit weight (g)	Stone weight (g)	Pulp weight (g)	Pulp/Stone	Fruit diameter (cm)		Shape index (E/P)
						Polar	Equatorial	
1	Gola CAZRI (S1-1)	22.02	1.50	20.52	13.68	3.50	3.42	0.98
2	Gola Jodhpur (S1-21)	21.05	1.40	19.65	14.04	3.62	3.50	0.97
3	Gola Gudgaon, (S3-5)	22.70	1.50	21.20	14.13	3.37	3.30	0.98
4	Gola Kakrola (S4-6)	19.28	1.33	17.95	13.50	3.13	3.20	1.02
5	Gola CIAH(N-1)	22.80	1.60	21.20	13.25	3.44	3.39	0.98
6	Gola by cuttings CIAH(N-2)	19.66	1.40	18.26	13.04	3.18	3.09	0.97
7	Gola CIAH (N-3)	22.61	1.60	21.01	13.13	3.40	3.35	0.98
8	Gola CIAH (Diggi No.1)	28.80	1.90	26.90	14.16	3.68	3.50	0.95
9	Gola HAU (S1-25)	21.50	1.50	20.00	13.33	3.38	3.33	0.98
10	Gola Laddu (S2-28)	22.08	1.50	20.58	13.72	3.34	3.40	1.02
11	Gola CIAH(R-1)	21.20	1.45	19.75	13.62	3.22	3.24	1.01
12	Gola Kakrola, (Rahuri S4-7)	18.50	1.21	17.29	14.29	2.96	2.99	1.01
13	Gola popular-Hisar (S6-9)	18.60	1.20	17.40	14.50	2.79	2.76	0.98
14	Gola MPKV (S6-13)	19.20	1.30	17.90	13.77	2.71	2.62	0.97
15	Gola Kakrola (Bhadurgarh S8-17)	20.40	1.40	19.00	13.57	2.99	2.95	0.99
16	Gola IARI (S9-17)	20.40	1.40	19.00	13.57	3.00	2.96	0.99
SEm±		2.23	0.35	1.88	1.79	0.39	0.35	0.27
CD (p=0.05)		6.69	1.05	5.64	5.37	NS	1.05	NS

Table 3: Evaluation of *Ber* cv. Gola for biochemical quality traits

S. No	Genotype Gola and Site of plantation	TSS (°B)	Acidity (%)	TSS/Acidity ratio	Total sugar (%)	Reducing sugars (%)	Non reducing sugars (%)	Ascorbic acid (mg/100g)
1	Gola CAZRI (S1-1)	30.60	0.38	80.53	16.40	7.55	9.71	78.80
2	Gola Jodhpur (S1-21)	25.50	0.42	60.71	13.23	6.09	7.84	82.20
3	Gola Gudgaon (S3-5)	27.10	0.41	66.10	14.06	6.48	8.33	80.60
4	Gola Kakrola (S4-6)	28.80	0.40	72.00	14.95	6.88	8.85	79.00
5	Gola CIAH(N-1)	27.40	0.41	66.83	14.22	6.55	8.42	80.60
6	Gola by cuttings CIAH(N-2)	29.10	0.40	72.75	15.10	6.95	8.94	80.00
7	Gola CIAH (N-3)	26.30	0.40	65.75	13.65	6.29	8.08	79.00
8	Gola CIAH (Diggi No.1)	28.10	0.40	70.25	14.58	6.72	8.64	78.70
9	Gola HAU (S1-25)	28.70	0.40	71.75	14.90	6.86	8.82	78.60
10	Gola Laddu (S2-28)	25.20	0.41	61.46	13.08	6.02	7.74	80.60
11	Gola CIAH(R-1)	27.20	0.41	66.34	14.12	6.50	8.36	80.60
12	Gola Kakrola (Rahuri S4-7)	29.80	0.39	76.41	15.47	7.12	9.16	77.40
13	Gola Popular-Hisar (S6-9)	28.00	0.40	70.00	14.53	6.69	8.60	79.00
14	Gola MPKV (S6-13)	30.10	0.38	79.21	16.14	7.43	9.56	76.80
15	Gola Kakrola (Badurgarh S8-17)	28.50	0.40	71.25	14.79	6.81	8.76	79.00
16	Gola IARI (S9-17)	26.00	0.41	63.41	13.49	6.21	7.99	80.60
SEm±		2.07	0.02	3.78	1.07	0.49	0.61	2.09
CD (p=0.05)		6.21	NS	11.34	3.22	1.48	1.83	NS

CAZRI (S1) (74.20 kg/plant). Additionally, fruit quality-wise, *ber* genotypes Gola CAZRI (S1-1) and Gola MPKV (S6-13) were found to be better than the rest of the genotypes. Moreover,

these tagged genotypes (Gola CAZRI, Gola CIAH (N-1 & 3) and Gola CIAH (D-1) can be used for further propagation due to better yield and quality of *ber* fruits.

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RESEARCH ARTICLE

Comparative Assessment of Different Earth System Models for Habitat Suitability of *Cuminum cyminum* (Linn.) Crop: A Machine Learning Evaluation from Arid and Semi-Arid Hot Areas of the India

Manish Mathur¹ and Preet Mathur^{2*}

Abstract

Crop species modeling is limited to prime crops like wheat, rice, maize, soyabean, etc., and few attempts have been made for spices' crops grown in arid and semi-arid regions. This study examines the habitat suitability of *Cuminum cyminum* (cumin), a crop grown in Rajasthan and Gujarat, India. The widely used WorldClim dataset and CMCC-BioClimInd bioclimatic dataset were tested for predictive and elucidative power. This evaluation calculated AUC and omission rate using the MaxEnt entropy method. The WorldClim dataset includes three-time frames and four greenhouse gas scenarios. The CMCC-BioClimInd dataset included five Earth System Models (ESMs): GFDL-ESM2M, HadGEM2-ES, IPSL-CM5A-LR, MIROC-ESM-CHEM, and NorESM1-M, which included two emission scenarios (4.5 and 8.5). The results indicate that both data sets have similar predictive accuracy. However, the optimal predictive areas for this crop differed significantly between the two model types. Annual precipitation, precipitation seasonality, precipitation during the coldest quarter, and potential evapotranspiration Hargreaves are the main factors affecting this crop's growth and expansion into new regions. Our research offers a novel standpoint for the implementation of this crop in potential new areas within Rajasthan and Gujarat.

Keywords Agricultural Production and Innovation, *Cuminum cyminum*, Climate Action, CMCC-BioClimInd, Maxent, Precipitation, Species Distribution Modelling.

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Introduction

India is the world's largest producer, exporter, and consumer of spices, leveraging its rich spice cultivation history. India has about 63 spices, 20 of which are seed spices. Cumin, fenugreek, coriander, and fennel are grown in India. A popular spice is cumin, scientifically known as *Cuminum cyminum* L. and it contributes Rs 2884.80 crore to the annual export value of seed spice crops, which total Rs 19505.81 crore. India satisfactorily meets 50% of global demand, demonstrating its importance in the global market (Sharma *et al.*, 2018; Kumar *et al.*, 2023). Rajasthan and Gujarat produce over 80% of India's cumin. Despite a smaller scale, Uttar Pradesh, Delhi, Uttarakhand, Madhya Pradesh, Chhattisgarh, and West Bengal have also grown this aromatic spice. Over a decade (2011–2020), this crop production in Gujarat and Rajasthan showed significant spatial differences. Cumin cultivation, production, and productivity are highest in Surendranagar, Gujarat. Banaskantha, Patan, Kutch, Rajkot, Junagadh, Porbandar, and Ahmedabad follow. Barmer had the most cumin land in Rajasthan, followed by Jodhpur, Jalore, Nagaur, Jaisalmer, and Ajmer (Kumar *et al.*, 2023).

Cumin, a small annual herbaceous plant in the Apiaceae (Umbelliferae) family (Fig. 1A and B), is susceptible to frost damage,

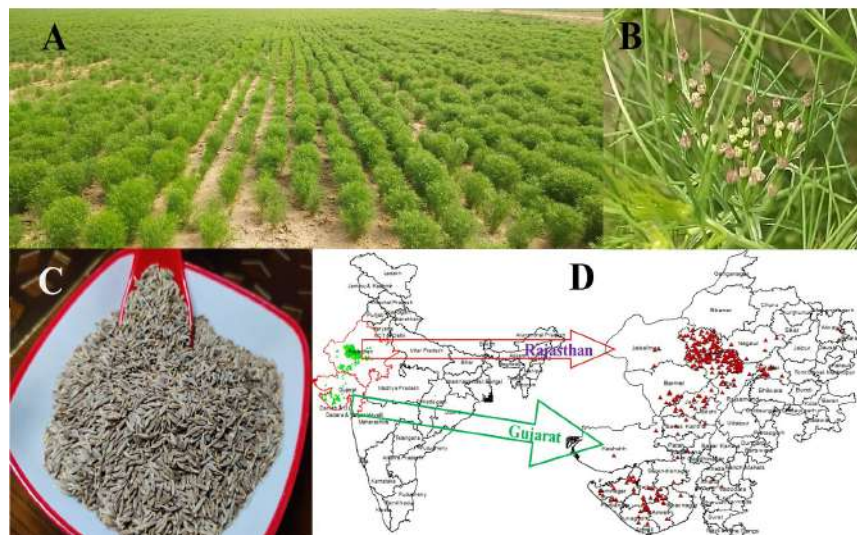


Fig. 1: Crops of *Cuminum cyminum* at vegetative stage (a), *C. cyminum* at fruiting stage (b), *C. cyminum* seeds (c), and spatially thinned sampling locations at Rajasthan and Gujarat states of India (D)

especially during flowering and seed formation. Aroma and pharmaceutical uses are valued in its seeds (Fig. 1C). The cumin plant, known for its resilience in arid climates, thrives in tropical or subtropical climates. The growth phase lasts 100–120 days. This species grows best at 20–30°C and 30–270 cm of annual precipitation. Sowing is best between mid-November and the first week of December. These climatic influences on cumin crops require scientific inventories to determine the reactions of various climatic models that predict the spatial and temporal fluctuations in the suitability of habitats for this crop, which is mostly found in Rajasthan and Gujarat.

Species Distribution Modelling (SDM) predicts and explains the geographical parameters that help species survive in the face of climate change (Mathur and Mathur, 2023). Scholars are using many global bioclimatic index datasets due to climate data accessibility (Morales-Barbero and Vega-Alvarez, 2019). The WorldClim dataset (Fick and Hijmans *et al.*, 2017; Poggio *et al.*, 2017) is a leading global climate dataset used in many fields. It is useful in ecological hydrology, climatology comparisons, carbon stock and temporal variation evaluation, and niche modelling of endangered plant species.

The dataset contains 19 bioclimatic variables related to temperature and precipitation, along with historical, current, 2050, and 2070 temporal frames. Additionally, the dataset includes Representative Concentration Pathways (RCPs) for Green House Gas Scenarios. The WorldClim dataset offers four spatial resolutions from 30 seconds (~1 km²) to 10 minutes (~340 km²), making it valuable. However, the limited scope of the 19 bioclimate variables makes it difficult to simulate plant and organism distribution because flora growth and proliferation require considerations beyond precipitation and temperature. As shown, some

regions have high evaporation despite high precipitation. Thus, plants transfer water through various mechanisms. Climate change is increasingly projected using Earth system models (ESMs) that combine atmospheric and oceanic dynamics with cryosphere and biosphere models (Asch *et al.*, 2021). Future climate data depend on CO₂ emission scenarios, which are usually represented by representative concentration pathways (RCPs) or shared socioeconomic pathways (SSPs) that represent optimistic and pessimistic global technological, economic, and social development (Mathur and Mathur, 2023).

Noce *et al.* (2020) introduced the CMC-BioClimInd, a 35-variable bio-climatic indicator with a 0.5° grid resolution, for five Earth System Models (ESMs) in CMIP5: GFDL-ESM2M, HadGEM2-ES, IPSL-CM5A-LR, MIROC-ESM-CHEM, NorESM1-M. This new data set covered 1960–1999 and 2040–2079 and 2060–2099. The inclusion of spatial information that is missing from the WorldClim dataset allows for the precise assessment of climate change's impact on the protection and management of fauna, flora, and ecological assets across various spatial scales and domains of study. The CMCC-BioClimInd can quickly and easily determine the correlation between the species under investigation and the climatic variables, mitigating fluctuations from the future trajectory and the physical attributes of various indicators. Noce *et al.* (2020) examined the interrelationships between climate variables in two models, CMCC-BioClimInd and WorldClim, and the causes of their differences. CMCC-BioClimInd predicts species distribution and simulates climate change with high accuracy, comprehensiveness, and efficacy.

In SDM analysis, bioclimatic model types like GCMs and ESMs, as well as RCPs and SSPs, cause predictive variabilities in area and production. These factors, alone or together,

shape SDM analysis results through complex interactions. According to our knowledge, CMCC-BioClimInd and WorldClim data have not been used to predict spice crop spatial extents. The current study compares WorldClim and CMCC-BioClimInd's RCPs and time frames on *Cuminum cyminum* crops habitat suitability in India's arid and semi-arid regions. Five ESMs, two time periods (2050 and 2070), and four Green House Gas Scenarios (GHS)—RCP 2.6, 4.5, 6.0, and 8.5 of WorldClim dataset were associated with two horizons. Both models were compared to WorldClim version 2 bio-climatic predictors.

Materials and Methods

Distribution data-set

Distributional records for this species were obtained from data repositories such as GBIF (<https://www.gbif.org>), published literature like Chandawat *et al.* (2008); Meena *et al.* (2012); Verma and Kumar (2015); Kant *et al.* (2017); Mehriya and Ramesh (2018); Pagaria and Sharma (2019) as well as information collected from Thasildar (revenue) office of Jodhpur (20221) and Jalor (2023), outreach activities carried out by different Kissan Vigyan Kendras (KVKs) like Amereli <http://www.jau.in/attachments/SeedVillage/KVK-Amreli.pdf>; Khaat <http://www.jau.in/attachments/SeedVillage/KVK-Khapat.pdf>;

Targhadia <http://www.jau.in/attachments/SeedVillage/KVK-Targhadia.pdf> and Jamnagar <http://www.jau.in/attachments/SeedVillage/KVK-Jamnagar.pdf> of Junagarh Agricultural University worked under Gujarat seed village programme (<http://www.jau.in/index.php/extension-40/>

seed-village-programme) and KVKs of Barmer (<https://www.aujodhpur.ac.in/kvk-ma.php?office=9>) operated under Agriculture University, Jodhpur. The coordinates of sites/village mentioned in published literature as well as government sources were identified through google earth and projected them with GIS ArcMAP on a WGS84 coordinate system (Coban *et al.*, 2020). In order to lessen the effects of spatial autocorrelation and eliminate redundant entries, we employed the spatial thin window of the "Wallace Software", a Graphical User Interface based on the R programming language (Kass *et al.*, 2018), with a thinning distance of 10 kilometres.

Bio-Climatic Variables

CMCC-BioClimInd is available in full from PANGAEA.1 (<https://doi.pangaea.de/10.1594/PANGAEA.904278?format=html#download>). There are 805 NetCDF4 files with a $0.5 \times 0.5^\circ$ grid resolution and global coverage (excluding Antarctica) (Noce *et al.*, 2020). As mentioned, we used five ESMs: CMIP5: GFDL-ESM2M, HadGEM2-ES, IPSL-CM5A-LR, MIROC-ESM-CHEM, NorESM1-M, and two RCPs: 4.5 and 8.5 for 2040-79 (60) and 2060-99 (80). We used ArcGIS 10.2 spatial analysis function to extract the simulated values of 35 bioclimatic indicators for India and clipped the data set using geoprocessing (Wang *et al.*, 2023). Observational data from WorldClim version 2.0 (Fick and Hijmans, 2017) predicted species distributions. 19 bioclimatic variables (Hijmans *et al.*, 2005) were downloaded at 30 seconds ($\sim 1 \text{ km}^2$) and converted to ASCII (or ESRI ASCII) in DIVA-GIS version 7.5 (Coban *et al.*, 2020) for current and two future climatic scenarios (2050- and 2070-time frames, respectively;

Table 1: Variables Importance Values of different bio-climatic variables studied with specific models using Maxent tool

Bioclimatic Models		Bio-Climatic Variables (BC)												
		1	2	3	4	6	7	11	12	15	19	27	29	34
Current		8.9	2.5	0.7	5.1	0.2	0.9	3.9	21	40.2	16.2	-	-	-
	2.6	9.4	7.4	1.3	1.6	4.5	0	6.8	23	27.7	18.9	-	-	-
2050 RCPs	4.5	10	7.5	1.1	1.3	6.9	0.7	1.3	19	41.9	10.8	-	-	-
	6	10.9	7.1	3.7	0.1	6.6	0.8	6.5	16	27.5	20.9	-	-	-
	8.5	8.6	6.3	1.5	2.2	5.7	0.6	4.9	18	36.3	16.4	-	-	-
2070 RCPs	2.6	1.9	5.5	0.1	2.5	3.5	0.4	0.8	9.9	64.2	11.2	-	-	-
	4.5	4.9	6.7	1.3	1.7	8.7	1	3.2	14	38.7	20.1	-	-	-
	6	15	7.9	1.9	1.5	0.9	0	6.2	17	27.3	22.7	-	-	-
	8.5	3.1	8.7	0.1	1.8	9.3	0.6	1.2	14	43.4	17.4	-	-	-
	45 (60)	8.3	0	0.2	5.5	0.8	0.2	1.1	10	7	41.4	2.1	7	16.3
GFDL	45 (80)	4.2	0	0.2	5.7	4.5	0.1	0.5	13	6.6	35.3	2.3	1.1	26.6
	85 (60)	10	0.6	0.4	2.4	0.3	0.9	7.6	20	13.2	21	1.4	1.9	20.4
	85 (80)	12.3	0.3	0.4	5.8	0.1	0.6	4.3	16	1.9	34	0.2	3.4	20.4

	45 (60)	1.6	0.4	0	1	0.8	0.9	1	13	58.5	13.2	0.4	1.9	7.6
HADGAM	45 (80)	4.5	0.4	0	0.7	0.4	0.2	1.6	16	58.9	5.3	1.2	1	10.1
	85 (60)	15.1	0.2	0	1.4	3.5	0.8	1.1	18	41.6	5.6	0.7	0.5	11.1
	85 (80)	3.9	0.3	0.3	0.1	4.7	0.3	0.6	13	21.4	36.1	0.3	3.3	16.3
IPSL	45 (60)	10.2	0	2.5	5.1	0.8	3.3	0.3	14	21.5	30.9	3.7	0.3	7.3
	45 (80)	14.9	1	0.1	4.6	0	0.4	1.3	16	16.8	29.2	0.7	4.5	10.7
	85 (60)	1	0.3	0.4	5.3	3.8	2.5	1.1	20	21.1	10.7	26.1	2.9	4.5
MIRCOS	85 (80)	12.1	0.5	0.9	3.1	1.4	1	17	30	16.9	0.6	3.9	1	11.6
	45 (60)	7.2	0.7	0	0.7	1.1	0.7	1.2	6.1	21.8	37.3	3.1	1.1	19.7
	45 (80)	3.4	0.1	0.1	6.8	1	0.9	1.7	9.5	11.2	38.4	1.2	4.7	21
NORSEM	85 (60)	7.2	0	0.1	1.1	5	0.2	1.3	7.1	37.5	19.3	0.4	0.5	20.4
	85 (80)	2.7	0.3	0.2	2.4	0.2	0.6	0.7	8.2	32.9	21	0.4	11	19.6
	45 (60)	2.1	2.9	0	0.8	2.3	2.1	5.8	3.8	44.7	24.3	0.1	0.8	10.3
	45 (80)	3.5	0.7	0	1	3.7	2.7	5.7	6.3	41.1	22	0.5	2.3	10.5
	85 (60)	3.6	0.2	0.8	0.8	0.5	0.2	1.1	3.3	73	8.8	0.7	2	5.6
	85 (80)	3.6	0.2	0.9	2.4	1.3	0.8	7.6	4.7	54.8	17	0.4	0.5	5.8

BC-1 Annual mean temperature; BC-2 Mean diurnal range; BC-3 Isothermality; BC-4 Temperature seasonality; BC-6 Minimum temperature of coldest month; BC-7 Temperature annual range; BC-11 Mean temperature of coldest quarter; BC-12 Annual precipitation; BC-15 Precipitation seasonality; BC-19 Precipitation of coldest quarter; BC-27 Simplified continentality index; BC-29 Mean temperature of coldest month and BC-34 Potential Evapotranspiration Hargreaves

Zhang *et al.*, 2022). RCPs 2.6, 4.5, 6.0, and 8.5 are represented by these datasets. The four RCPs range from high (RCP 8.5) to low (RCP 2.6) planned concentrations. In RCP 2.6, aggressive mitigation and low emissions reduce greenhouse gas (GHG) concentrations. The maximum emission scenario is RCP 8.5, while intermediate scenarios are RCP 4.5 and 6.0. In GHG concentration pathways, radioactive forcing (global energy imbalance) stabilizes 2.6, 4.5, 6.0, and 8.5 W/m² (Chaturvedi *et al.*, 2012). The units and mathematical expressions for each bio-climatic parameter are in supplementary Table 1.

The climate during this crop's cultivation greatly affects its distribution and production. Instead of using a multi-collinearity test, we only consider bio-climatic variables that occur during cultivation, have an annual influence, and follow this rationale. So, 13 variables were chosen. annual mean temperature (BC-1), mean diurnal range (BC-2), isothermality (BC-3), temperature seasonality (BC-4), minimum temperature of coldest month (BC-6), temperature annual range (BC-7), mean temperature of coldest quarter (BC-11), annual precipitation (BC-12), precipitation seasonality (BC-15), precipitation of coldest quarter (BC-19), simplified continentality index (BC-27), mean temperature of coldest month (BC-29), and potential evapotranspiration Hargreaves

Projection transformation

It is critical to standardize the projections of the Bio-Climatic variables obtained from disparate sources and at varying resolutions prior to extracting data and generating

forecasts using the MaxEnt model. Our analytical approach included ArcMap and ArcToolbox to follow a predefined methodology. The projection delineation was originally explained in the "projection and transformation" section of the Data Management Tools interface (Jijon *et al.*, 2021). We used the World Geodetic System 1984 EASE Geographic Coordinate System (GCS) for this. We converted the habitat class raster file projections to WGS 1984 web Mercator (auxiliary sphere-3857) in order to use Arc Map's "calculate geometry" window to quantify area under each habitat suitability class (see below).

Species Distribution Modelling

The present study used Maxent 3.4.1 (<http://www.cs.princeton.edu/schapiro/Maxent/>) to simulate and predict *C. cyminum* plausible geographic distribution likelihood using bio-climatic models of different timeframes and RCPs. This tool's discrete execution with each predictor in isolation allows us to accurately measure their impact on the species' distributional pattern. During modeling, 70% of this crop's spatially thinned distribution data were randomly selected as training data and 30% as testing data. The random background point count was 10,000 (Zhang *et al.*, 2021). We set the regularization multiplier to 0.1 to avoid test data overfitting. (Phillips *et al.*, 2006). Linear, quadratic, and hinge properties were used. The environment parameters were configured using the Jackknife method, while the rest were left at their software defaults. threshold-independent receiver-operating characteristic (ROC) analyses and an

area under the receiver operating curve (AUC) were used to calibrate and validate Maxent model evaluation and estimate model prediction accuracy (Elith *et al.*, 2006). The performance of the model was classified as failing (0.5-0.6), poor (0.6-0.7), fair (0.7-0.8), good (0.8-0.9), or excellent (0.9-1). The closer the AUC value to 1, the farther away from the random distribution, the greater the correlation between environmental variables and the predicted geographical distribution of species, and the more accurate the performance of the model (Mathur *et al.*, 2023).

The effects of bio-climatic variables on species distribution were assessed using Variable Importance values and response curves (Mathur and Mathur, 2023). We then used ArcGIS to convert the Maxent output ASCII file into raster format and used this to categorize the habitat areas as optimal (1.0 to 0.80), moderate (0.80 to 0.60), marginal (0.60 to 0.40), low (0.40 and 0.20), and absent (< 0.20) for this species.

Area -Yield Estimation

A simple regression analysis with Minitab (2016) software was used to compare the projected areas and their effects on cumin yield under the optimum habitat type. The relationships between village/site area (ha.) and cumin average yield (kg/ha.) were examined. For both parameters, Rajasthan and Gujarat government offices provided 225 site data sets. Raster file transformation into Keyhole Markup Language (KML) configuration allowed comparative visualization of projected changes in the optimum habitat type. Current and specific bio-climatic model KML files were superimposed, transformed in ArcMap, and saved as tiff files (Mathur and Mathur, 2023).

We used WorldClim dataset with two RCPs, 4.5 and 8.5, with 2050- and 2070-time frames and five ESMs with both RCPs and time frames to study the impact of model types and individual use of WorldClim and ESMs on area under different habitat suitability classes.

Results

Models performances

Through a comprehensive analysis of diverse sources, we found 387 species occurrence sites. After using Wallace Software's spatial thin window feature (Kass *et al.*, 2018), we eliminated all but one instance of a record within a specific region using a 10-kilometre thinning distance. SDM development was completed by including 300 spatially autocorrelation-free *C. cyminum* presence points (Fig. 1D).

Omission-commission plots show cumulative threshold selection's effect on anticipated area and sample point autocorrelation (test and training). The test sample omission rate should match the projected rate (Mathur *et al.*, 2023). With most predictors, test omission lines are well matched or above with projected omission (Supplementary Figs 1 to

7), indicating no sampling autocorrelation and no sampling bias in our model attributes. The area under the receiver operating curve (AUC) was used to evaluate the Maxent model's performance for predicting distribution of this species. Result revealed that this machine learning method performs excellently (AUC > 0.94) with all the studied predictors and their AUC curves are depicted in Supplementary Figs 8 to 14.

Variable Importance and Response curves

Table 1 shows the variable importance value of bio-climatic variables with different climatic model datasets and RCPs, and Supplementary Figs 15 to 29 show the response curves of the most influential two variables. WorldClim models for current, 2050, and 2070 and future RCPs scenarios showed that BC-12 (annual precipitation), BC-15 (precipitation seasonality), and BC-19 (coldest quarter precipitation) were the most important variables for this species' habitat suitability, with precipitation seasonality being the most dominant. In this study, the response curve for precipitation seasonality showed that cumin presence increases up to 150 mm, so this species will survive better in intermediate precipitation conditions. Annual precipitation (BC-12) response curves with current, 2050 (RCP 2.5, 4.5, 8.5) also indicated that the area receiving 450 to 550 mm rainfall had the highest probability of its occurrence. Precipitation during the coldest quarter (BC-19), the second most influential factor for this crop in 2050 (RCP 6.0) and 2070 (RCP 2.6, 4.5, 6.0, and 8.5), and their response curves show that this species prefers 5-8 mm of rainfall.

With other ESMs, like NORSEM, HADGAM [45(60), 45 (80), 85(60)] and MIRCOS [(85(60) and 85(80))], precipitation seasonality was also recorded as most influential factor. Among these ESM, NORSEM and MIRCOS showed the identical response curves for these variables as with WorldClim data set, however occurrence probability of cumin is lesser than that of with WorldClim data set. Interestingly, HADGAM 45 (60) and 45 (80) exhibited very less range for peak occurrence probability as compared to other models. Similarly, precipitation of the coldest quarter (BC-19) was found to be most influential variable with GFDL-ESM as well as with IPSL [45(60), 45(80) and 85(60)] and with MIRCOS-ESM particularly with 45(60) and 45 (80) and their response curve range are similar to WorldClim data set, however, the peak of occurrence probability is smaller with these ESMs. With GFDL, MIRCOS, NORESM and HADGEM 45 (80) and 85 (80), potential evapotranspiration Hargreaves was also recorded as influential factor for occurrence probabilities of this species.

Area (km²) of different habitat suitability class with various bio-climatic models.

Spatial extents of different habitat suitability classes identified for *C. cyminum* with help of MaxENT modelling

are depicted in Fig. 2 (with current and 2050 time-frames its four RCPs), Fig. 3 (2070 time-frames its four RCPs), Fig. 4 (GFDL ESM), Fig. 5. (HADGAM ESM); Fig. 6 (IPSL ESM); Fig. 7 (MIRCOSESM) and Fig. 8 (NORSEM ESM). Based on cell values in raster output processed with ArcMap, we can categorize four types of suitability classes with 0.20-point break and these were designated as optimum, moderate, marginal and low (Mathur and Mathur 2023) and the area under these classes (km²) are depicted in Table 2.

Graphical representation of spatial extent of optimum suitable habitats was found to be higher in Rajasthan state compared to Gujrat state under current and 2050 and 2070 WorldClim data set. Within these data-set, single continuous larger area was observed with current climatic conditions which is de-fragmented into several small populations extending at both the states (Fig. 2 and 3) with future time-frames. Among the such data-set, higher area under optimum (89623.33 km²; 16.64 Per cent optimum cumin area with reference to total geographical areas of Rajasthan and Gujarat), and moderate (104171.7 km²) suitability classes were recorded with 2050 RCP 8.5 and 4.5, respectively. While lower areas under these suitability classes were also recorded with same time-frame (2050) but with RCP 6.0 and 2.6, respectively. Interestingly, results revealed the area under the optimum class are higher with RCP 8.5 with both the future time-frame in comparison to other RCPs scenarios (Table 2) and with such result it can be said that this crop

will show good performance against higher greenhouse gas conditions. Higher areas under marginal (108433.9 km²) and low (91999.91 km²) class were recorded with current bio-climatic conditions (Fig. 2, Table 2).

Similarly, with ESMs, higher area under optimum (246962.1 km²; 45% of total geographical study areas) and moderate (186496.4 km²) habitat classes were recorded with MIRCOSESM (60) ESM (Fig. 7), while the lowest areas under these classes (134068.9 km² and 76626 km²) were recorded with GFDL85(60); Fig. 4) and HADGSM 85(80); Fig. 5), respectively. Higher (125005.7 km²) as well as lower (54959.87 km²) areas under marginal class were recorded with NORESM85(60) and NORESM 45 (80) ESM, respectively (Fig. 8). This analysis indicated the decrease of non-suitable areas for this crop with other ESM compared to WorldClim data set encompassing both current and two future time-frames and four respective RCPs. Further, range of per cent geographical areas of cumin under optimum habitat was recorded higher (24.90 to 45.86) with five studied earth system models, in comparison to WorldClim (10.38-16.64) data sets (Table 2).

Area -Yield Estimation

Regression analysis yield significant linear relationships between area of villages/sites (Ha.) and cumin average yield (kg/ha.) and this relationships (Supplementary figure 30) can be equate as

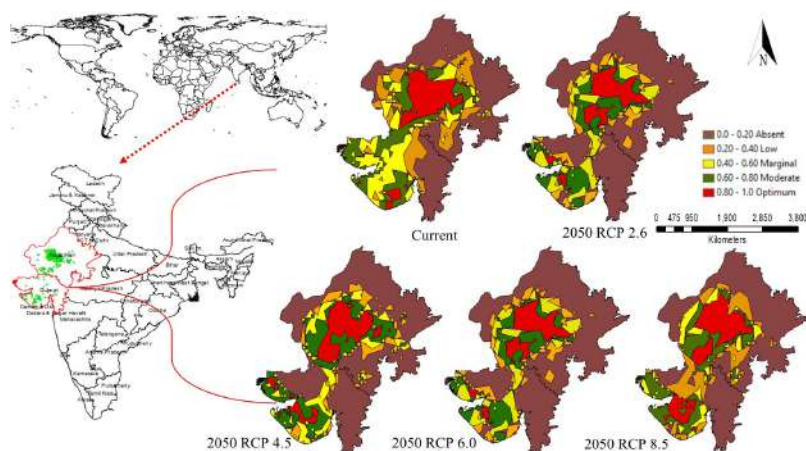


Fig. 2: Habitat suitability of *C. cyminum* with current and 2050 time-frames its four RCPs

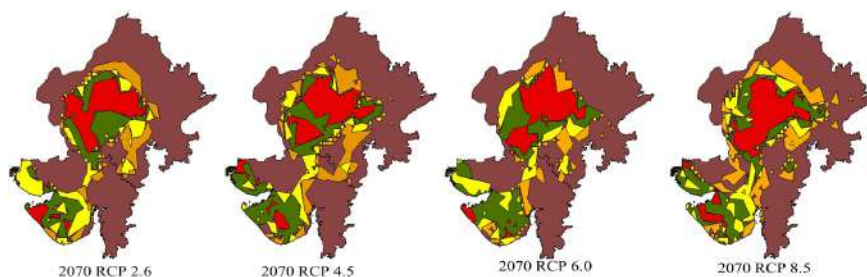


Fig. 3: Habitat suitability of *C. cyminum* with current and 2070 time-frames its four RCPs

Table 2: Area (km²) under different habitat suitability classes calculated with different bio- climatic models. Total geographical area of Rajasthan (342239) and Gujarat (196244) is 538483 Km²

Bio-Climatic Models	Optimum	Moderate	Marginal	Low	Per cent of optimum cumin area with reference to total geographical area
WorldClim Current	72827.01	97553.31	108433.9	91999.91	13.52
2050 RCP 2.6	58283.43	76823.04	72802.89	54599.65	10.82
2050RCP 4.5	77621.71	104171.7	65469.76	47075.38	14.41
2050 RCP 6.0	55911.12	102284.6	76407.01	77864.04	10.38
2050 RCP 8.5	89623.33	86964.15	51211.94	83803.57	16.64
2070RCP2.6	74349.43	77433.97	69356.89	53908.02	13.81
2070 RCP4.5	74145.89	94801.41	48913.03	63967.24	13.77
2070 RCP6.0	74500	94360.5	60744.76	45558.55	13.84
2070 RCP 8.5	84050.36	89353.67	59695.58	78311.14	15.61
GFDL45(60)	173676	109573.2	87584.69	145716.1	32.25
GFDL45(80)	178782.5	97022.43	69942	185083.4	33.20
GFDL85(60)	134068.9	173401.4	76822.86	130838	24.90
GFDL85(80)	170104.7	153232.6	79503.79	94690.18	31.59
HADGSM 45(60)	155075.8	144953.3	71598.04	119254.3	28.80
HADGSM 45(80)	141438.4	123043.7	83335.18	130652.2	26.27
HADGSM 85(60)	176950.4	133939.3	85391.74	73551.69	32.86
HADGSM 85(80)	198980.2	76626	97975.61	121631.6	36.95
IPSL45(60)	184419.9	102888.2	95343.54	103708	34.25
IPSL45(80)	203160	106134.1	90498.76	100448.1	37.73
IPSL85(60)	160706.6	146080.3	95123.81	95123.81	29.84
IPSL85(80)	219438.6	97785.15	97856.31	121072.6	40.75
MIRCOS45(60)	246962.1	186496.4	112656.9	56140.29	45.86
MIRCOS45(80)	197463.4	108102.4	89920.51	115849.4	36.67
MIRCOS85(60)	183633.3	109833.8	87886.21	111462.4	34.10
MIRCOS85(80)	164490.5	110256.4	118375.8	143026.3	30.55
NORESM45(60)	217094.2	149136.3	114966.2	79519.77	40.32
NORESM45(80)	209111.3	148829.2	54959.87	106591.8	38.83
NORESM85(60)	155563.1	109752.6	125005.7	102631.7	28.89
NORESM85(80)	188765.7	141083	86779.29	56786.85	35.06

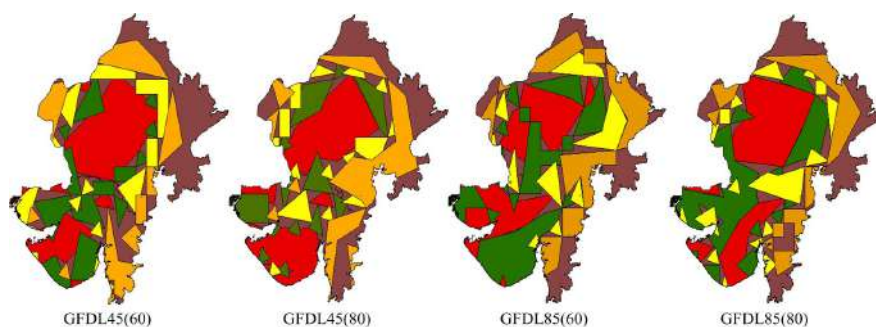
**Fig. 4:** Habitat suitability of *C. cyminum* with GFDL earth system model its four RCPs

Table 3: Two-way Analysis of Variance for area (km²) of cumin with different model number settings

ESMs models and their RCPs	ANOVA Variables	Area	
		Model	Habitat types
Both Model	F calculated	2.94*	26.54*
	F critical	1.62	2.71
CMCC-BioClimInd only	F calculated	0.29 ^{NS}	36.77*
	F critical	1.77	2.76
WorldClim only	F calculated	1.42 ^{NS}	6.5*
	F critical	2.35	3

*and Bold number represents the significant values at 5% level; NS = non-significant

Table 4: Two-way Analysis of Variance for area (km²) for examining the effect of EMS and RCP 4.5 and 8.5)

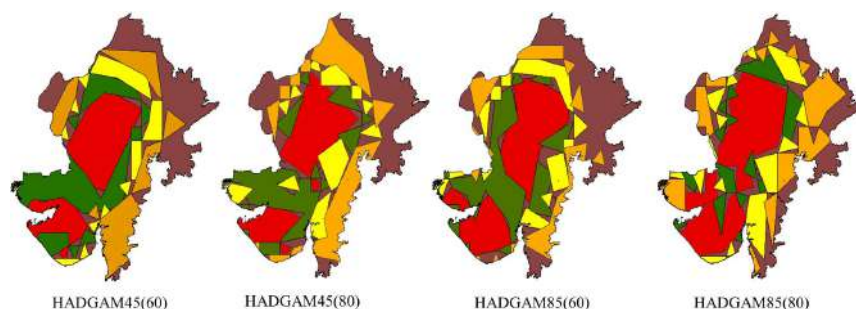
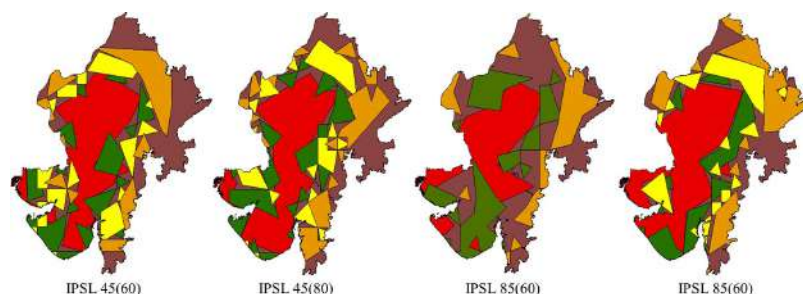
Habitat Suitability	Area (Calculated F values)	
	ESMs	RCP (4.5 and 8.5)
	F calculated	F calculated
Optimum	9.9*	5.05*
Moderate	2.39 ^{NS}	4.77 ^{NS}
Marginal	1.79 ^{NS}	1.0 ^{NS}
Low	3*	3.54 ^{NS}

F critical for EMS = 2.81 and 4.84 for RCP; *and Bold number represents the significant values at 5% level; NS = non-significant

$$\text{Area of villages or sites (ha)} = 515.4 + 0.026 \times \text{cumin average yield (Kg per ha)} \quad R^2 = 0.75^* \quad n = 225$$

Comparative results are depicted in Figs 9 to 15 pertaining to 2050 RCPs, 2070 RCPs, GFDL, HADGEM, IPSL, MIRCOS and NORSEM ESMs, respectively. With current scenario, maximum optimum areas come under Jodhpur, Pali, Ajmer, Jalor, Nagaur and Parts of Barmer districts of Rajasthan and Amreli, Junagadh and parts of Jamnagar and Bhavnagar (Fig. 9). With 2050 RCP 2.6, we found -19.57 per cent reduction in area and -15.71 in yield as compared to current. With this specific time frame and RCP, reduction was mostly observed at Jodhpur, Pali and Amreli and Junagadh areas. However, some new areas of optimum habitat are occurred at Kachchh and Jamnagar areas of Gujarat and Barmer and Jalor and Bikaner areas of Rajasthan (Fig. 9a). With RCP 4.5 of the same time-frame, 6.57 and 5.18 per cent increase in area and yield were recorded. Major gain was recorded at Kachchh, Jamnagar, Rajkot, Surendra Nagar of Gujarat and Barmer and Jalor areas of Rajasthan (Fig. 9b). Similar to RCP 2.6 of this time-frame, -23.23 and -18.27 per cent reduction were recorded in area and yield, respectively, with RCP 6.0. Specifically, with this time frame, highest area and yield gain +23.06 and +18.14, respectively were recorded with RCP 8.5 (Fig. 9d) and new areas were majorly occurred at Rajkot, Jamnagar, Surendranagar of Gujarat state and Bikaner, Jaisalmer and Jalor districts of Rajasthan.

Opposite to 2050, current study revealed the gain in area and yield with all four RCPs belongs to 2070 time-frame (Fig. 10 a

**Fig. 5:** Habitat suitability of *C. cyminum* with HADGAM earth system model its four RCPs**Fig. 6:** Habitat suitability of *C. cyminum* with IPSL earth system model its four RCPs

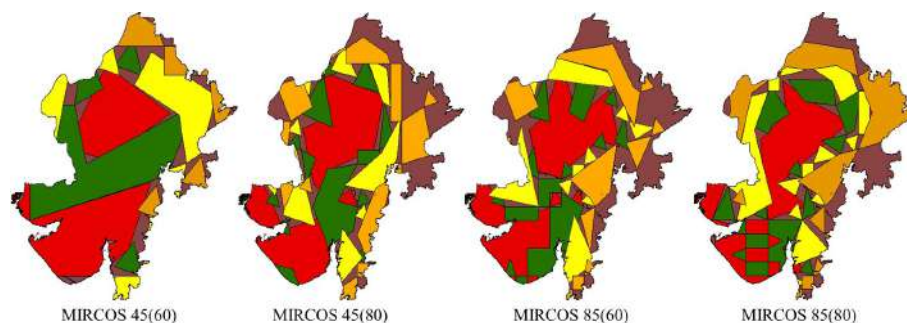


Fig. 7: Habitat suitability of *C. cyminum* with MIRCOS earth system model its four RCPs

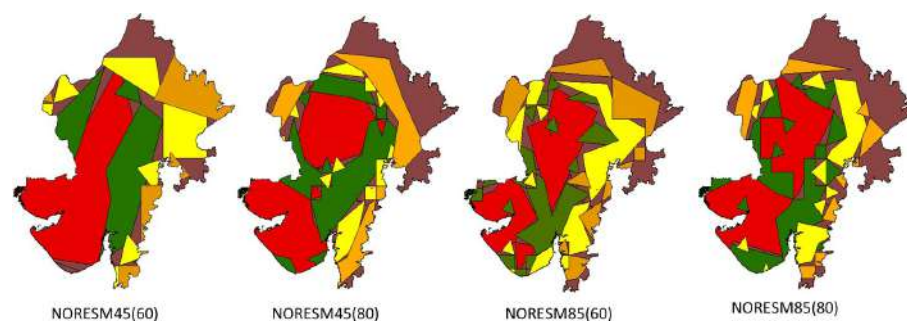


Fig. 8: Habitat suitability of *C. cyminum* with NORSEM earth system model its four RCPs

to d) and the maximum + 11.29 and + 8.86 gain in area and yield, respectively was recorded with RCP 8.5, and such gains were highlighted at Jamnagar, Rajkot, Kachchh and Banas Kantha districts of Gujarat and Barmer and Jalor district of Rajasthan. With GFDL-ESM, except 85 (60) more than twice the area gain was recorded (Fig. 11 a to d) and among them, maximum area +145.49 and excepted yield +114.45 were recorded with RCP 4.5 (Fig. 11b). Such new areas are covering most parts of the Gujarat covering entire Jamnagar, Rajkot, Amreli, Bhavnagar, parts of Surendranagar and Sabar Kantha, while at Rajasthan it covering most parts of Barmer, some parts of Bikaner, Nagaur, Ajmer and Pali districts. Similar higher gain +173.22, +136.27 in area and yield, respectively was recorded with HADGEM 85 (80 Fig. 12d). However, compared to GFDL 45 (80), additional extension of optimum habitat was recorded at Kachchh, Banas Kantha areas of Gujarat and Bikaner, Jaisalmer, Sirohi and Rajsamand districts of Rajasthan. With IPSL 85(80) +201.31 % increase in area and +158.37 % increase in yield (Fig. 13d) were recorded and such area increase were observed both at Gujarat (Jamnagar, Rajkot, Kachchh, Surendranagar, Patan, Banas Kantha and Gandhinagar) and Rajasthan (entire Barmer and Jalor, Pali and Jodhpur district and some parts of Jaisalmer and Sirohi). Among all studied ESM, 239.11% area gain and 188.10% gain in yield were recorded with MIRCOS45(60) covering entire areas of Jamnagar, Junagadh, Amreli, Bhavnagar, Rajkot, Surendranagar, Anand, Gandhinagar, Sagar Kantha, Kheda, and some parts of Patan and Bharuch

in Gujarat state (Fig. 14a) and similarly areas of Jodhpur, and parts of Barmer, Nagaur, Pali, Ajmer and Jaisalmer. Similarly, results of NORSEM ESM are depicted in Fig. 15 a to d.

ANOVA analysis suggested significant variabilities in areas brought by use of combination of both model types only, while their individual use were non-significant for areas. In comparison to this, impacts of habitat types with different setting of model numbers were statistically significant with all variables (Table 3). Similar statistical analysis was also carried out to examine the impact of ESMs and two RCPs namely 4.5 and 8.5 on area under different habitat suitability classes. ANOVA result revealed the statistically significant result for area under optimum habitat with both types of climatic data-sets and their RCPs, while area under moderate and marginal habitats these predictors were non-significant (Table 4).

Discussion

Climate-based geographic crop modeling is essential for predicting the best cultivation sites and yield. This step helps sustain food production (Alsafadi *et al.*, 2023). This study examined how climatic projections affect cumin's current and future geographical distribution in India's arid and semi-arid regions. This study compares four greenhouse gas scenarios (RCP 2.6, 4.5, 6.0, and 8.5) using the WorldClim data set for two future time frames and five ESMs, CMIP5: GFDL-ESM2M, HadGEM2-ES, IPSL-CM5A-LR, MIROC-ESM-CHEM, and NorESM1-M, from the recently

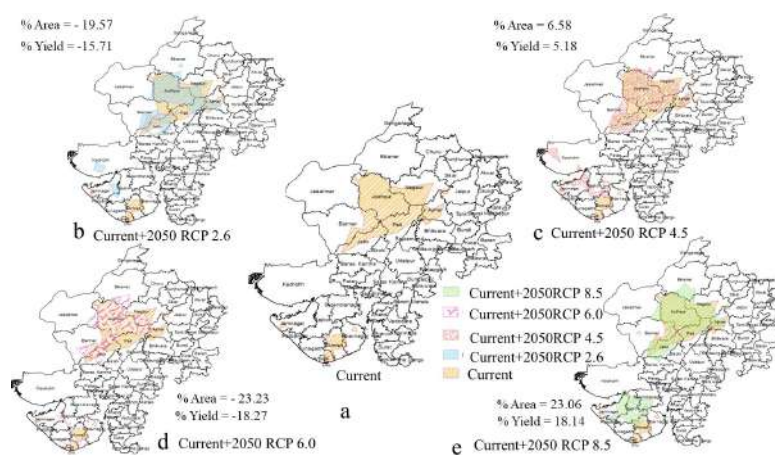


Fig. 9: Superimposition of current optimum suitability sites with different RCPs of 2050 time-frames

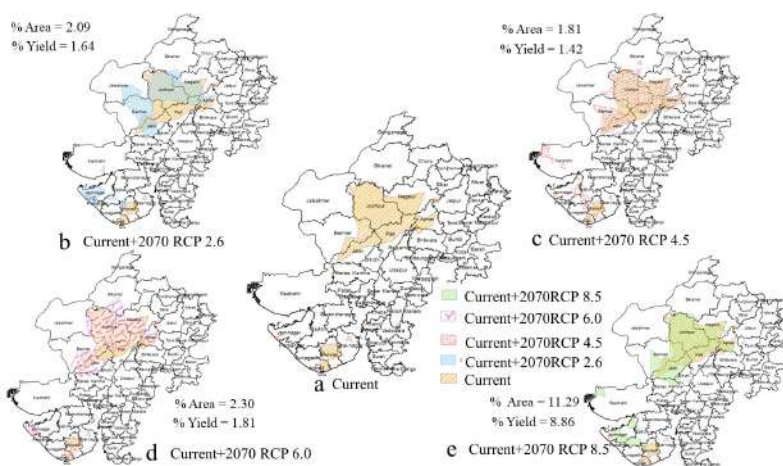


Fig. 10: Superimposition of current optimum suitability sites with different RCPs of 2070 time-frames

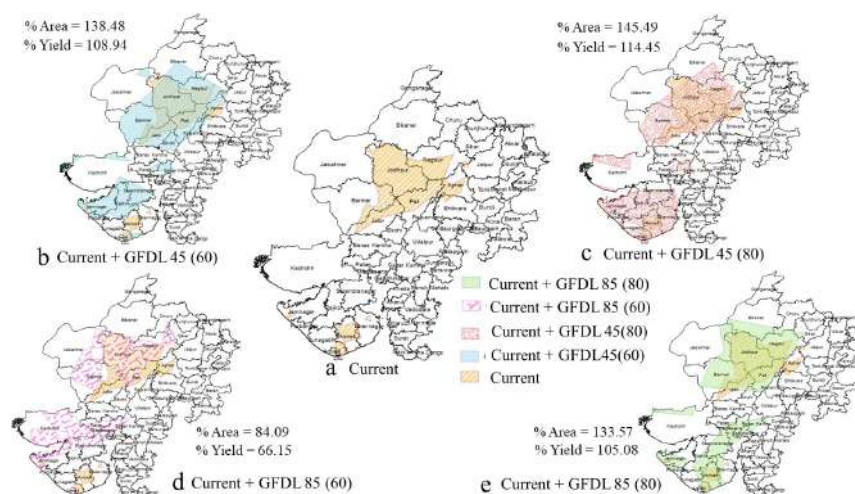


Fig. 11: Superimposition of current optimum suitability sites with GFDL ESM

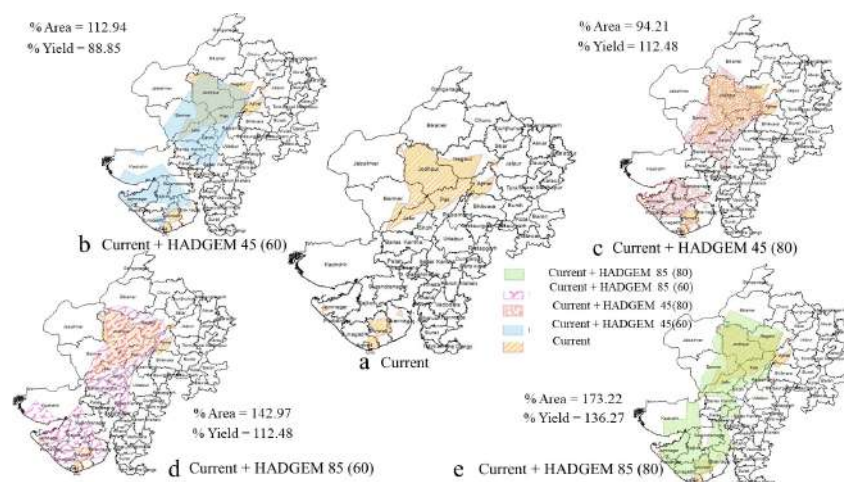


Fig. 12: Superimposition of current optimum suitability sites with HADGEM ESM

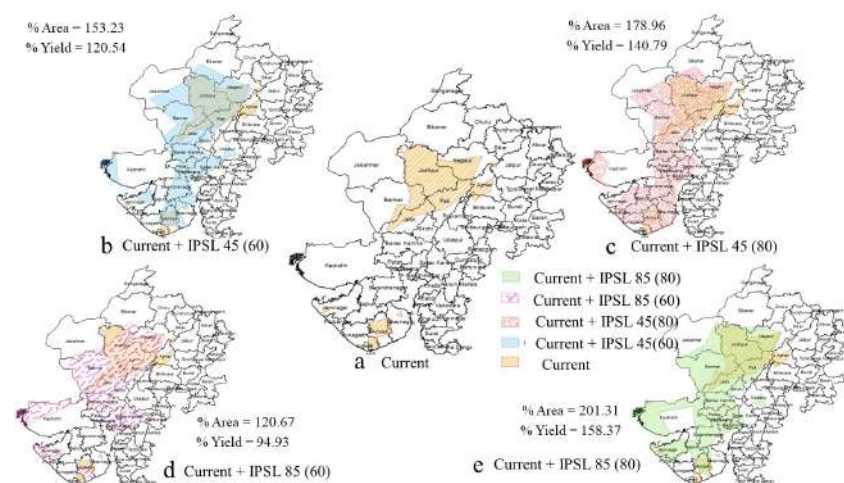


Fig. 13: Superimposition of current optimum suitability sites with IPSL ESM

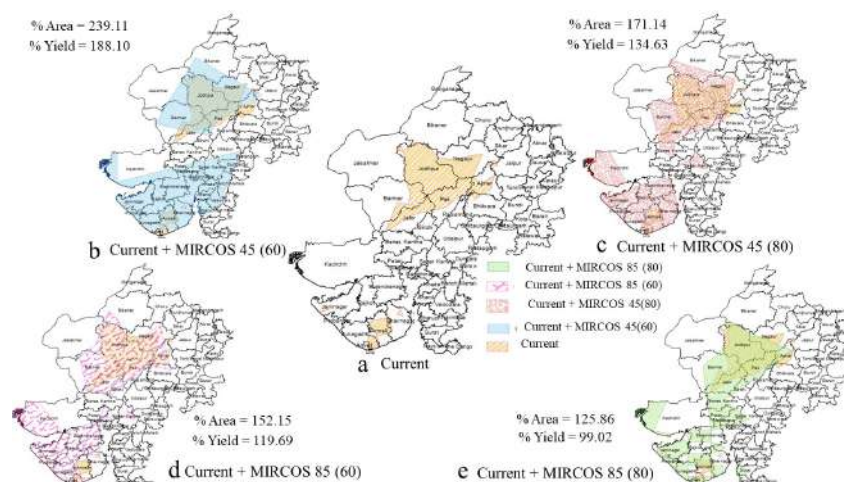


Fig. 14: Superimposition of current optimum suitability sites with MIRCOS ESM

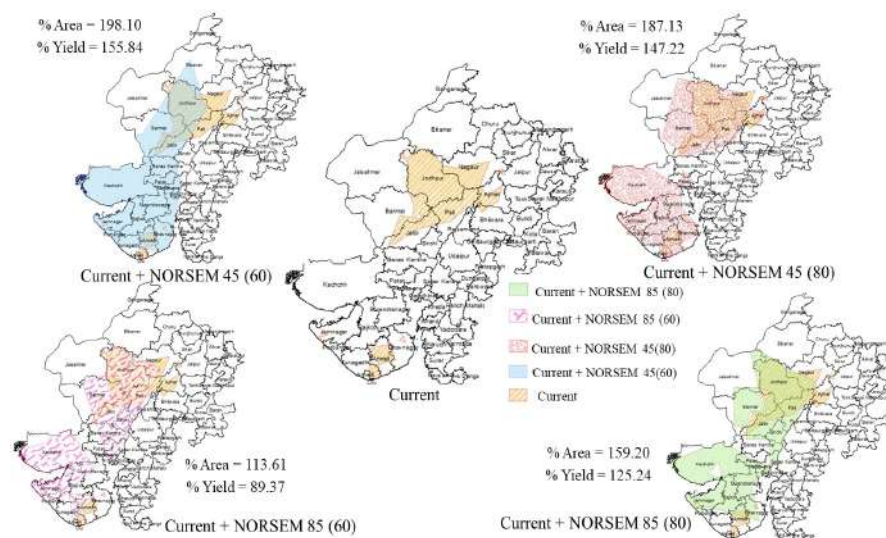


Fig. 15: Superimposition of current optimum suitability sites with NORSEM ESM

developed BioClimInd dataset for two RCPs, 4.5 and 8.5, of two-time frames, 2040-79 (60) and 2060-99 (80). Kogo *et al.* (2019a) used HAdGEM2-ES and CCM4 GCM models with RCP 4.5 and 8.5 to compare the current and future maize distribution in Kenya. CCSM4 showed minor differences. Zhang *et al.* (2017), Xu *et al.* (2021) examined how different climatic models affect maize in China, eastern Africa, and respectively. Kogo *et al.* (2019b) examined how climate change affects maize growth and productivity across regions. They found several effective general circulation models (GCMs): ECHAM5, CCSM, HadCM3, CSIRO-MK3, CGCM3.1, UKLO, and MIROC3.2. They also recommended multi-model ensemble GCMs for crop modeling to improve accuracy and reliability. In contrast, Alsafadi *et al.* (2023) used three models from the Coupled Model Intercomparison Project Phase 6 (CMIP6): BCC-CS2-MR (Beijing), CanESM5 (Canadian model), and IPSL-CM6A_LR (France) to simulate the effects of predicted climate change on wheat cultivation in Syria's semi-arid southwest. Their study found that the Beijing model was more accurate than the others, especially for wheat production in the examined area. Koo *et al.* (2017) recommend climatic multi-models with diverse temporal frameworks to reduce climate pattern ambiguity. Liu *et al.* (2023) used five GCMs and three CO₂ levels for *Calligonum mongolicum*, an arid shrub. GCMs and emission scenarios introduce uncertainty, which they discuss.

The dataset WorldClim 2.1 is widely cited in academic literature. This dataset contains 19 bio-climatic variables with varying resolutions. These variables cover recent climate conditions from 1970 to 2000 and projected future climate scenarios for 2021-2040, 2041-2060, 2061-2080, and 2081-2100. WorldClim uses 9 Generative Circulation and Earth System Models to project future climate. They are part of the Coupled Model Intercomparison Project Phase 6 (CMIP6),

which compares and evaluates climate models globally. Note that WorldClim has limitations. A major drawback is the lack of spatial details needed by models to accurately assess climate change impacts. This limitation limits the dataset's ability to predict and account for climate patterns and processes' spatial heterogeneity (Zhang *et al.*, 2023a). When considering spatial heterogeneity, a medium-resolution grid cell can encompass a climate environment with hundreds of meters of elevation difference (Poggio *et al.*, 2017). The complex process of plant growth requires considerations beyond precipitation and temperature, which may limit the 19 bioclimate variables' ability to simulate plant and organism distribution (Mathur and Mathur, 2023). As shown, evaporation increases in some regions despite heavy precipitation.

The CMCC-BioClimInd dataset improves community-valued spatial information accessibility in two ways. First, it includes 35 historical and future bioclimatic indicators. Second, it uses models and other analytical methods to ensure climate change impact assessments are reliable and accurate, taking into account uncertainties.

In this study, under most predictors and according to the interpretation criterion, our omission lines are well matched or above with projected omission, indicating no sampling autocorrelation and no sampling bias in our model attributes. Perhaps this is because we cleaned the spatial locations of 387 cumin cultivation sites across the study area using a spatial auto-correlation filter before performing our SDM on a subset of 300 of these sites.

Receiver operating characteristic (ROC) test is threshold-independent and does not require thresholds for presence versus absence predictions (Peterson *et al.*, 2007). The area under the curve (AUC) of ROC measures a model's ability to distinguish between species-rich and species-poor sites

(Elith *et al.*, 2006). The machine learning method excels in this study (AUC > 0.94; Mean 0.97 ± 0.01) for all studied predictors. Chao-Yun *et al.* (2012) reported similar SDM results for Asian Piper nigrum spice. Zhang *et al.* (2023b) used the AUC tool to compare the WorldClim and CMCC-BioClimInd datasets efficacy. The researcher classified their study using numerical bio-climatic variables. These variables included 19 WorldClim variables, CMCC-BioClimInd (1–19 bio-climatic predictors), CMCC-BioClimInd (1–35 bio-climatic predictors), and CMCC. 11 global invasive species were studied using these classifications, yielding average AUC values of 0.948, 0.951, 0.963, and 0.969. They found that the simplified continentality index (the divergence between the mean values of the most hot and cold months across a given span of years) and modified Kira warmth index (energy inputs in the warmest portion of the year), derived from CMCC-BioClimInd, were effective explanatory tools.

Response curves have helped quantify the logistic probability of species presence and environmental factors. This has greatly improved our understanding of the species' complex ecological niche (Mathur *et al.*, 2023). This study found that WorldClim dataset annual precipitation (BC-12), seasonal precipitation (BC-15), and coldest quarter precipitation (BC-19) are key factors in cumin species habitat suitability. This analysis included the present, 2050-, and 2070-time frames and four RCP scenarios. Precipitation seasonality had the greatest impact on cumin species habitat suitability.

Precipitation seasonality measures and analyzes monthly precipitation variability over a year. The index is the ratio of monthly aggregate precipitation standard deviation to mean precipitation (Noce *et al.*, 2020). In fragile ecological systems like arid and semi-arid regions, crop productivity depends on precipitation timing and quantity during the growth cycle. This study stressed the importance of precipitation during the growth cycle. Numerous researchers emphasize the importance of precipitation in rainfed wheat cultivation (Aixia, 2022; Zhang, 2022). Murugan *et al.* (2022) examined how precipitation seasonality affects cardamom distribution in southern India under RCP 4.5 and 8.5. Their findings show that a significant change in precipitation seasonality negatively affects spice cultivation, maturation, and productivity. Buckland *et al.* (2022) reported similar effects of precipitation seasonality on *Garcinia indica* distribution in India, *Dalbergia latifolia* in Nepal, and *Opuntia ficus-indica* and *Euphorbia tirucalli* worldwide.

Response curves for three bio-climatic variables help us identify cumin's optimal growing conditions. These areas have a precipitation seasonality threshold of 150 mm (BC-15), an annual precipitation range of 450 to 550 mm (BC-12), and 5 to 8 mm of rainfall during the coldest quarter (BC-19). Precipitation seasonality was also the most influential factor for NORSEM, HADGAM [45(60), 45 (80),

85(60)], and MIRCOS [(85(60) and 85(80)], whose response curves were identical to WorldClim but had lower peak occurrence probabilities. GFDL, MIRCOS, NORESM, and HADGEM 45 (80) and 85 (80) also showed that potential evapotranspiration Hargreaves affected species occurrence probabilities. The temperature-based Hargreaves equation calculates potential evapotranspiration (Hargreaves *et al.*, 1985). Mean monthly maximum temperature - mean monthly minimum temperature for the month of interest and mean air temperature were included.

The cumin plant's physiological processes and secondary metabolite production are affected by many environmental stresses. These responses help the plant develop tolerance and survive harsh conditions (Pandey *et al.*, 2015). The physiological responses of *C. cyminum* to water stress include the synthesis of proline, soluble sugars, and essential oils (Kazemi *et al.*, 2018). Water-related variables in cumin cultivation have been extensively studied. Under experimental control conditions (Mehriya *et al.*, 2020), irrigation water and crop evapotranspiration (IW/Etc) increased plant height, branch count, and photosynthetic rate. Cumin seed yield increased significantly due to these improvements. Cumin crop yield attributes and increase are due to optimal precipitation and soil moisture levels. These conditions promote efficient nutrient uptake and a good soil-water air relationship with higher root zone oxygen (Mathur, 2013). The root zone's optimal moisture levels boost plant physiological activity and dry matter accumulation. The correlation between a consistent precipitation pattern throughout the year and cumin development during the colder season is linked to many complex physiological mechanisms. These mechanisms reduce leaching, increase photosynthetic rates, and help plants absorb essential nutrients. Due to these factors, cumin seed yield increases. Water-related climatic variables boost root development and soil moisture extraction. This phenomenon may promote abundant vegetative growth in water-rich conditions (Mathur and Sundaramoorthy, 2013). Based on the data, water-related factors rather than temperature-related factors determine the likelihood of this important crop in the studied reg

MaxENT modelling has revealed the spatial distribution of *C. cyminum* habitat suitability classes. Using the WorldClim dataset, the optimal class had greater spatial coverage under the RCP 8.5 scenario compared to other RCP scenarios and across future time frames. *C. cyminum* may benefit from the "CO₂ fertilization effect" and increase crop production (e.g., wheat; Alsafadi *et al.*, 2023) under high CO₂ conditions.

Our comparison of optimum habitat spatial extension and yield provided useful crop information. Currently, Jodhpur, Pali, Ajmer, Jalor, Nagaur, and parts of Barmer in Rajasthan and Amreli, Junagadh, and parts of Jamnagar and Bhavnagar are the best areas. These findings are supported

by Singh and Kumar (2016); Sharma *et al.* (2018); Pagaria and Sharma (2020).

With comparative analysis, areas and yield fall under gain (+) or loss (-) with different RCPs and datasets could also be marked. Area and yield only decreased with 2050 RCP 2.6 and 6.0. MIRCOS45(60) again recorded maximum area and yield in Jamnagar, Junagadh, Amreli, Bhavnagar, Rajkot, Surendranagar, Anand, Gandhinagar, Sagar Kantha, Kheda, and some parts of Patan and Bharuch in Gujarat, and most of western Rajasthan, including Jodhpur, Barmer, Nagaur, Pali, and Jaisalmer.

ANOVA analysis of areas affected by models (both ESMs and WorldClim, ESMs only, and WorldClim only) and their RCPs and habitat types (optimum, moderate, marginal, and low) showed significant variabilities in areas caused by both model types, but not by their individual use. In contrast, habitat types with different model numbers affected all variables statistically (Table 3). The effects of ESMs and RCPs 4.5 and 8.5 on habitat suitability class area were also examined. ANOVA showed that ESMs and RCPs were significant for optimum habitat but not moderate or marginal habitats (Table 4).

This species cannot be grown in Rajasthan's irrigated northwest plain (Sriganganagar and Hanumangarh), sub-humid southern plain (Bhilwara, Udaipur, and Chittorgarh), humid southern plain (Dungarpur, Banswara, and Chittorgarh), or humid southeastern plain. The agro-climatic zonation of Gujarat shows which areas are best for cumin cultivation. Salve *et al.* (2017) reported rising cumin area and yield in Banaskantha district, Gujarat.

WorldClim and CMCC-BioClimInd models had excellent model accuracy, but their optimum areas were statistically different. Two-way ANOVA analysis is used in previous studies to identify the major cause of area uncertainty. Real *et al.* (2010) suggested that GCMs, not CO₂ emission scenarios, cause climate change modeling uncertainty. In their modeling, Hamit *et al.* (2018), and Zhu (2019) found that RCPs or SSPs caused such uncertainty, while GCMs/ESMs were ignored. Thus, climate change studies must assess the relative contributions of ESMs and RCPs/SSPs. Liu *et al.*, (2023) used two-way ANOVA to examine the significance of 5 GCMs and 3 SSPs for range dynamics of an arid shrub *Calligonum mongolicum*. Their analysis showed that GCMs had a significant impact on gain area, loss area, and total area, indicating that selecting these GCMs is uncertain for all aspects of range change. SSPs scenarios only affected gain and loss areas, not total area. It shows that SSP selection did not affect total area simulation but did affect gain and loss area estimation.

The current, 2050, and 2070 WorldClim data sets showed that Rajasthan had more optimally suitable habitats than Gujarat. Under current climate, a single continuous larger area was observed in these datasets, but it is expected to

break up into several small populations across both states (Figs 2 and 3). The aforementioned analysis found fewer unsuitable regions for growing this crop using alternative Earth System Models (ESMs) than the WorldClim dataset. Two-way ANOVA analysis showed that using both model types (WorldClim + five EMS of CMCC-BioClimInd) together caused statistically significant area differences, while using either model type alone did not. However, different habitat types had statistically significant effects under different model numbers. Statistics were also used to examine how ESMs and two RCPs, 4.5 and 8.5, affected habitat suitability classes. In an ANOVA, both types of climatic data-sets and their RCPs showed statistically significant results for the area under optimum habitat, but not moderate or marginal habitats (Table 4).

Conclusion

With this research, we simulated the habitat suitability and estimated climate thresholds for *C. cyminum* (cumin) crop in arid and semi-arid parts of the India. We also projected changes in the suitable habitat (particularly optimum) under various climate change model and emission scenarios. The obtained climatically suitable habitat map and habitat change maps could be guiding us to introduce this species in new areas of Rajasthan and Gujarat. The obtained climate thresholds possess the potential to be utilized by policymakers in deducing the appropriateness of the habitat for cumin, thereby fortifying the management of said regions.

Supplementary Materials: Table 1 and figs 1 to 30

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Competing interests

The authors declare that they have no competing interests

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The data that support the findings of this study specifically geo-coordinates of the species are available on request from the corresponding author, [Preet Mathur]. The data are not publicly available due to avoid the duplication of the work within the same geographical area

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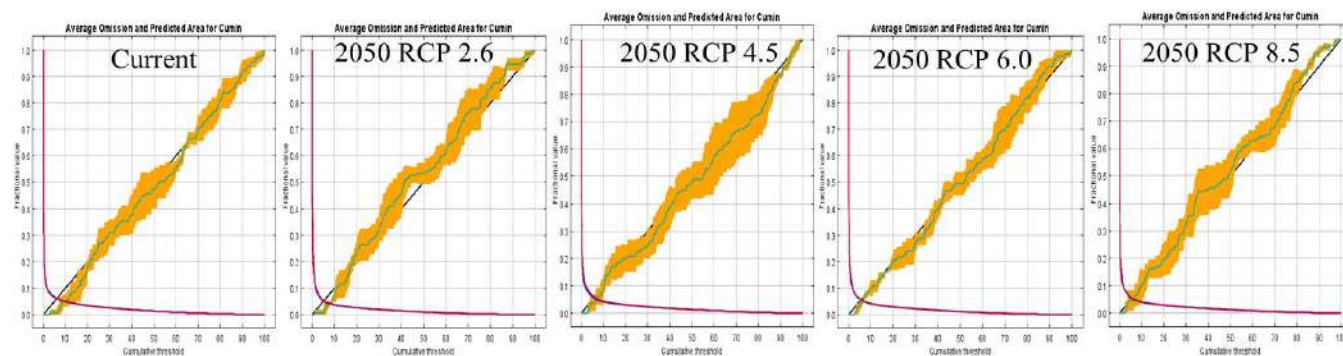
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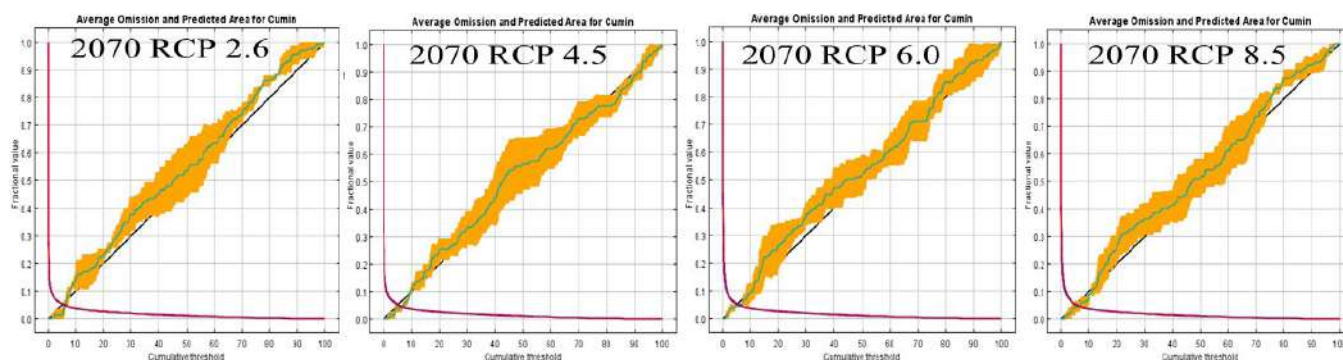
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Supplementary Table 1: Details of 35 bio-climatic variables opted from five earth system models and WorldClim dataset. (Details of each bio-climatic variables can be found in Noce, S., Caporaso, L. and Santini, M. 2020. A new global dataset of bioclimatic indicators. Scientific Data, 7: 398 <https://doi.org/10.1038/s41597-020-00726-5>)

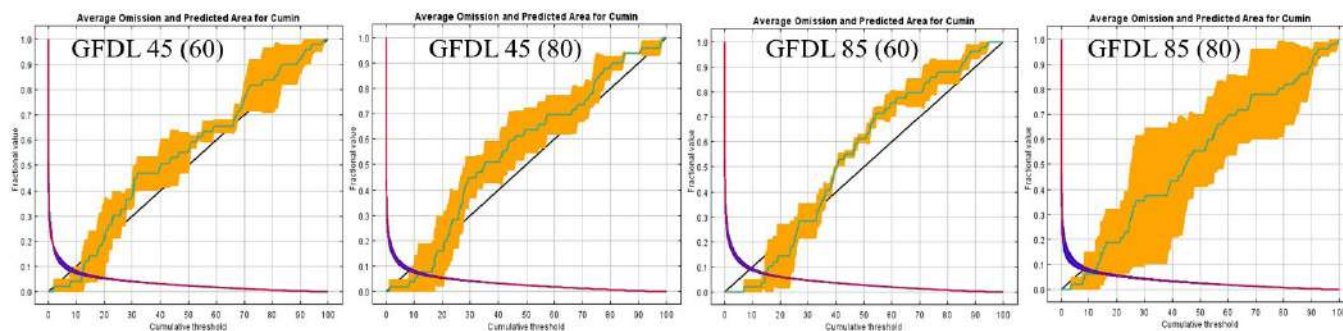
Code	Name	Unit
Bio1	Annual mean temperature	°C
Bio2	Mean diurnal range	°C
Bio3	Isothermality	%
Bio4	Temperature seasonality	°C
Bio5	Maximum temperature of warmest month	°C
Bio6	Minimum temperature of coldest month	°C
Bio7	Temperature annual range	°C
Bio8	Mean temperature of wettest quarter	°C
Bio9	Mean temperature of driest quarter	°C
Bio10	Mean temperature of warmest quarter	°C
Bio11	Mean temperature of coldest quarter	°C
Bio12	Annual precipitation	mm
Bio13	Precipitation of wettest month	mm
Bio14	Precipitation of driest month	mm
Bio15	Precipitation seasonality	%
Bio16	Precipitation of wettest quarter	mm
Bio17	Precipitation of driest quarter	mm
Bio18	Precipitation of warmest quarter	mm
Bio19	Precipitation of coldest quarter	mm
Bio20	Ellenberg quotient	°C/mm
Bio21	Yearly positive temperature	°C
Bio22	Sum of annual temperature	°C
Bio23	Ombrothermic index	mm/°C
Bio24	Yearly positive precipitation	mm
Bio25	Modified Kira coldness index	°C
Bio26	Modified Kira warmth index	°C
Bio27	Simplified continentality index	°C
Bio28	Mean temperature of warmest month	°C
Bio29	Mean temperature of coldest month	°C
Bio30	Mean temperature of driest month	°C
Bio31	Mean temperature of wettest month	°C
Bio32	Modified Thermicity index	°C
Bio33	Ombrothermic index of summer and the previous month	mm/°C
Bio34	Potential Evapotranspiration Hargreaves	mm
Bio35	Potential Evapotranspiration Thornthwaite	mm



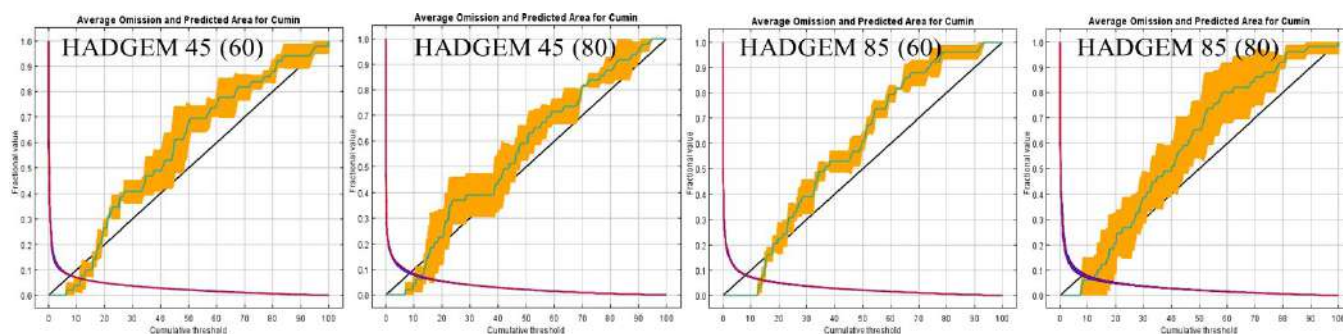
Supp. Fig. 1: Analysis of omission/commission with current and 2050 bio-climatic timeframe and its RCPs projection



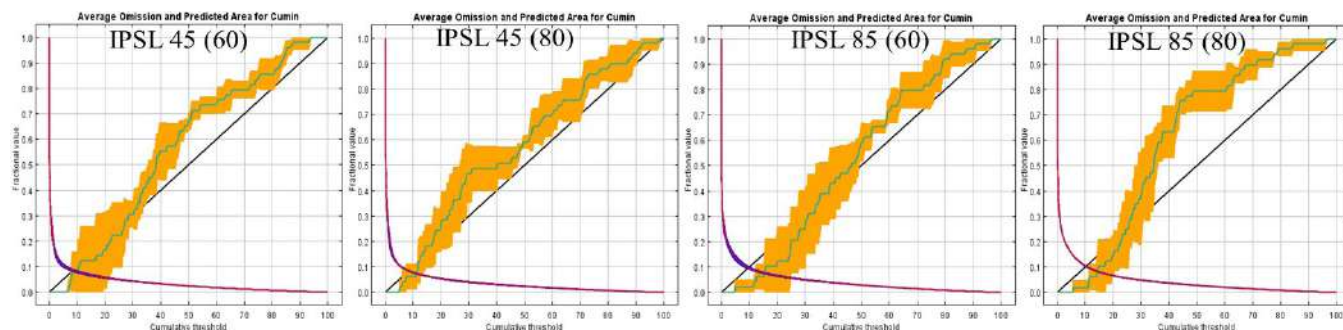
Supp. Fig. 2: Analysis of omission/commission with current and 2070 bio-climatic timeframe and its RCPs projection



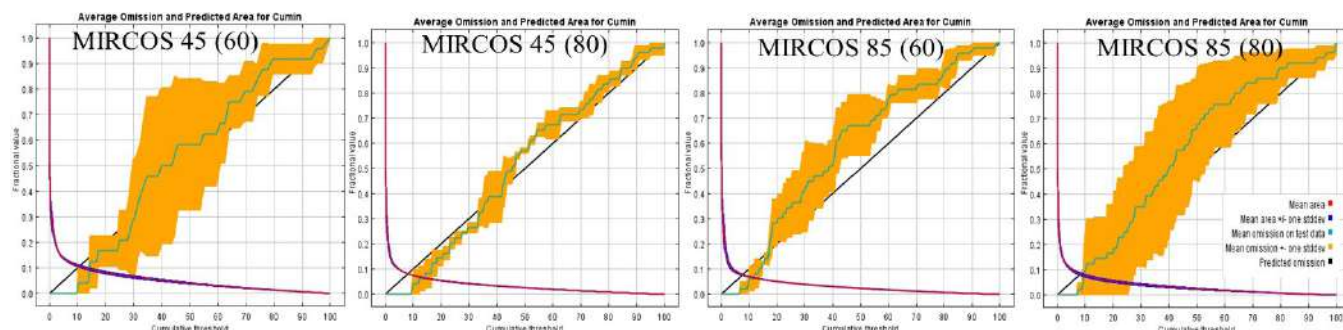
Supp. Fig. 3: Analysis of omission/commission with GFDL earth system model with two time frames and its projections



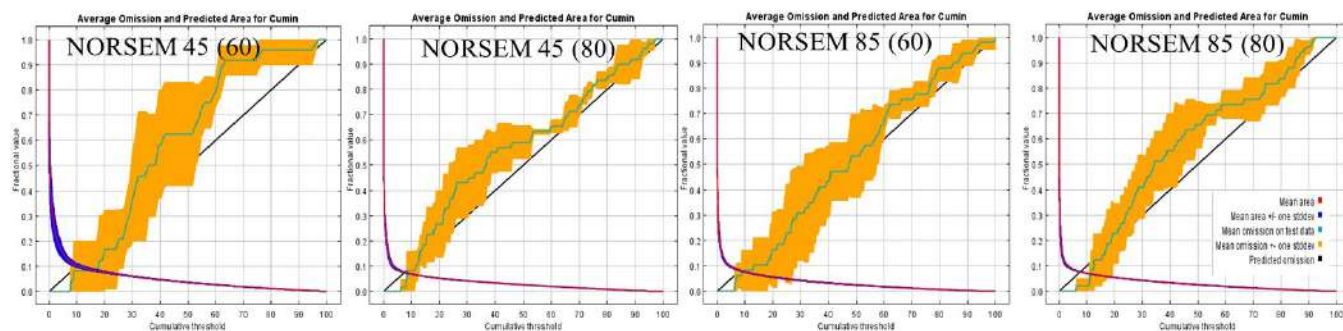
Supp. Fig. 4: Analysis of omission/commission with HADGEM earth system model with two time-frames and its projections



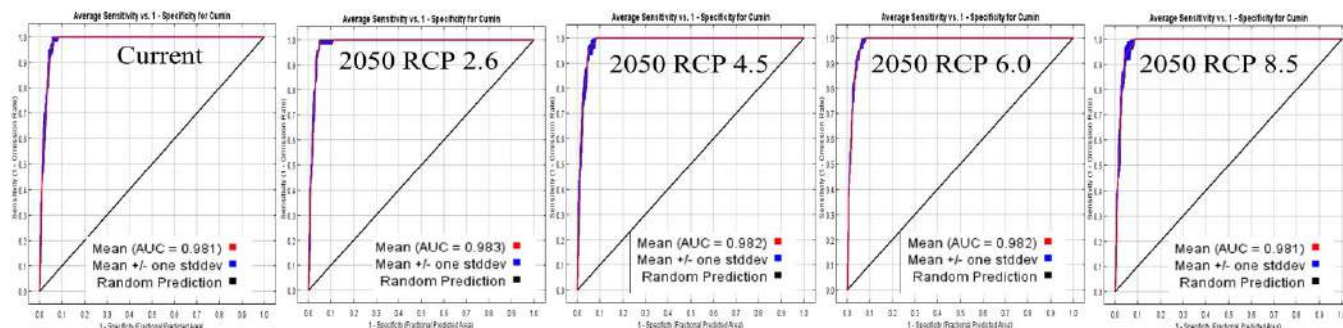
Supp. Fig. 5: Analysis of omission/commission with IPSL earth system model with two time-frames and its projections



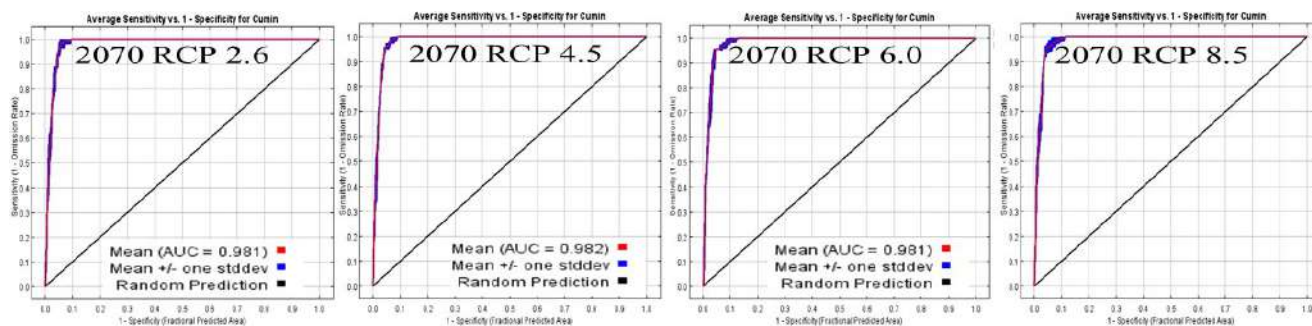
Supp. Fig. 6: Analysis of omission/commission with MIRCOS earth system model with two time-frames and its projections



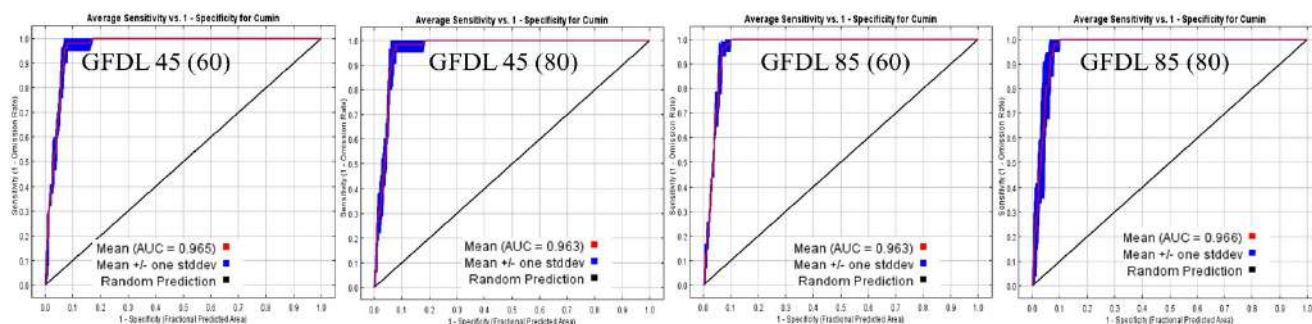
Supp. Fig. 7: Analysis of omission/commission with NORSEM earth system model with two time-frames and its projections



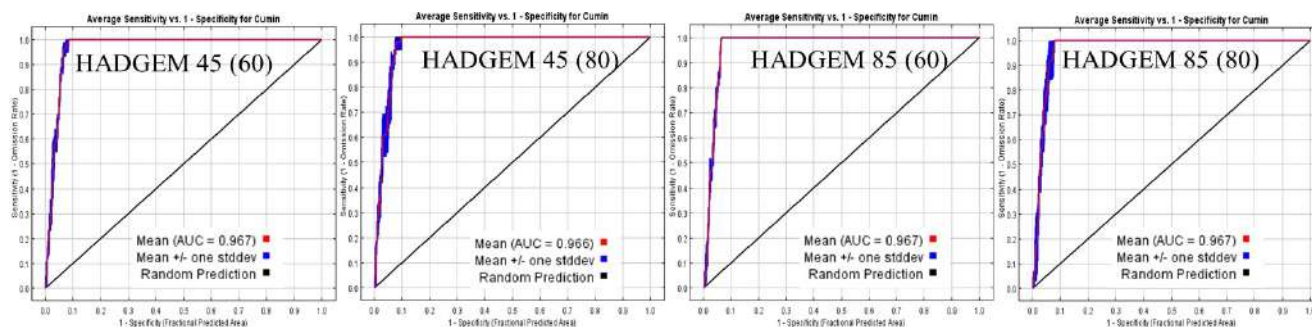
Supp. Fig. 8: Receiver Operating Characteristic (ROC) curves: with current and 2050 bio-climatic timeframes and RCPs projection



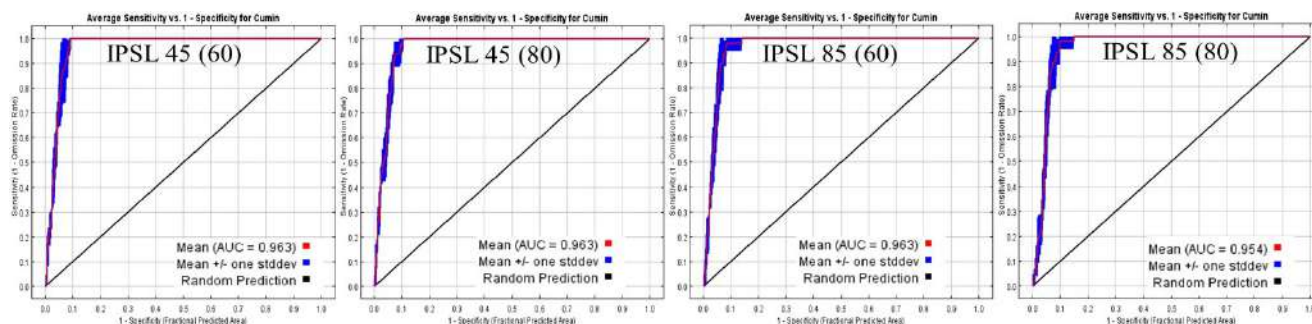
Supp. Fig. 9: Receiver Operating Characteristic (ROC) curves: with 2070 bio-climatic timeframes and RCPs projection



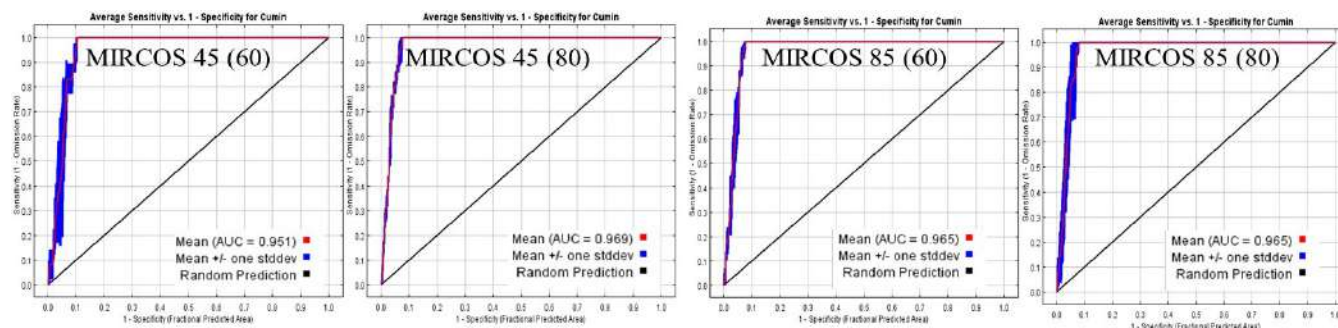
Supp. Fig. 10: Receiver Operating Characteristic (ROC) curves: with GFDL earth system model using two time frames



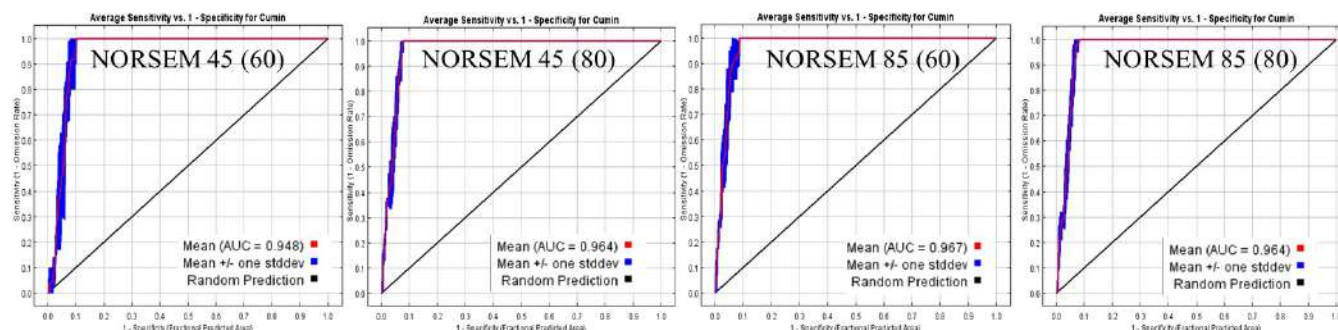
Supp. Fig. 11: Receiver Operating Characteristic (ROC) curves: with HADGEM earth system model using two time frames



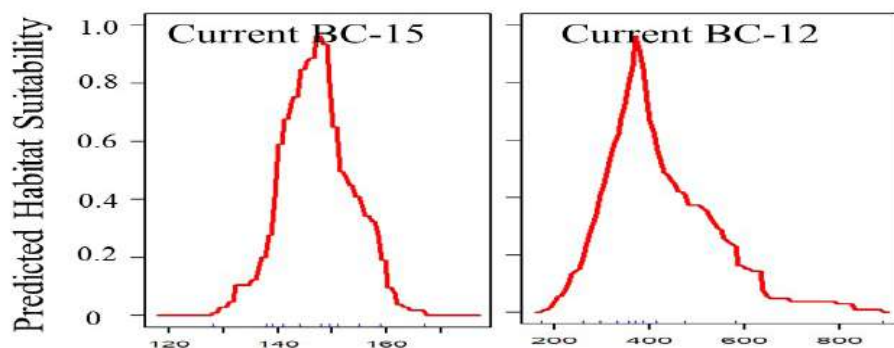
Supp. Fig. 12: Receiver Operating Characteristic (ROC) curves: with IPSL earth system model using two time frames



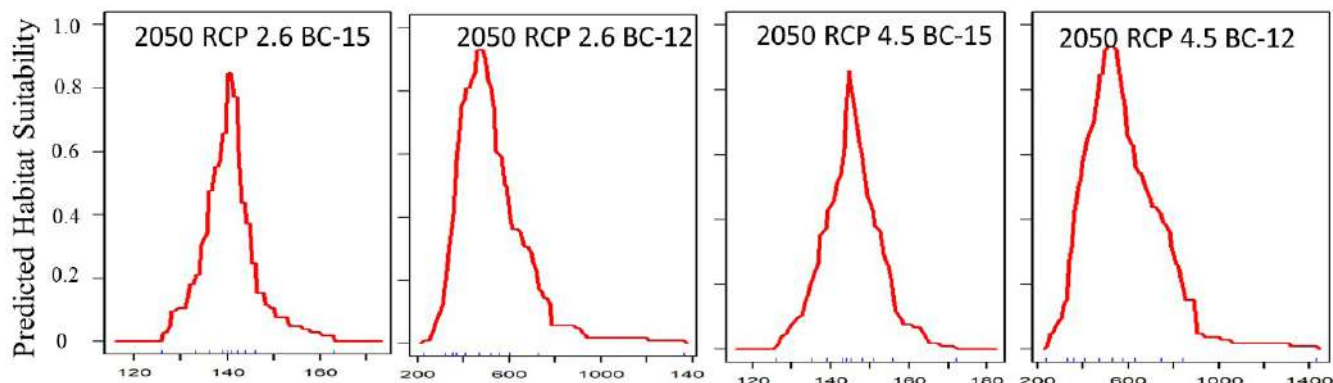
Supp. Fig. 13: Receiver Operating Characteristic (ROC) curves: with MIRCOS earth system model using two time frames



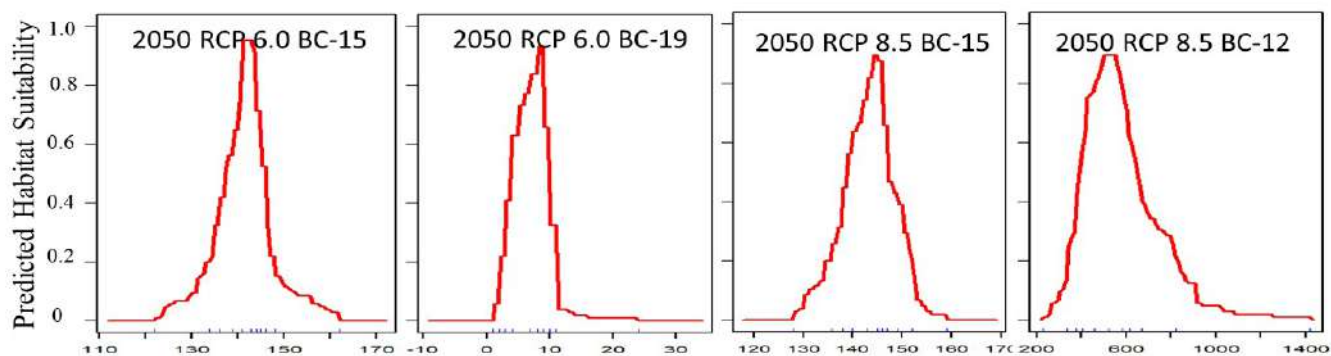
Supp. Fig. 14: Receiver Operating Characteristic (ROC) curves: with NORSEM earth system model using two time frames



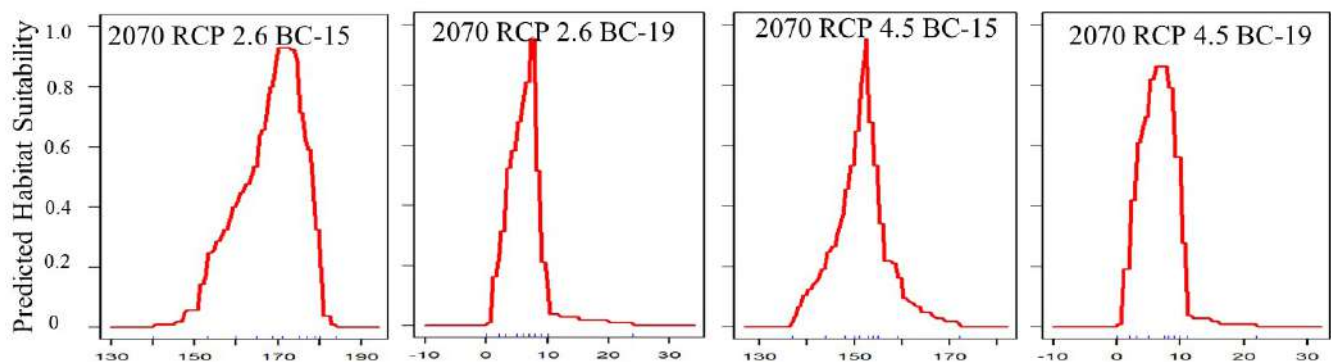
Supp. Fig. 15: Response curves with current-climatic parameters illustrating the likelihood of *Cuminum cyminum* habitat suitability



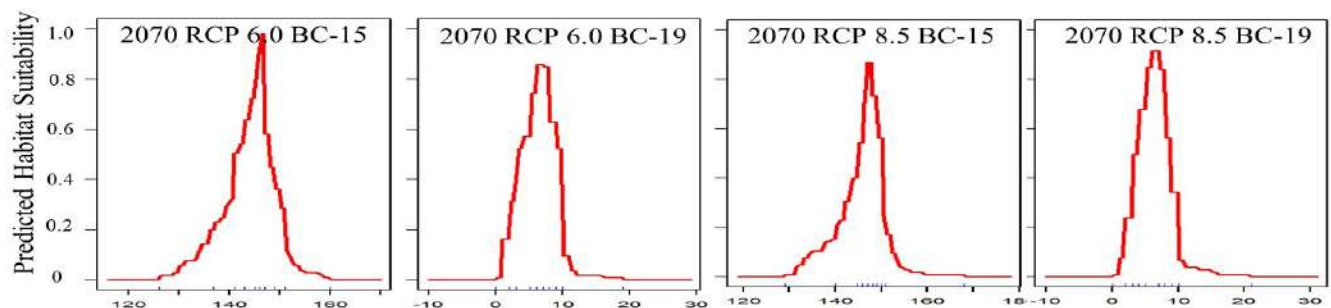
Supp. Fig. 16: Response curves with RCP 2.6 and 4.5 of 2050 bio-climatic time-frame illustrating the likelihood of *Cuminum cyminum* habitat suitability



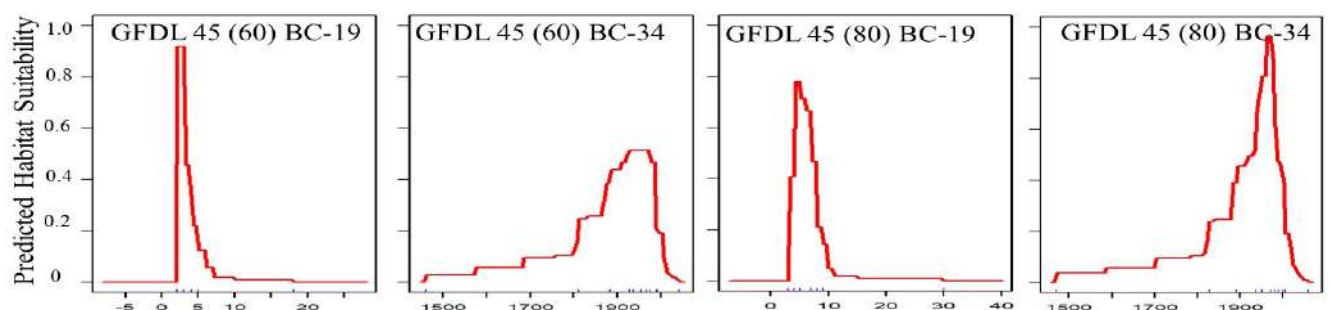
Supp. Fig. 17: Response curves with RCP 6.0 and 8.5 of 2050 bio-climatic time-frame illustrating the likelihood of *Cuminum cyminum* habitat suitability.



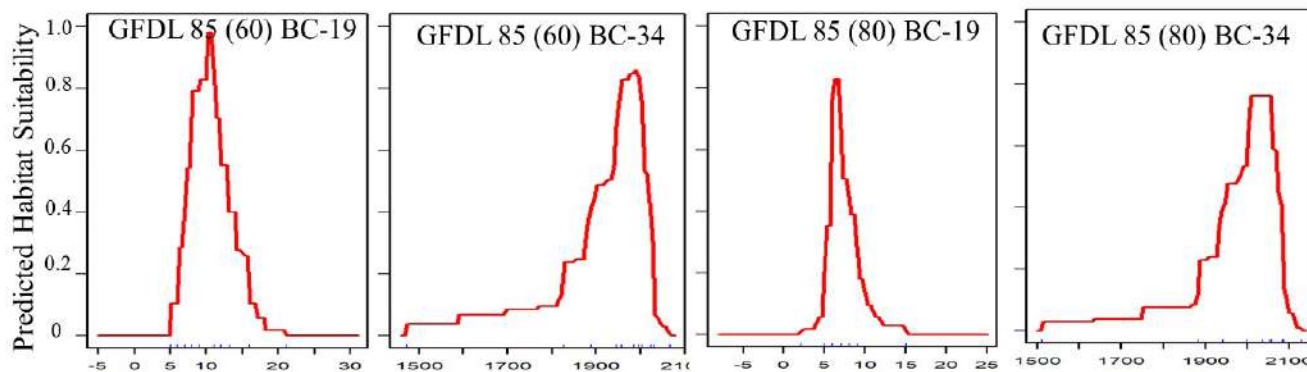
Supp. Fig. 18: Response curves with RCP 2.6 and 4.5 of 2070 bio-climatic time-frame illustrating the likelihood of *Cuminum cyminum* habitat suitability.



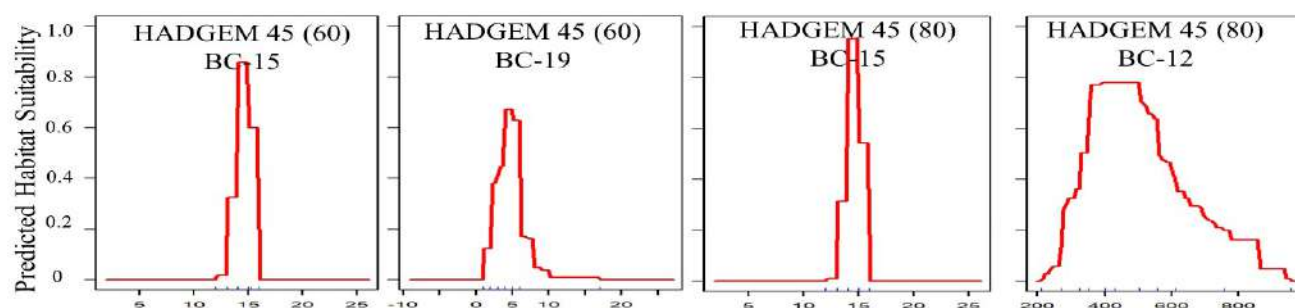
Supp. Fig. 19: Response curves with RCP 6.0 and 8.5 of 2070 bio-climatic time-frame illustrating the likelihood of *Cuminum cyminum* habitat suitability.



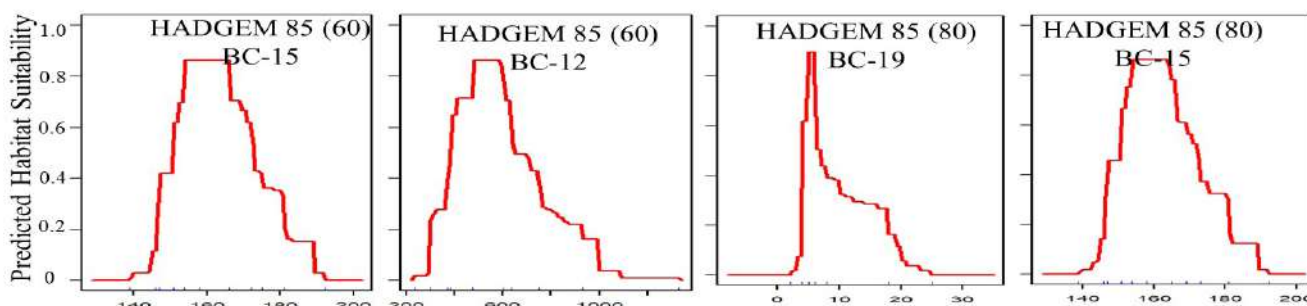
Supp. Fig. 20: Response curves with GFDL earth system model with 45 scenario and two time-frame illustrating the likelihood of *Cuminum cyminum* habitat suitability.



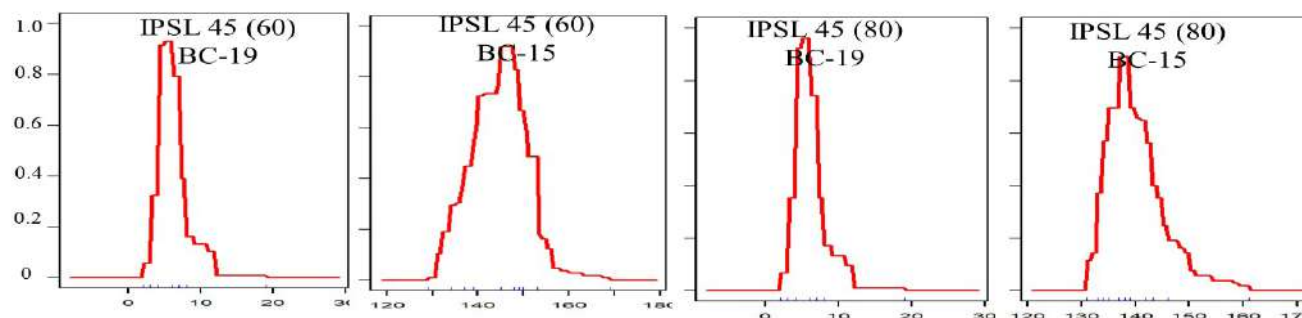
Supp. Fig. 21: Response curves with GFDL earth system model with 85 scenario and two time-frame illustrating the likelihood of *Cuminum cyminum* habitat suitability



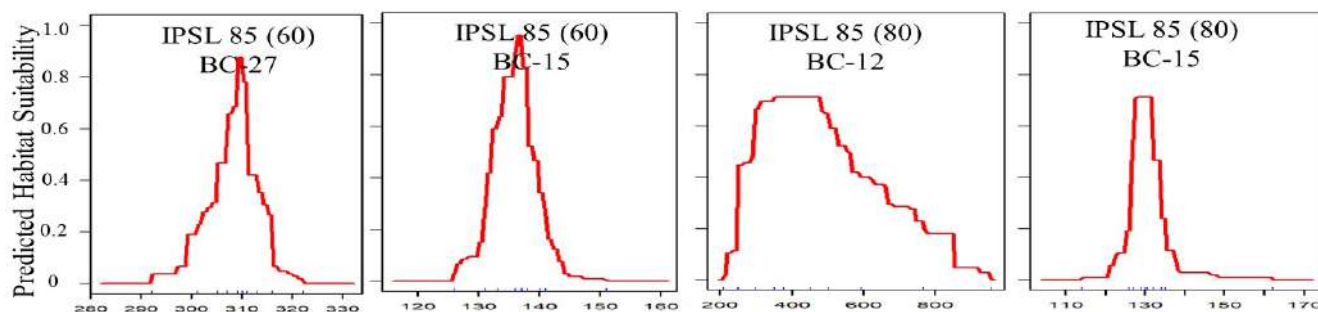
Supp. Fig. 22: Response curves with HADGEM earth system model with 45 scenario and two time-frame illustrating the likelihood of *Cuminum cyminum* habitat suitability



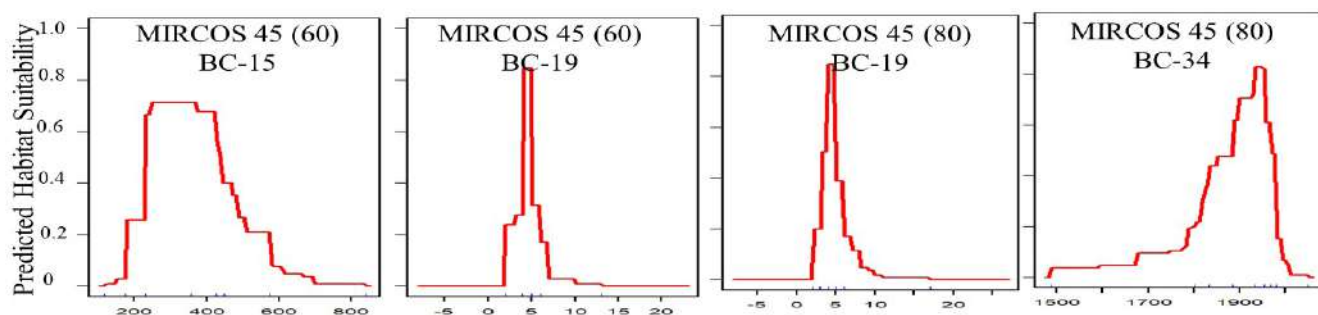
Supp. Fig. 23: Response curves with HADGEM earth system model with 85 scenario and two time-frame illustrating the likelihood of *Cuminum cyminum* habitat suitability



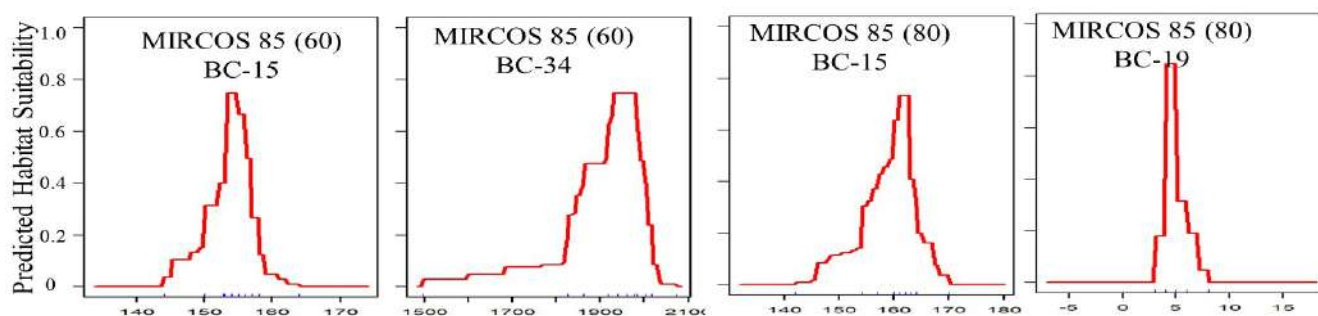
Supp. Fig. 24: Response curves with IPSL earth system model with 45 scenario and two time-frame illustrating the likelihood of *Cuminum cyminum* habitat suitability



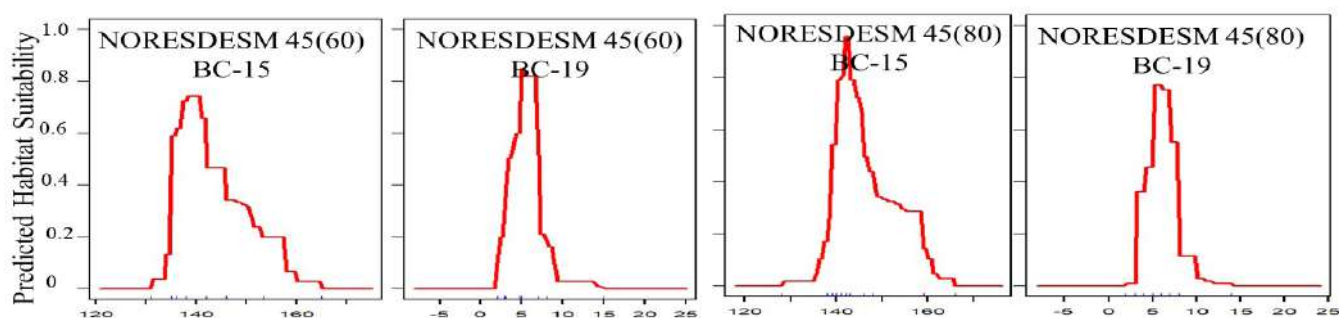
Supp. Fig. 25: Response curves with IPSL earth system model with 85 scenario and two time-frame illustrating the likelihood of *Cuminum cyminum* habitat suitability



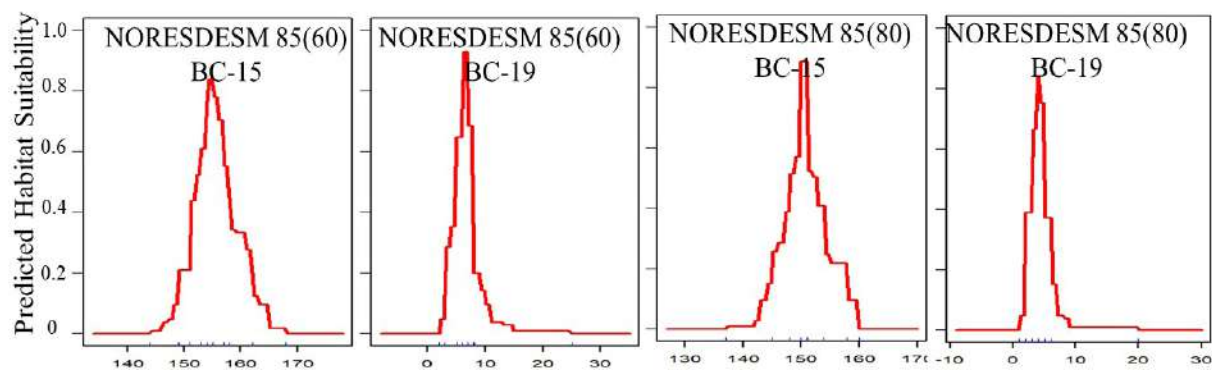
Supp. Fig. 26: Response curves with MIRCOS earth system model with 45 scenario and two time-frame illustrating the likelihood of *Cuminum cyminum* habitat suitability



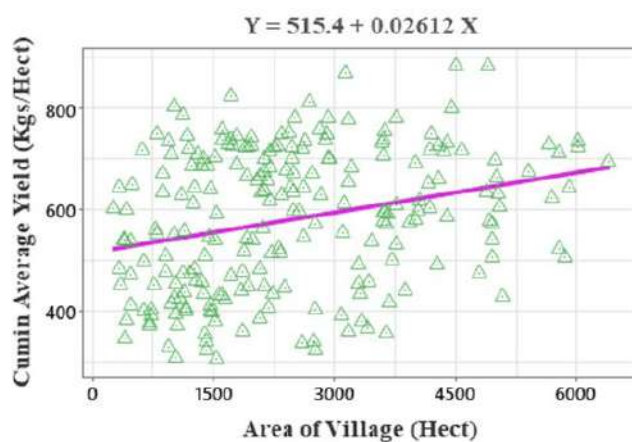
Supp. Fig. 27: Response curves with MIRCOS earth system model with 85 scenario and two time-frame illustrating the likelihood of *Cuminum cyminum* habitat suitability



Supp. Fig. 28: Response curves with NORESDESM earth system model with 45 scenario and two time-frame illustrating the likelihood of *Cuminum cyminum* habitat suitability



Supp. Fig. 29: Response curves with NORESDESM earth system model with 85 scenario and two time-frame illustrating the likelihood of *Cuminum cyminum* habitat suitability



Supplementary Fig. 30: Simple regression analysis between area of village (Ha.) and *Cuminum cyminum* average yield (Kg/ha.) $n = 225$; $R^2 = 0.75^*$

Plant Germplasm Registration Notice¹

The Plant Germplasm Registration Committee (PGRC) of ICAR held its XXXXVIIth meeting on March 29, 2022 in virtual mode. 128 proposals were received and examined at ICAR-NBPGR out of which 90 proposals complete in all respects and reviewed by experts were considered for registration. 63 proposals with unique/novel features belonging to 26 species were finally recommended for registration. The information on registered germplasm is published with the purpose to disseminate the information to respective crop breeders for utilization of these genetic stocks in their crop improvement programmes.

1. SBTIL121 (IC643999; INGR22001), a rice (*Oryza sativa*) germplasm for *sd1* gene

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Crosses were attempted using marker- assisted backcross breeding (MABB) approach to introgress two BB resistance genes (*xa13*, *Xa21*) and one dwarfing gene (*sd1*) from Punjab Basmati 4 (PAU148) into Ranbir Basmati. Genotype namely SBTIL121 identified as “ideal” genotypes, which can be recommended for release and exploited in a resistance breeding program for the region confronting bacterial blight disease alongwith lodging resistance.

Morpho-agronomic characteristics

This genotype is possessing *xa13*, *Xa21* and *sd1* bacterial blight and lodging resistant genes and good yield potential (40-45 qt/ha). The genotype SBTIL121 is semi-tall type (130-135 cm) and early maturing having 90-95 days for flowering and 125-130 days for maturity. The genotype has 35-40 number of effective tillers alongwith 1000-grain weight of 23 gm.

Associated characters and cultivated practices

The genotype also has good grain quality characteristics of basmati rice. The genotype is having grain length of 7.97 mm with KLAC value of 13.98. It can be grown in on soils suitable for rice cultivation; however, it gives best performance in

basmati rice growing areas following under geographical indication (GI). The field should be well prepared for nursery by two to three ploughings and transplanting should be done at 3-4 leaves stage in well ploughed and puddled soils. Apply entire quantity of DAP with 1/3rd of N from Urea at the time of puddling. Fertilizers should be applied @ 30:20:10 NPK kg/ha. Remaining quantity of nitrogen through urea was applied in two equal splits - at tillering stage (30 DAT) and before panicle initiation stage (60 DAT). Apply Butachlor granules 5 G @ 30 kg/ha. Within 24 hours of transplanting. Do not drain the water from the field for one week after application of granules. The seed treatment should be done with fungicide like Thirum/Captan/Carbendazim @ 2.0 g/kg seed before nursery sowing. The optimum nursery sowing time is 1st week of June to 15th of June and transplanting is 1st week of July to 15th of July. The seed rate for nursery sowing is 20-25 kg/ha. If available, about 20 tonnes of organic manures should be incorporated in the soil at the time of land preparation. This genotype has good worth as a donor in future field rice breeding programme towards development of basmati rice varieties with good level of resistance against bacterial blight and in other basic studies.

2. DCMS 9A & DCMS 9B (IC640658 & IC640659; INGR22002), wheat (*Triticum aestivum*) cytoplasmic genetic male sterile line in DBW 17 background with CMS source Chuan 13A alongwith maintainer (B) line

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The proposed genetic stock DCMS 9A was developed using CHUAN 13A based CMS 5A (CHUAN13A/CHUAN13B/4/7*KAUZ/PFAU//VEE#5/3/KAUZ) as female parent in first cross with Indian advanced variety DBW 17 as male parent. DBW 17

was a landmark variety for north western plains zone as well as north eastern plains zone for timely sown irrigated condition. After initial cross, 8 generations of backcrosses were made with DBW 17 as recurrent parent in order to

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recover its agronomic background. In this process, more than 100 spikes were pollinated. In every generation, five spikes of recipient population were bagged just after emergence from the flag leaf in order to ensure complete male sterility as indicated by no seed set.

After eight backcross generations, the resultant male sterile line was planted in the field along with recurrent parent as maintainer line. The newly developed CMS lines were pollinated with maintainer lines for getting seeds for next generation. For the purpose, the CMS line was planted along with maintainer line in 2B: 4A: 2B row ratio and all the recommended agronomic practices were adopted to raise a good crop during four consecutive crop seasons of 2015-16, 2016-17, 2017-18 and 2018-19. Five randomly selected spikes of these CMS lines were bagged at just after emergence from flag leaf and the seed set was observed in these un-pollinated spikes for calculating seed set percentage. The data were also recorded on days to heading and plant height for evaluating their suitability to hybrid wheat programme. The seed set was observed among these lines and the results indicated no seed set in the bagged un-pollinated spikes of the CMS lines. This indicated complete male sterility in the new CMS line DCMS 9A (Table 1).

In hybrid development programme based on three-line system, the maintenance of CMS lines is very crucial. The similarity in days to heading and plant height in CMS and maintainer lines is needed to facilitate pollination and get maximum seed set in the CMS line. The heading period in DCMS 9A ranged from 92-104 days with mean of 97 days whereas average plant height was 89 cm with range of 85-95 cm. Compared to this, the maintainer line DCMS 9B showed similar days to heading and plant height which is helpful in synchronised flowering and pollen movement for maximum seed set.

In addition, few additional agro-morphological traits namely coleoptile colour, growth habit, foliage colour, awn colour, spikelet number per spike, spike length, spike colour,

Table 1: Performance of DCMS 9A and its maintainer for quantitative traits

Year	DCMS 9A (CMS- A line)			DCMS 9B (DBW 17: B line)	
	Male sterility(%)	Days to heading	Plant height(cm)	Days to heading	Plant height (cm)
2015-16	100	97	85	97	92
2016-17	100	93	85	93	86
2017-18	100	92	91	94	88
2018-19	100	104	95	102	91
Mean	100	97	89	97	89

Table 2: Performance of DCMS 9A and its maintainer DCMS 9B for morphological traits

S. No.	Traits	DCMS 9A	DCMS 9B (DBW 17)
1	Coleoptile Colour	Absent	Absent
2	Growth habit	Semi erect	Semi erect
3	Foliage Colour	Green	Green
4	Spike colour	white	white
5	Spike shape	Tapering	Tapering
6	Awn colour	White	White
7	Grain colour	Amber	Amber
8	Spike length (cm)	10	9
9	Spikelet number	20	20
10	Days to maturity	147	139

spike shape and grain colour were observed over these years which showed similar pattern to the parental maintainer line DBW 17 (Table 2). The results also indicated more maturity duration in DCMS 9A which is indicative of more grain filling duration leading to better seed development.

It may be concluded that the proposed genetic stock DCMS 9A may be used intensively for development of hybrid wheat for Indian conditions so that new breakthrough may be achieved in wheat productivity.

3. DCMS 44A & DCMS 44B (IC640660 & IC640661; INGR22003), wheat (*Triticum sativum*) cytoplasmic genetic male sterile line in CBW 38 background with CMS source MTSA 2A

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The proposed genetic stock DCMS 44A was developed using MTSA 2A based CMS 2A (MTSA 2A/8*RAYON) as female parent in first cross with Indian advanced variety CBW 38 as male parent. CBW 38 was released variety for north eastern plains zone for timely sown irrigated condition. After initial cross, 8 generations of backcrosses were made with CBW 38 as recurrent parent in order to recover its agronomic background. In this process, more than 100 spikes were pollinated. In every

generation, five spikes of recipient population were bagged just after emergence from the flag leaf in order to ensure complete male sterility as indicated by no seed set.

After eight backcross generations, the resultant male sterile line was planted in the field along with recurrent parent as maintainer line. The newly developed CMS lines were pollinated with maintainer lines for getting seeds for next generation. For the purpose, the CMS line was planted

along with maintainer line in 2B: 4A: 2B row ratio and all the recommended agronomic practices were adopted to raise a good crop during four consecutive crop seasons of 2015-16, 2016-17, 2017-18 and 2018-19. Five randomly selected spikes of these CMS lines were bagged at just after emergence from flag leaf and the seed set was observed in these un-pollinated spikes for calculating seed set percentage. The data were also recorded on days to heading and plant height for evaluating their suitability to hybrid wheat programme (Table 1). The seed set was observed among these lines and the results indicated no seed set in the bagged un-pollinated spikes of the CMS lines. This indicated complete male sterility in the new CMS line DCMS 44A (Table 2).

In hybrid development programme based on three-line system, the maintenance of CMS lines is very crucial. The similarity in days to heading and plant height in CMS and maintainer lines is needed to facilitate pollination and get maximum seed set in the CMS line. The heading period in DCMS 44A was ranged from 99-104 days with mean of 98 days whereas average plant height was 106 cm with range of 101-115 cm. Compared to this, the maintainer line DCMS 44B (CBW 38) showed average days to heading of 99 days and plant height of 110 cm which is in tune to CMS line. This similar heading is helpful in synchronised flowering whereas more height in maintainer line facilitates better pollen movement for maximum seed set.

It may be concluded that the proposed genetic stock DCMS 44A may be used intensively for development of hybrid wheat for Indian conditions so that new breakthrough may be achieved in wheat productivity.

Table 1: Performance of DCMS 44A and its maintainer DCMS 44B for agro-morphological traits

S. No.	Traits	DCMS 44A	DCMS 44B (CBW 38)
1	Coleoptile Colour	Absent	Absent
2	Growth habit	Semi erect	Semi erect
3	Foliage Colour	Green	Green
4	Spike colour	white	white
5	Spike shape	Tapering	Tapering
6	Awn colour	White	White
7	Grain colour	Amber	Amber
8	Spike length (cm)	10	9
9	Spikelet number	20	20
10	Days to maturity	150	148

Table 2: Performance of DCMS 44A and its maintainer for quantitative traits

Year	DCMS 44A (CMS- A line)			DCMS 44B (CBW 38: B line)	
	Male sterility (%)	Days to heading	Plant height (cm)	Days to heading	Plant height (cm)
2015-16	100	99	102	99	105
2016-17	100	94	101	94	107
2017-18	100	94	115	95	121
2018-19	100	104	104	106	108
Mean	100	98	106	99	110

4. DCMS 35A & DCMS 35B (IC640662 & IC640663; INGR22004), wheat (*Triticum sativum*) cytoplasmic genetic male sterile line in DBW 55 background with CMS source Chuan 13A

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The proposed genetic stock DCMS 35A was developed using CHUAN 13A based CMS 5A (CHUAN13A/CHUAN13B/4/7*KAUZ/PFAU//VEE#5/3/KAUZ) as female parent in first cross with Indian advanced variety DBW 55 as male parent. DBW 55 was a high yielding elite line evaluated in NIVT 1A meant for north western plains zone as well as north eastern plains zone under timely sown irrigated condition. Under timely sown irrigated condition. After initial cross, 8 generations of backcrosses were made with DBW 55 as recurrent parent in order to recover its agronomic background. In this process, more than 100 spikes were pollinated. In every generation, five spikes of recipient population were bagged just after emergence from the flag leaf in order to ensure complete male sterility as indicated by no seed set.

After eight backcross generations, the resultant male sterile line was planted in the field along with recurrent

parent as maintainer line. The newly developed CMS lines were pollinated with maintainer lines for getting seeds for next generation. For the purpose, the CMS line was planted along with maintainer line in 2B: 4A: 2B row ratio and all the recommended agronomic practices were adopted to raise a good crop during four consecutive crop seasons of 2015-16, 2016-17, 2017-18 and 2018-19. Five randomly selected spikes of these CMS lines were bagged at just after emergence from flag leaf and the seed set was observed in these un-pollinated spikes for calculating seed set percentage. The data were also recorded on days to heading and plant height for evaluating their suitability to hybrid wheat programme. The seed set was observed among these lines and the results indicated no seed set in the bagged un-pollinated spikes of the CMS lines. This indicated complete male sterility in the new CMS line DCMS 35A (Table 1).

In hybrid development programme based on three-line system, the maintenance of CMS lines is very crucial. The similarity in days to heading and plant height in CMS and maintainer lines is needed to facilitate pollination and get maximum seed set in the CMS line. The heading period in DCMS 35A was ranged from 99-104 days with mean of 98 days whereas average plant height was 96 cm with range of 90-104 cm. Compared to this, the maintainer line DCMS 35B (DBW 55) showed similar days to heading of 98 days with plant height of 98 cm which is in tune to CMS line. These features in DCMS 35A are helpful in synchronised flowering and pollen movement for maximum seed set.

In addition, few additional agro-morphological traits namely coleoptile colour, growth habit, foliage colour, awn colour, spike colour and shape and grain colour were observed over these years which showed similar pattern to the parental maintainer line DBW 55 (Table 2). The results also indicated comparable spikelet number per spike, spike length and maturity period in DCMS 35A as compared to the maintainer line DBW 55. The maturity days was more in the DCMS 35A which is beneficial to get good grain development in the CMS line as well as seed set while attempting hybrid combinations.

It may be concluded that the proposed genetic stock DCMS 35A may be used intensively for development of hybrid wheat for Indian conditions so that new breakthrough may be achieved in wheat productivity.

5. DCMS 52A & DCMS 52B (IC640664 & IC640665; INGR22005), a wheat (*Triticum aestivum*) cytoplasmic genetic male sterile line in UP 2338 background with CMS source MTSA 2A

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The proposed genetic stock DCMS 44A was developed using MTSA 2A based CMS 2A (MTSA 2A/8*RAYON) as female parent in first cross with Indian advanced variety UP 2338 as male parent. Variety UP 2338 was released for north western plains zone for timely sown as well as late sown irrigated condition and very popular due to its flexibility in sowing time. After initial cross, 8 generations of backcrosses were made with CBW 38 as recurrent parent in order to recover its agronomic background. The resultant male sterile line was planted in the field along with recurrent parent as maintainer line. The newly developed CMS lines were pollinated with maintainer lines for getting seeds for next generation. For the purpose, the CMS line was planted along with maintainer line in 2B: 4A: 2B row ratio and all the recommended agronomic practices were adopted to raise a good crop during four consecutive crop seasons of 2015-16, 2016-17, 2017-18 and 2018-19. Five randomly selected spikes of these CMS lines were bagged at just after emergence from flag

Table 1: Performance of DCMS 35A and its maintainer for quantitative traits

Year	DCMS 35A (CMS- A line)			DCMS 35B (DBW 55: B line)	
	Male sterility (%)	Days to heading	Plant height (cm)	Days to heading	Plant height (cm)
2015-16	100	99	94	99	98
2016-17	100	94	94	94	96
2017-18	100	95	90	97	100
2018-19	100	104	104	103	99
Mean	100	98	96	98	98

Table 2: Performance of DCMS 35A and its maintainer DCMS 35B for morphological traits

S. No.	Traits	DCMS 35A	DCMS 35B (DBW 55)
1	Coleoptile Colour	Absent	Absent
2	Growth habit	Semi erect	Semi erect
3	Foliage Colour	Green	Green
4	Spike colour	White	White
5	Spike shape	Tapering	Tapering
6	Awn colour	White	White
7	Grain colour	Amber	Amber
8	Spike length (cm)	12	11
9	Spikelet number	24	23
10	Days to maturity	148	146

leaf and the seed set was observed in these un-pollinated spikes for calculating seed set percentage. The seed set was observed among these lines and the results indicated no seed set in the bagged un-pollinated spikes of the CMS lines. This indicated complete male sterility in the new CMS line DCMS 52A (Table 1).

Table 1: Performance of DCMS 52A and its maintainer for quantitative traits

Year	DCMS 52A (CMS- A line)			DCMS 52B (UP 2338: B line)	
	Male sterility (%)	Days to heading	Plant height (cm)	Days to heading	Plant height (cm)
2015-16	100	99	97	99	104
2016-17	100	86	100	86	102
2017-18	100	96	114	99	111
2018-19	100	108	120	111	113
Mean	100	97	108	99	108

Table 2: Performance of DCMS 52A and its maintainer DCMS 52B for agro-morphological traits

S. No.	Traits	DCMS 52A	DCMS 52B (UP 2338)
1	Coleoptile Colour	Absent	Absent
2	Growth habit	Semi erect	Semi erect
3	Foliage Colour	Dark Green	Dark Green
4	Spike colour	White	White
5	Spike shape	Tapering	Tapering
6	Awn colour	White	White
7	Grain colour	Amber	Amber
8	Spike length (cm)	12	11
9	Spikelet number	20	22
10	Days to maturity	149	146

The heading period in DCMS 52A was ranged from 986-108 days with mean of 97 days whereas average plant

height was 108 cm with range of 97-120 cm. Compared to this, the maintainer line DCMS 52B (UP 2338) showed average days to heading of 99 days with similar plant height to CMS line.

In addition, few additional agro-morphological traits were observed over these years which showed similar pattern to the parental maintainer line UP 2338 (Table 2). The results indicated comparable spikelet number per spike, spike length and maturity period in DCMS 52A as compared to the maintainer line UP 2338. The maturity days was more in the DCMS 52A which is beneficial to get good grain development in the CMS line as well as seed set while attempting hybrid combinations.

It may be concluded that the proposed genetic stock DCMS 52A may be used intensively for development of hybrid wheat for Indian conditions so that new breakthrough may be achieved in wheat productivity.

6. DWAP 18-12 (IC640668; INGR22006), a wheat (*Triticum aestivum*) germplasm highly tolerant to water stress conditions of warmer areas; low SSI; low yield reduction under stress

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The germplasm line DWAP 1812 was developed from the cross DBW 87/5EGPSN 82//HI 1583 following modified pedigree method. The segregating generations were evaluated at Karnal and 8 shuttle breeding centres in CZ and PZ for target oriented selection. It was evaluated at 10 locations in all four zones i.e., Karnal in NWPZ, Ayodhya in NEPZ, Jabalpur, Junagadh, Vijapur and Bilaspur in CZ and Akola, Niphad and Dharwad in PZ under timely sown irrigated condition and timely sown restricted irrigation condition in replicated trials. The check varieties are the latest commercial cultivars of all the zones for various

production conditions. The yield data was analysed location wise to estimate the stress susceptibility index (Fischer & Maurer, 1978) and percent reduction of yield under stress conditions (Choukan *et al.*, 2006). The pooled analysis of SSI and Red (%) indicated wide range of variability among the genotypes in the experiment (Table 1.). In general, the genotypes having SSI <1.0 is considered as stress tolerant genotypes. The proposed genotype DWAP 1812 showed least SSI of 0.15 alongwith least reduction in yield under stress conditions (3.96) which reflects its high tolerance nature to water scarcity conditions (Table 2).

Table 1: Stress susceptibility index

S. No.	Entry	Stress susceptibility index (SSI)											Reduction in yield under stress (%)
		Akola	Ayodhya	Jabalpur	Niphad	Dharwad	Junagadh	Powarkheda	Vijapur	Bilaspur	Karnal	Pooled	
1	DWAP 18-12	-0.75	0.91	-22.06	1.07	-0.08	0.79	3.49	0.95	0.96	-0.86	0.15	3.96
2	DBW 110 ©	-0.13	0.66	4.43	1.16	0.80	1.16	4.97	1.13	1.04	-3.62	0.66	17.04
3	DBW 93 ©	1.25	0.59	-24.67	0.79	0.90	0.83	2.68	1.52	1.23	0.53	0.70	18.05
4	HD 2967 ©	-3.13	1.42	-6.52	1.06	1.30	0.89	-1.30	-0.13	1.10	1.06	1.04	26.59
5	HD 2932 ©	1.84	0.59	8.84	0.91	0.98	0.86	0.74	1.00	0.73	1.31	1.12	28.79
6	GW 322 ©	0.84	2.15	3.58	1.01	1.07	1.11	3.46	1.77	1.11	2.65	1.25	31.99
7	MACS 6222 ©	1.57	0.68	1.72	1.09	1.05	1.15	-0.93	0.79	0.89	-0.44	1.28	32.78
8	DBW 222 ©	0.05	2.23	3.61	1.18	1.01	1.43	1.27	1.18	0.77	2.02	1.29	33.05

8. IC279317 (IC279317; INGR22008), a wheat (*Triticum sativum*) germplasm for better yield traits and heat stress indices

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Introduction

The temperature on earth is increasing due to global warming and supposed to rise 1 and 3°C by 2025 and 2100, respectively (IPCC). The gradual increase of temperature substantially reduces the productivity of major crops. The effect of high temperature stress is clearly observed in wheat as late planting is very common in India due to intensive cropping system (Agarwal *et al.*, 2021). Delayed sowing hastens the seedling emergence, tiller initiation, emergence of flag leaf and spike that shortens the total growth duration of plant (Singh and Pal, 2003). The enhanced wheat production can be achieved only by growing heat tolerant wheat genotypes. To identify the heat tolerant wheat germplasm for hyper-arid regions, a set of 102 germplasm of wheat was collected from National Bureau of Plant Genetic Resources (NBPGR), New Delhi (India) under RKVY project and grown in normal (15 November) as well as in late (15 December) sowing conditions at Instructional farm of Agricultural Research Station, Swami Keshwanand Rajasthan Agricultural University, Bikaner (India).

Morpho-agronomic Characteristics

The diverse germplasm of wheat were tested for four

consecutive years (2010-11 to 2013-14) and then in year 2016-17 on various visual, morpho-physiological characters, biochemical traits, stress responsive characters, yield and yield attributes.

Associated characters and cultivated practices

Out of 102 wheat germplasm, a set of eleven germplasm were screened out after three successive years (2010-11 to 2012-13) which was further tested in 2013-14. In 2013-14, three germplasm were selected as heat tolerant. The experiment was repeated with a set of 11 wheat germplasm in *rabi* season 2016-17 and confirmed the results that three germplasm viz., entry no. 26 (IC279317), 43 (IC335971) and 58 (IC336816) were the most suitable heat tolerant wheat germplasm which might be utilized for further breeding programmes to develop heat tolerant wheat varieties. The screened germplasm are multiplied in the *rabi* season 2020-21.

References

Agarwal VP, NK Gupta, S Gupta and G Singh (2021) Screening of wheat germplasm for terminal heat tolerance under hyper-arid conditions. *Cereal Res. Commun.* 49: 375-383.

9. IC336816 (IC336816; INGR22009), a wheat (*Triticum aestivum*) germplasm heat tolerance nature

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References

- Agarwal VP, NK Gupta, S Gupta and G Singh (2021) Screening of wheat germplasm for terminal heat tolerance under hyper-arid conditions. *Cereal Res. Commun.* 49: 375-383.
- Singh S and M Pal (2003) Growth, yield and phenological response of wheat cultivars to delayed sowing. *J. Plant Physiol.* 8: 277-286.

10. IC212176 (IC212176; INGR22010), a wheat (*Triticum aestivum*) germplasm Gigas plant with reduced number of tillers (69 Tillers/m row) and long spike (12 cm)

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Plant architecture of wheat plant depends upon the number of tillers. The extent of tillering is governed by number of factors including genetical factors, time of sowing, temperature, and rainfall. Majority of spring wheat varieties produces 8-10 tillers per plant but IC212176 (a local collection from Bhavnagar, Gujarat) is identified as Gigas plant type with 1-2 tillers per plant with long spike length, high number of grains per spike and thick stem. The IC212176 evaluated in NGSN at 31 locations during 2020-21 (Table 1). In NGSN, the average number of tillers per metre in IC212176 was 69 as compared to 81 in IC296729 (INGR 99005) already registered genetic stock for "GIGAS" plant. The check varieties namely Sonalika, HD2967 and HI 8713 recorded a value of 97, 97, and 89 respectively for number of tillers per metre. This

Table 1: IC212176 in NGSN 2020-21

S. No.	Genotype	Tillers/ m row	Grains/ spike	Sp. length (cm)	1000-gr. wt. (g)
1	IC212176	69	57	12	43
2	IC296729 (Already registered genetic stock)	81	48	10.8	44
3	Sonalika (Check)	97	53	9.31	39
4	HD 2967 (Check)	97	53	10.3	39
5	HI 8713 (d) (Check)	89	53	9.17	41
	SD±	10.39	3.84	1.02	3.41

genotype also recorded higher number of grains per spike (57 grains per spike) and long spike (12 cm).

11. QBI19-09 (IC643956; INGR22011), bread wheat (*Triticum aestivum* subsp. *aestivum*) germplasm with high grain zinc concentration (48.6 ppm)

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Deficiency of iron leads to reduced hemoglobin and red cells resulting in anemia. Deficiency of Zinc is associated with diarrhea, loss of cognitive function and reduced immunity. The current levels of grain micronutrients in wheat, a staple for Indians, are not sufficient to provide even 50% of the RDA for adults. Therefore, zinc and iron are important nutritional traits to be improved by plant breeding. In this effort, high yielding line QBI19-09 having high grain Zn and Fe concentration was developed at ICAR-IARI. QBP19- 9 was evaluated along with 52 entries and check varieties in nine locations over two years

(2019-20 and 2020-21) in the QCWBN conducted by IIWBR, Karnal. This entry recorded higher values of grain zinc and iron and test weight over the Biofortified variety WB-02 and the checks in the respective zones as shown in Table 1. It also excelled in grain yield over biofortified check WB02 and the yield check GW322 in CZ and PZ during 2020-21 and in CZ during 2019-20. The genotype QBI19-09 was also found resistant, in IPPSN screening by IIWBR Karnal, to stripe rust and leaf rust in all the zones thus making it a valuable resource for high grain Fe and grain Zn concentration.

Table 1: Grain yield, test weight and nutritional quality traits of QBI19-09 along with quality and yield checks

Genotype	NWPZ 20-21 (4 LOCATIONS)					NWPZ 2019-20 (5 LOCATIONS)				
	Grain yield (t/ha)	Iron (mg/kg)	Zinc (mg/kg)	GPC (%)	TW (kg/hl)	Grain yield (t/ha)	Iron (mg/kg)	Zinc (mg/kg)	GPC (%)	TW (kg/hl)
QBI19-09	64.8	42.6	51.4	13.4	80.4	57.7	40.1	50.9	13	77.5
WB 02	56.2	40.3	44.5	13.9	77.7	54.8	38.3	37.8	12.5	75.8
HD3086 (C)	73.4	38.3	40.6	12.9	78.1	60.9	37.3	35.3	11	78.1
Genotype	NEPZ 20-21 (2 LOCATIONS)					NEPZ 2019-20 (2 LOCATIONS)				
	Grain yield (t/ha)	Iron (mg/kg)	Zinc (mg/kg)	GPC (%)	TW (kg/hl)	Grain yield (t/ha)	Iron (mg/kg)	Zinc (mg/kg)	GPC (%)	TW (kg/hl)
QBI19-09	46.4	38	49.9	10.9	77.4	26.9	39.4	34	12.3	74.9
WB 02	47	34	38	11.6	75.1	30	38.3	33.1	10.9	78.2
DBW 187 (C)	57.6	32	32.4	10	75.4	34.5	35.5	26.4	11.2	74.9
Genotype	CZ 20-21 (2 LOCATIONS)					CZ 2019-20 (2 LOCATIONS)				
	Grain yield (t/ha)	Iron (mg/kg)	Zinc (mg/kg)	GPC (%)	TW (kg/hl)	Grain yield (t/ha)	Iron (mg/kg)	Zinc (mg/kg)	GPC (%)	TW (kg/hl)
QBI19-09	52.6	41.9	55.5	13.2	79.8	59.8	38.9	51.4	12.4	80
WB 02	50.9	43.4	53.6	14.6	77.5	63.1	36.7	44.4	12	77.7
GW 322 (C)	51.7	40.2	48.4	11	75.9	50.8	36.3	31.9	10.3	73.9
Genotype	PZ 20-21 (1 LOCATION)					PZ 20-21				
	Grain yield (t/ha)	Iron (mg/kg)	Zinc (mg/kg)	GPC (%)	TW (kg/hl)	Grain yield (t/ha)	Iron (mg/kg)	Zinc (mg/kg)	GPC (%)	TW (kg/hl)
QBI19-09	37.5	50.1	48.8	13.0	82	-	-	-	-	-
WB 02	29.0	46.7	45.1	15.1	79.5	-	-	-	-	-
GW 322 (C)	36.6	40.1	40.7	11	81	-	-	-	-	-
Genotype	All India mean values					All India mean values				
	Grain yield (t/ha)	Iron (mg/kg)	Zinc (mg/kg)	GPC (%)	TW (kg/hl)	Grain yield (t/ha)	Iron (mg/kg)	Zinc (mg/kg)	GPC (%)	TW (kg/hl)
QBI19-09	50.325	43.15	51.4	12.625	79.9	41.17	39.47	45.43	12.6	77.5
WB 02	45.775	41.1	45.3	13.8	77.4	40.5	37.77	38.43	11.8	77.2

12. QBI20-20 (IC643957; INGR22012), a wheat (*Triticum aestivum* subsp. *aestivum*) germplasm for Low Hardness Index (32) (Soft endosperm); low SDS-Sedimentation value (40.5 mL)

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India is the second largest producer of wheat in the world. It has become more than self-sufficient in wheat production in the last two decades. India has also seen an upsurge in the value based industry. The milling and baking industry of India is rapidly expanding and is valued at ~Rs 69 billion. India has become the third largest producer of biscuits next to USA and China. Ready to make pre-mixes for products viz cake, muffins, pretzels, etc all require a specific flour. Grain softness is an important trait that can yield a flour highly suitable for these products. However, all commercially used varieties in India are hard grained. Therefore, genetic improvement to produce soft grained wheat suited for soft textured products has both a domestic and export potential. ICAR-IARI have developed a new strain QBI20-20 for grain softness. This

line was evaluated in 2020-21 in 11 locations in Quality Component and Wheat Biofortification Nursery (QCWBN) conducted by IIWBR covering all the four zones. QBI20-20 recorded commercially exploitable value (32) of GHI in all the locations as summarized in Table 1 below. In addition, QBI20-20 also recorded high grain zinc concentration of 45.9 mg/Kg which is similar to biofortified variety WB02. This genotype is also free of rusts as per the IPPSN screening of the AIWBIP 2020-21. Thus with its high grain yield (56.9 q/ha) in NWPZ, lower GHI (30), low sedimentation value (40.4ml) and good amount of grain Fe and Zn make it highly suitable as a donor in soft wheat breeding programme. It also has commercial value as it is free of rusts and can be used by Industry for making bakery products.

Table 1: Summary of Grain yield, grain hardness index, hectoliter weight and nutritional quality of QBI20- 20 along with yield and quality checks in QCBWN of AIWBIP 2020-21

<i>NWPZ 20-21 (4 Locations)</i>						
	<i>GHI</i>	<i>SDS-Sed (ml)</i>	<i>IRON (mg/Kg)</i>	<i>ZINC (mg/Kg)</i>	<i>YIELD (t/ha)</i>	<i>TW (Kg/hl)</i>
QBI-20-20	30	41.875	38.5	46.4	56.9	76.3
HD3226	82	60.275	38.3	40.6	73.4	78.1
WB02	76	61.325	40.3	44.5	56.2	77.65
HS490	30	40	36.5	40.7	51.7	74.28
<i>NEPZ 20-21 (2 Locations)</i>						
QBI-20-20	31	40.15	37.9	41.9	49.7	73.45
DBW 187	74	53.25	32	32.4	57.6	75.4
WB02	81	53.25	34	38	47	75.1
HS490	24	37	33.6	33.9	51.1	73.35
<i>CZ 20-21 (2 Locations)</i>						
QBI-20-20	36	38.43	43.5	55.6	49.1	76.5
GW 322	89	39.43	40.2	48.4	51.7	75.9
WB02	72	61.3	43.40	53.6	50.9	77.53
HS490	44	40.10	39.8	46.2	46	74.15
<i>PZ 20-21 (3 Locations)</i>						
QBI-20-20	31	41.3	45.2	42.9	37.8	78.35
GW 322	83	39.2	40.1	40.7	36.6	80.9
WB02	74	66.15	46.7	45.1	29.0	79.45
HS490	40	40.5	40.8	35.6	34.7	76.47
<i>Mean values (National basis)</i>						
QBI-20-20	32	40.4	40.5	45.9	38.925	76.2
WB02	73	61.8	41.7	45.1	38.5	77.5
HS490	35	39.6	37.5	39.5	37.2	74.7

13. DWRBG 2 (IC641989; INGR22013), a barley genotype (*Hordeum vulgare*) germplasm with high hectoliter weight (66.7 kg/hl) coupled with higher protein content 13.0% (dwb) and bold grains (>2.5 mm size)

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DWRBG-2 (tested as BCU 4966) is a two-row hulled barley genotype, selected from 10th EMBSN nursery (entry-29) supplied by ICARDA/CIMMYT. DWRBG-2 has high hectolitre weight of 66.7 kg/hl which is higher than the released malt type varieties (59.3 to 65.4 kg/hl) and the previously registered germplasm DWR 38 (64.0 kg/hl) for this trait. DWRBG-2 has average protein content of 13.0 % (dwb), which is in very desirable range of current specifications by malt industry. Besides these two attributes DWRBG-2 has bold grains (>2.5 mm size) percentage of more than 96.2% (Table 1). Barley malt is the traditional and key raw material

for beer (some speciality whisky also) making besides several malt-based products like energy drinks, confectionary and bakery products. Barley has been the grain of choice for malt making due to certain grain physical and biochemical parameters. The percent use of barley of total barley production is increasing for malting and brewing and the global malt ingredient market is projected to register a CAGR of 7.1% from 2000 to 2025. Test weight or specific weight or Hectolitre weight indicates the density of grains in particular volume at a standardized moisture level and normally reported as kilogram per hectolitre (kg/hl). Test

Table 1: Grain quality traits in DWRBG-2 (tested as BCU-4966) at different locations during 2019-20 and 2020-21

Genotype	Hisar	Karnal	Pantnagar	Hisar	Karnal	Ludhiana	Durgapura	Pantnagar	Kanpur	Mean
Hectolitre wt (kg/hl)	2019-20			2020-21						
BCU-4966	61.0	69.8	64.0	63.4	70.9	66.2	69.9	68.4	NA	66.7
DWR 38 (c)*	NT	NT	NT	62.7	63.7	64.8	65.6	65.2	62.0	64.0
DWRUB 52 (c)	60.7	66.7	60.5	61.3	68.7	57.9	70.8	64.5	62.3	63.7
DWRB 101 (c)	60.6	66.5	63.0	59.8	66.3	65.3	70.7	67.7	64.7	65.0
DWRB 123 (c)	57.5	62.6	63.2	60.1	67.0	63.7	69.3	67.3	57.5	63.1
DWRB 92 (c)	56.5	61.3	59.6	57.5	66.0	61.7	67.1	65.1	59.8	61.6
DWRB 91 (c)	62.0	66.9	60.6	58.3	66.4	61.8	69.2	65.1	62.5	63.6
RD 2849 (c)	NT	NT	NT	60.4	69.7	63.8	68.6	65.4	64.3	65.4
DWRB-160 (c)	58.7	62.2	57.5	52.6	62.5	58.5	67.6	63.8	57.4	60.1
DWRB-182 (c)	55.9	62.3	57.7	54.1	59.8	55.6	69.2	63.2	56.1	59.3
DWRB 137 (c)	NT	NT	NT	54.8	63.5	59.5	65.3	61.4	57.2	60.3
Protein content (%)	2019-20			2020-21						
BCU-4966	13.9	11.4	12.3	13.1	14.0	14.4	14.7	10.5	NA	13.0
DWR 38 (c)	NT	NT	NT	14.2	16.0	13.4	15.0	14.1	15.2	14.7
DWRUB 52 (c)	13.4	10.6	9.8	12.0	11.6	14.5	12.0	9.7	12.5	11.8
DWRB 101 (c)	13.0	11.1	10.2	12.1	11.7	12.8	11.7	9.6	11.1	11.5
DWRB 123 (c)	13.3	10.4	9.8	12.5	12.1	12.6	11.6	9.4	10.1	11.3
DWRB 92 (c)	14.9	11.9	11.5	12.5	14.1	13.5	11.9	10.7	12.7	12.6
DWRB 91 (c)	11.9	11.2	9.5	11.7	12.1	13.4	12.0	10.3	10.3	11.4
RD 2849 (c)	NT	NT	NT	11.6	12.2	11.1	10.4	11.2	9.5	11.0
DWRB-160 (c)	11.6	11.1	9.8	11.8	12.2	12.4	11.3	10.9	10.3	11.3
DWRB-182 (c)	12.8	11.7	11.5	12.2	11.6	14.2	13.2	12.1	10.4	12.2
DWRB 137 (c)	NT	NT	NT	12.8	13.5	12.9	11.2	14.6	10.2	12.5
Bold grain (%)	2019-20			2020-21						
BCU-4966	98.1	96.0	96.9	89.1	96.9	97.0	98.2	97.5	NA	96.2
DWR 38 (c)	NT	NT	NT	92.4	92.9	95.8	92.9	96.7	94.1	94.1
DWRUB 52 (c)	97.9	76.0	89.0	89.3	81.0	46.5	93.6	75.0	61.0	78.8
DWRB 101 (c)	97.1	79.9	95.7	91.9	87.0	87.5	96.6	73.2	77.9	87.4
DWRB 123 (c)	98.3	81.3	94.5	92.8	91.2	88.5	96.4	88.0	78.8	90.0
DWRB 92 (c)	98.7	89.5	97.4	98.0	98.3	91.3	98.7	98.2	94.7	96.1
DWRB 91 (c)	98.0	85.6	96.2	94.8	97.4	75.6	98.4	96.4	70.5	90.3
RD 2849 (c)	NT	NT	NT	84.5	74.8	79.8	95.1	64.2	79.0	79.6
DWRB-160 (c)	98.7	95.6	99.0	94.9	83.8	85.4	98.4	96.1	92.5	93.8
DWRB-182 (c)	93.2	75.9	68.8	70.6	74.9	63.2	93.3	67.0	73.3	75.6
DWRB 137 (c)	NT	NT	NT	88.9	86.1	82.6	96.0	87.6	83.4	87.4

*Registered stock for the trait

weight depends upon grain weight, shape, size/plumpness and their packing (AACC 55-10, 2000). Test weight is an indirect or crude method to assess the suitability of grains for malting quality. Usually higher test weight grains (> 65 kg/hl) are preferred for malting as the higher test weight normally indicates bigger endosperm and lower husk content. Probability of higher starch content is more in grains with

higher values of test weight. There is a positive correlation between hectolitre weight and hot water extract (malt extract) and this trait can serve as good criteria for selection of good malt quality lines in early generation of breeding programme. Thus, DWRBG-2 is a potential genotype which can be used as donor parent for improving the malting quality in barley.

14. QβLM11 (IC644007; INGR22014), a maize (*Zea mays*) germplasm with enhanced beta-carotene (7.46 ppm), lysine and tryptophan (0.298% & 0.080%)

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Introduction

Malnutrition affects growth and development in humans and causes socio-economic losses. Normal maize is deficient in essential amino acids, lysine and tryptophan; and vitamin-A. Crop biofortification is a sustainable and economical approach to alleviate micronutrient malnutrition. The present scenario necessitates developing maize genotypes with a combination of QPM and proA so that value-added product reaches poor communities. Buland and PMH1 are the popular maize hybrids released for cultivation in the North-Western Plain Zones of India. Our previous investigation has led to the development of QPM versions viz., BulandQ (QLM11 × QLM12) and PMH1Q (QLM13 × QLM14). The present study was thus aimed to (i) pyramid the favorable alleles of crtRB1 and lcyE genes into QPM background using marker-assisted backcross breeding (MABB), (ii) evaluate the nutritional quality of the introgressed lines and reconstituted hybrids, and (iii) assess the agronomic performance and yield potential of the MAS-derived genotypes. The present experiment was conducted at three locations viz. (i) Punjab Agricultural University (PAU), Ludhiana, (ii) Regional Research Station (PAU) Gurdaspur and (iii) IARI, New Delhi, India during the years from 2018 to 2019.

QβLM11 inbred line was developed through backcross selection (twicely backcrossed with recurrent female parent) and inbreeding for six generations. Molecular assisted backcross breeding strategy was used for the introgression of opaque-2 (*o2*), β-carotene hydroxylase (*crtRB1*) and Lycopene-ε-cyclase (*lcyE*) genes into the elite maize inbred

LM11. At the end of six selfing and evaluation, uniform progenies were identified and their selfed seeds were bulked which were multiplied in isolation. This is a late maturing line.

Morpho-agronomic Characteristics

The plant length is tall with medium ear placement. Its tassel is semi-compact with strongly curved and drooping branches. Leaves are long light green and strongly curved and drooping. Cobs are conico-cylindrical and were fully filled with grain & top of the cobs were tightly enclosed with the husk. Grains are yellow orange, flint with caps and are arranged in straight rows on the cob. This improved QβLM11 line with elevated lysine, tryptophan and provitamin A concentration can be used in maize biofortification programs. In addition, on the basis of multilocation trials the introgressed QβLM11 gave 3538.9 kg/hac grain yield which was at par with the original check and beta-carotene content is 7.46 ppm whereas lysine and tryptophan is 0.298% and 0.080%, respectively (Singh *et al.*, 2021). This shows that the nutritionally enriched QβLM11 can be used as a potential donor for the development of biofortified maize cultivars in future breeding programmes.

Reference

Singh J, S Sharma, A Kaur, Y Vikal, AK Cheema, BK Bains, N Kaur, GK Gill, PK Malhotra, A Kumar, P Sharma, V Muthusamy, A Kaur, JS Chawla and F Hossain (2021) Marker-assisted pyramiding of lycopene-ε-cyclase, β-carotene hydroxylase1 and opaque2 genes for development of biofortified maize hybrids. *Sci. Rep.* 11:12642 <https://doi.org/10.1038/s41598-021-92010-8>.

15. QβLM12 (IC644008; INGR22015), a maize (*Zea mays*) germplasm with enhanced beta-carotene (7.40 ppm) lysine and tryptophan (0.333% and 0.068%)

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Introduction

Malnutrition affects growth and development in humans and causes socio-economic losses. Normal maize is deficient in essential amino acids, lysine and tryptophan; and vitamin-A. Crop biofortification is a sustainable and economical approach to alleviate micronutrient malnutrition. The present scenario necessitates developing maize genotypes with a combination of QPM and proA so that value-added product reaches poor communities. Buland and PMH1

are the popular maize hybrids released for cultivation in the North-Western Plain Zones of India. Our previous investigation has led to the development of QPM versions viz., BulandQ (QLM11 × QLM12) and PMH1Q (QLM13 × QLM14). The present study was thus aimed to (i) pyramid the favorable alleles of crtRB1 and lcyE genes into QPM background using marker-assisted backcross breeding (MABB), (ii) evaluate the nutritional quality of the introgressed lines and reconstituted hybrids, and (iii) assess the agronomic performance and

yield potential of the MAS-derived genotypes. The present experiment was conducted at three locations viz. (i) Punjab Agricultural University (PAU), Ludhiana, (ii) Regional Research Station (PAU) Gurdaspur and (iii) IARI, New Delhi, India during the years from 2018 to 2019. QβLM12 inbred line was developed through backcross selection (twicely backcrossed with recurrent female parent) and inbreeding for six generations. Molecular assisted backcross breeding strategy was used for the introgression of opaque-2 (*o2*), β-carotene hydroxylase (*crtRB1*) and Lycopene-ε-cyclase (*lycE*) genes into the elite maize inbred LM12. At the end of six selfing and evaluation, uniform progenies were identified and their selfed seeds were bulked which were multiplied in isolation. This is a late maturing line.

Morpho-agronomic Characteristics

The plant length is tall with medium ear placement. Its tassel is open and lateral branches of tassel are straight. Leaves are short, dark green and straight. Cobs are small & conical in shape and were fully filled with grain & top of

the cobs were tightly enclosed with the husk. Grains are bold orange, flint and are arranged in straight rows on the cob. This improved QβLM12 line with elevated lysine, tryptophan and provitamin A concentration can be used in maize biofortification programs. In addition, on the basis of multilocation trials, the introgressed QβLM12 gave 3451.0 Kg/hac grain yield which was at par with the original check and beta-carotene content is 7.40 ppm whereas lysine and tryptophan is 0.333% and 0.068%, respectively (Singh *et al.*, 2021). This shows that the nutritionally enriched QβLM12 can be used as a potential donor for the development of biofortified maize cultivars in future breeding programmes.

Reference

Singh J, S Sharma, A Kaur, Y Vikal, AK Cheema, BK Bains, N Kaur, GK Gill, PK Malhotra, A Kumar, P Sharma, V Muthusamy, A Kaur, JS Chawla and F Hossain (2021) Marker-assisted pyramiding of lycopene-ε-cyclase, β-carotene hydroxylase1 and opaque2 genes for development of biofortified maize hybrids. *Sci. Rep.* 11: 12642 <https://doi.org/10.1038/s41598-021-92010-8>.

16. QβLM13 (IC644009; INGR22016), a maize (*Zea mays*) germplasm with enhanced beta-carotene (6.89 ppm) lysine and tryptophan (0.383% & 0.074%)

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Introduction

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Molecular assisted backcross breeding strategy was used for the introgression of opaque-2 (*o2*), β-carotene hydroxylase (*crtRB1*) and Lycopene-ε-cyclase (*lycE*) genes into the elite maize inbred LM13. At the end of six selfing and evaluation, uniform progenies were identified and their selfed seeds were bulked which were multiplied in isolation. This is a medium maturing line.

Morpho-agronomic Characteristics

The plant length is tall with medium ear placement. Its tassel is dense and lateral branches of tassel are straight. Leaves are long green having straight attitude of blade. Brace roots, anthers and silks have anthocyanin pigmentation. Cobs are conico- cylindrical and were fully filled with grain & top of the cobs were tightly enclosed with the husk. Grains are orange, flint and are arranged in straight rows on the cob. This improved QβLM13 line with elevated lysine, tryptophan and provitamin A concentration can be used in maize biofortification programs. In addition, on the basis of multilocation trials, the introgressed QβLM13 gave 3735.0 kg/ha grain yield which was at par with the original check and beta-carotene content is 6.89 ppm whereas lysine and tryptophan is 0.383% and 0.074%, respectively (Singh *et al.*, 2021). This shows that the nutritionally enriched QβLM13 can be used as a potential donor for the development of biofortified maize cultivars in future breeding programmes.

Reference

Singh J, S Sharma, A Kaur, Y Vikal, AK Cheema, BK Bains, N Kaur, GK Gill, PK Malhotra, A Kumar, P Sharma, V Muthusamy, A Kaur, JS Chawla and F Hossain (2021) Marker-assisted pyramiding of

lycopene- ϵ -cyclase, β -carotene hydroxylase1 and opaque2 genes for development of biofortified maize hybrids. *Sci. Rep.* 11:12642 <https://doi.org/10.1038/s41598-021-92010-8>.

17. Q β LM14 (IC644010; INGR22017), a maize (*Zea mays*) germplasm with enhanced beta-carotene (6.12 ppm), lysine and tryptophan (0.336% & 0.079%).

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Introduction

Malnutrition affects growth and development in humans and causes socio-economic losses. Normal maize is deficient in essential amino acids, lysine and tryptophan; and vitamin-A. Crop biofortification is a sustainable and economical approach to alleviate micronutrient malnutrition. The present scenario necessitates developing maize genotypes with a combination of QPM and proA so that value-added product reaches poor communities. Buland and PMH1 are the popular maize hybrids released for cultivation in the North-Western Plain Zones of India. Our previous investigation has led to the development of QPM versions viz., BulandQ (QLM11 $\times$ QLM12) and PMH1Q (QLM13 $\times$ QLM14). The present study was thus aimed to (i) pyramid the favorable alleles of crtRB1 and lcyE genes into QPM background using marker-assisted backcross breeding (MABB), (ii) evaluate the nutritional quality of the introgressed lines and reconstituted hybrids, and (iii) assess the agronomic performance and yield potential of the MAS-derived genotypes. The present experiment was conducted at three locations viz. (i) Punjab Agricultural University (PAU), Ludhiana, (ii) Regional Research Station (PAU) Gurdaspur and (iii) IARI, New Delhi, India during the years from 2018 to 2019. Q β LM14 inbred line was developed through backcross selection (twicely backcrossed with recurrent female parent) and inbreeding for six generations. Molecular assisted backcross breeding strategy was used for the introgression of opaque-2 (o2), β -carotene hydroxylase (crtRB1) and Lycopene- ϵ - cyclase (lcyE) genes into the elite

maize inbred LM14. At the end of six selfing and evaluation, uniform progenies were identified and their selfed seeds were bulked which were multiplied in isolation. This is a late maturing line.

Morpho-agronomic Characteristics

The plant length is tall with medium ear placement. Its tassel is semi-compact and lateral branches of the tassel are curved. Leaves are long green, curved and drooping and the stem is zigzag in nature. Cobs are conico-cylindrical in shape & top of the cobs were not enclosed with the husk. Grains are yellow, flint and are arranged in straight rows on the cob. This improved Q β LM14 line with elevated lysine, tryptophan and provitamin A concentration can be used in maize biofortification programs. In addition, on the basis of multilocation trials, the introgressed Q β LM14 gave 3648.8 kg/ha grain yield which was at par with the original check and beta-carotene content is 6.12 ppm whereas lysine and tryptophan is 0.336% and 0.079%, respectively (Singh *et al.*, 2021). This shows that the nutritionally enriched Q β LM14 can be used as a potential donor for the development of biofortified maize cultivars in future breeding programmes.

Reference

Singh J, S Sharma, A Kaur, Y Vikal, AK Cheema, BK Bains, N Kaur, GK Gill, PK Malhotra, A Kumar, P Sharma, V Muthusamy, A Kaur, JS Chawla and F Hossain (2021) Marker-assisted pyramiding of lycopene- ϵ -cyclase, β -carotene hydroxylase1 and opaque2 genes for development of biofortified maize hybrids. *Sci. Rep.* 11:12642 <https://doi.org/10.1038/s41598-021-92010-8>.

18. V 601 (IC643993; INGR22018), a maize (*Zea mays*) germplasm liguleless; early maturity; The stock is broadly in the genetic background of elite inbred V 407 (female parent of Vivek Maize Hybrid 53 and CMVL 55); known heterotic affinity of V 407 makes the stock directly usable maize hybrid breeding programmes

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Table 1: Morpho-agronomic characteristics of liguleless stock V601 and its parental lines

	Ligule	Days to 50% pollen shed	Days to 50% silking	Plant height (cm)	Ear height (cm)	Days to 75% brown husk	Cob length (cm)
PDH-3	Absent	49	50	165	90	86	8.4
V407	Present	53	55	135	70	91	15.8
V601	Absent	51	52	170	95	88	17.4
	Cob girth (cm)	Kernel rows	Grains /Row	1000-seed wt.	Grain type	Turcicum leaf blight (1-9)	Maydis leaf blight (1-9)
PDH-3	10.5	12-14	16	160	Yellow Dent	7.0	7.0
V407	14.8	14-16	23	302	Orange Flint	2.0	3.0
V601	13.5	10-12	26	280	Yellow Dent	3.0	3.0

Mutants with defective ligule and auricle development exhibit erect leaf architecture, and such leaf architecture facilitates dense and planting and contributes to higher yields (Duvick 2005). Since indigenous liguleless stocks are not available in Indian public maize breeding programme, liguleless line PDH-3 (EC928978) was obtained from University of Hohenheim, Germany and crossed with normal (liguled) inbred V 407 with the objective of transferring the trait in to locally adapted background. The cross was advanced following pedigree method and in F5 generation, liguleless line V 601 was identified on the basis of its agronomic performance (Table 1). The line V 601 has been developed and is being maintained at ICAR-Vivekananda Parvatiya Krishi Anusandhan Sansthan, Almora (Uttarakhand), India. V 601 is a liguleless line with upright leaves. Its plant height is 170 cm and days to 75% brown husk is 88 (early group) (Table 1). Tassel is medium large and semi-open. Colour of anthers is purple and anthocyanin pigmentation is present in the silk. The cob length and cob girth of V 601 are 17.4 and 13.5 cm, respectively. The grain

is yellow dent and test-weight is 280 g. V 601 is moderately resistant to Turcicum and Maydis leaf blight.

Liguleless trait can be phenotyped at seedling stage, which makes liguleless genotypes amenable for genetic studies such as gene-editing 'proof-of-concept' demonstration, and haploid induction rate (HIR) assessment of maize haploid inducer lines (Prigge *et al.* 2012). Moreover, since V601 is broadly in the genetic background of elite inbred V 407 (female parent of maize hybrids VMH53 and CMVL 55), it can also be directly used as a parental inbred in maize hybrid breeding programmes.

References

- Duvick DN (2005) The contribution of breeding to yield advances in maize (*Zea mays* L.) In: Donald LS (ed) *Advances in Agronomy*. Science Direct:Academic Press. pp 83–145.
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19. V 602 (IC643994; INGR22019), a maize (*Zea mays*) germplasm carrying liguleless trait

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The ligular region, which separates leaf blade and sheath, establishes the leaf angle and determines the overall architecture of maize plant. Mutants with defective ligule and auricle development exhibit erect leaf architecture, and such leaf architecture facilitates high density planting and contributes to higher yields (Duvick 2005). A liguleless line V 602 has been developed at ICAR-VPKAS, Almora, from a cross between an elite inbred V 407 and a liguleless donor line PDH-3 (EC928978) obtained from University of Hohenheim, Germany India. The cross was advanced following pedigree method and in F5 generation, liguleless line V 602 was identified on the basis of its agronomic performance.

V 602 is a liguleless line with upright leaves. Its plant height is 165 cm and days to 75% brown husk is 90 (early group) (Table 1). Tassel is medium large and semi-open. Colour of anthers is purple and anthocyanin pigmentation is present in the silk. The cob length and cob girth of V 602 are 15.8 and 13.2 cm, respectively. The grain is yellow with slight dent and test-weight is 265 g. V 602 is moderately resistant to Turcicum and Maydis leaf blight.

Owing to the ability of liguleless trait to be phenotyped at seedling stage, liguleless genotypes are useful for genetic studies such as gene-editing 'proof-of-concept' demonstration. Liguleless trait being recessive in nature,

Table 1: Morpho-agronomic characteristics of liguleless stock V602 and its parental lines

	Ligule	Days to 50% pollen shed	Days to 50% silking	Plant height (cm)	Ear height (cm)	Days to 75% brown husk	Cob length (cm)
PDH-3	Absent	49	50	165	90	86	8.4
V407	Present	53	55	135	70	91	15.8
V602	Absent	52	54	185	95	90	15.8
	Cob girth (cm)	Kernel rows	Grains/ Row	1000-seed wt.	Grain type	Turcicum leaf blight (1-9)	Maydis leaf blight (1-9)
PDH-3	10.5	12-14	16	160	Yellow Dent	7.0	7.0
V407	14.8	14-16	23	302	Orange Flint	2.0	3.0
V602	13.2	12-14	26	265	Yellow with Slight Dent	4.0	4.0

liguleless genotypes are also used to assess haploid induction rate (HIR) of maize haploid inducer lines (Prigge *et al.* 2012). V 602 carries the liguleless trait in the genetic background of elite inbred V 407 (female parent of maize hybrids VMH 53 and CMVL 55), which makes it directly useful as a parental inbred in maize hybrid breeding programmes.

References

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20. PML 46 (IC643958; INGR22020), a maize (*Zea mays*) germplasm with tolerance to high density planting, orange colored kernel and medium maturity

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Introduction

The PML 46 is a medium maturing field corn inbred line possessing tolerance to high-density planting under tropical conditions. The PML 46 was developed at ICAR-Indian Agricultural Research Institute, New Delhi (Mukri *et al.* 2020). A pedigree breeding method was followed to derive PML 46 from the field corn hybrid, SAFAL-X12. It is a highly potential germplasm line to develop high-density tolerant cultivars that would enhance productivity through high-density planting.

Morpho-agronomic characteristics

PML 46 has green foliage with semi-droopy leaves. Plant height ranges from 126-140 cm and bears the cob at the middle of the plant (≈ 75 cm). The average productivity of PML 46 is 2.82 tha⁻¹ under normal/recommended plant density. Under high-density planting, its grain yield potential increases up to 3.80 tha⁻¹ (Table 1). It expressed stable yielding ability across locations and years (Table 2). It has conico-cylindrical cob, and orange, flint kernels.

Table 1: Performance of PML 46 at different plant density

Plant density (plants/ha)	Plant height (cm)	Ear height (cm)	Cob length (cm)	Grain yield (t/ha)
66,666	152.5	72.5	14.2	2.82
83,333	162.5	77.5	15	3.06
88,888	175	82.5	12.3	3.8
1,11,111	137.5	67.5	12	3.54

Table 2: Performance of PML 46 at station trials across locations (Delhi and Dharwad) and seasons (2016-2019)

Location/Year	Plant Population	Grain Yield
2016-17- RRC-Dharwad	83,333 plants/ha	3.83 t/ha
2017- IARI-Delhi	66,666 plants/ha	2.50 t/ha
2017-18-RRC-Dharwad	88,888 plants/ha	4.10 t/ha
2018-IARI-Delhi	66,666 plants/ha	2.60 t/ha
2019-IARI-Delhi	83,333 plants/ha	3.70 t/ha

Associated characters and cultivation practices

PML 46 is resistant to curvularia leaf spot and rust disease and tolerant to lodging. It has field tolerance to stem borer and early water stress. However, to obtain the expected grain yield, PML 46 requires 120 N: 90 P: 60 K per ha. An entire dose of phosphorus, potash, and 20 % nitrogen along with 25 kg-1ha of ZnSO₄ should be applied to the soil at sowing and the remaining nitrogen should be given in 4 splits (Dass et al. 2010)

21. PAUFL 1 (IC643975; INGR22021), a cotton (*Gossypium arboreum*) mutant without Lint, Fuzz and trichomes

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Cotton is the most important source of natural fibre worldwide. Cotton fibre is the extension of ovule epidermal cells and develops in four overlapping stages namely fibre initiation, primary cell wall formation (fibre elongation), secondary cell wall thickening (mainly cellulose deposition), and maturation. Cotton seed fibres are of two types: short (fuzz) and long (lint). In addition to ovule, epidermal outgrowths known as trichomes are also present on almost all aerial plant parts. Here, we report a fibreless (fuzzless and lintless) and trichomeless mutant namely PAUFL 1 of *desi* cotton (*Gossypium arboreum* L.). It was identified as a spontaneous mutant in the plot of *desi* cotton variety LD 327 at PAU Regional Research Station, Faridkot (Punjab). LD 327 was developed from the intra-*arboreum* cross of G 57 × (G 27 × LD 124) following pedigree method and was released for commercial cultivation in the Punjab state during 1987. PAUFL 1 is characterized by lack of fuzz and lint fibres on the seed coat and trichomes on other aerial plant parts. Like its wild type parent LD 327, the mutant possesses pigmented (red) plant body, digitate leaves, and variegated (pink and white) petals. PAUFL 1 has consistently maintained its mutant phenotype since its identification in 2003 through 2021. Genetic analysis involving F₂ and backcross populations showed that the fuzzless-lintless-trichomeless phenotype was governed by a single recessive gene (Grover et al., 2016).

Monogenic recessive control of fibreless phenotype in *desi* cotton mutants has also been reported by (Rong et al. 2005). Tight linkage between fuzzless-lintless- trichomeless traits was observed in the segregating populations derived

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from PAUFL 1 × wild type *desi* cotton crosses. According to (Arpat et al., 2004) and Rong et al. (2005), lint fibres and trichomes shared a similar pathway in their early stages of development. Alternatively, they may be under the control of same gene that influences trichome and fibre development (Desai et al., 2008). *Desi* cotton mutant PAUFL 1 can be used for the identification of transcriptional regulators and metabolic pathways involved in initiation of cotton fibre and trichomes which has important implications for enhancing cotton fibre yield and quality. In a study involving this mutant, it was shown that a positive correlation existed between trichome density and whitefly population suggesting that lower trichome density favours low incidence of whitefly in cotton (Grover et al., 2016).

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22. VR 1141 (IC644006; INGR22022), a finger millet (*Eleusine coracana*) germplasm for banded blight resistance

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Finger millet, VR 1141 is banded blight resistant line developed by crossing VR 708 with GPU 48 during 2010. through pedigree method of selection. Single plant selections were made from the cross up to five generations and during, 2017 uniform bulk selections were promoted to preliminary yield trial and evaluated with uniform bulks from other crosses. Twenty-four promising lines along with local and national checks for yield; resistant and susceptible check for blast disease resistance were evaluated in Randomized block design with three replications during four consecutive years (*khari*, 2017 to 2020) at Agricultural Research Station, Vizianagaram (lat 18°10' N, long 83°39' E and altitude of 74 msl), Andhra Pradesh which is a hot spot for banded blight disease.

The entry, VR 1141 matures in 124 days and grows to

height of 125 cm and it is having more number of fingers per ear (10) with broad finger width (1.2cm). It recorded less incidence of banded blight (3.2% pooled data) among all the 30 entries tested for banded blast resistance. The score for banded blight was significantly lower compared to all the checks during four years of testing. It recorded 49.92%, 74.92% and 88.64% less incidence of finger blast over Resistant check, Local check and susceptible check respectively. It also showed moderate resistance to neck blast (12.4% pooled data) and finger blast (15.8% pooled data). Hence, VR 1141 is unique in terms of banded blight resistance and moreover it is an advanced breeding line which can be directly used for development of high yielding banded blight resistant varieties.

23. VR 1122 (IC0644004; 22023), a finger millet (*Eleusine coracana*) germplasm with finger blast resistance

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Finger millet, VR 1122 is finger blast resistant line developed by crossing VR 708 with GPU 48 during 2010. Pedigree method of breeding was followed. Single plant selections were made from the cross up to five generations and during, 2017 uniform bulk selections were promoted to preliminary yield trial and evaluated with uniform bulks from other crosses. Twenty four promising lines along with local and national checks for yield; resistant and susceptible check for blast disease resistance were evaluated in Randomized block design with three replications during four years (*khari*, 2017 to 2020) at Agricultural Research Station, Vizianagaram (lat 18°10' N, long 83°39' E and altitude of 74 msl), Andhra Pradesh which is a hot spot for blast disease.

VR 1122 belongs to medium maturity (117 days) with medium plant height (118 cm) and more number of productive tillers/plant (4). The entry, VR 1122 was screened

along with local variety (VR 847), resistant check (GE 4999) and susceptible check (VR 708) under high disease pressure under field conditions during consecutive four years from 2017 to 2020. It recorded significantly lower score of finger blast over all the checks during all four years of testing. It recorded 66.82%, 85.29% and 96.19% less incidence of finger blast over Resistant check, Local check and Susceptible check respectively. It recorded less incidence of Finger blast (2.1% pooled data) among all the 30 entries tested for finger blast resistance and is considered as resistant to finger blast. It also showed resistance to neck blast (4.6% pooled data) and banded blight (5.6% pooled data). Hence, VR 1122 is unique in terms of finger blast resistance and moreover, it is an advanced breeding line with resistance to neck blast and banded blight as well and can be used directly for development of high yielding blast resistant varieties.

24. SPV 2804 (IC0288432; INGR22024), a sorghum (*Sorghum bicolor*) germplasm with higher Leaf Stem Ratio (0.32) and low HCN content (40.9%)

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Sorghum (*Sorghum bicolor*) is the fourth most important cereal following rice, wheat and maize and staple food in the semi-arid parts of the world. Single cut forage sorghum has importance considering the demand for the fodder from the dairy sector of the country. Good quality fodder must have high leaf: stem ratio. Similarly, HCN is the antinutritional factor present in the sorghum fodder. So low value of HCN is desirable for better fodder quality in forage sorghum. Therefore, identification of the *Kharif* single cut forage sorghum genotype with more leaf: stem ratio and low HCN is of prime importance. Considering this, the *kharif* single cut genotype SPV 2804 which was found superior for both these traits in the National Level trial- Initial Varietal and Hybrid Trial (Single Cut) *Kharif* 2020 conducted by Indian Institute of Millets Research (IIMR), Hyderabad is proposed for registration.

In the National Level trial-Initial Varietal and Hybrid Trial (Single Cut)-*Kharif* 2020, the *Kharif* Single cut forage Sorghum Genotype SPV 2804 contributed by Sorghum Research Unit, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola (Maharashtra) was tested during *Kharif* 2020. Trial

consisted of the testing entries along with the checks CSH 40F, CSH 36F, CSV 21F, CSV30F, CSV 35F. The experiments were sown in randomized block design with three replications. The character leaf stem ratio was recorded at 10 locations across the India while the trait HCN content was estimated at four locations across India.

For the character leaf stem ratio, the genotype SPV 2804 ranked first at National level with the value of 0.32 while the checks were with low values i.e. CSH 40F (0.26), CSH 36F (0.29), CSV 21F (0.27), CSV30F (0.29), CSV 35F (0.30). While for the character HCN %, the genotype SPV 2804 recorded lower HCN content at National level with the value of 40.9% while the checks were with high values i.e. CSH 40F (41.3%), CSH 36F (42.5%), CSV 21F (41.9%), and CSV 35F (44.2%).

Thus, it was concluded from the present study that considering the more leaf: stem ratio and low HCN in the genotype SPV 2804, it need to be exploited in breeding for development of genotypes with better fodder quality in single cut forage sorghum as the donor parent in the crossing programme.

25. SPV 2805 (IC643757; INGR22025), a sorghum (*Sorghum bicolor*) germplasm with longer leaves (86.5 cm), greater plant height (276 cm) and more number of leaves per plant (12.9)

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Sorghum (*Sorghum bicolor*) is the fourth most important cereal following rice, wheat and maize and staple food in the semi-arid parts of the world. Single cut forage sorghum has importance considering the demand for the fodder from the dairy sector of the country. Leaf length is one of the important contributing trait in fodder yield. Similarly, higher plant height as well as more number of leaves per plant are also good supporting traits in improving the fodder yield. Therefore, identification of the *Kharif* single cut forage sorghum genotype with more leaf length along with the supporting traits like higher plant height and more number of leaves per plant is of prime importance. Considering this, the *kharif* single cut genotype SPV 2805 which was found superior for the trait i.e. more leaf length

in the National Level trial-Initial Varietal and Hybrid Trial (Single Cut)-*Kharif* 2020 conducted by Indian Institute of Millets Research (IIMR), Hyderabad is proposed for registration.

In the National Level trial-Initial Varietal and Hybrid Trial (Single Cut)-*Kharif* 2020, the *Kharif* Single cut forage Sorghum Genotype SPV 2805 contributed by Sorghum Research Unit, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola (Maharashtra) was tested during *Kharif* 2020. Trial consisted of the testing entries along with the checks CSH 40F, CSH 36F, CSV 21F, CSV30F, CSV 35F. The experiments were sown in randomized block design with three replications. The character leaf length, plant height and number of leaves per plant were recorded at 12 locations across the India.

For the character leaf length, the genotype SPV 2805 ranked first at National level with the value of 86.5 cm. while the checks were with low values *i.e.* CSH 40F (79.9 cm), CSH 36F (82.7 cm), CSV 21F (75.5 cm), CSV30F (79.0 cm), CSV 35F (80.7 cm). While for the character plant height, the genotype SPV 2805 ranked first at National level with the value of 276 cm while the checks were with low values *i.e.* CSH 40F (263 cm), CSH 36F (264 cm), CSV 21F (253 cm), CSV30F (265 cm), CSV 35F (230 cm). Similarly, for the character number of leaves per plant, the genotype SPV 2805 ranked third at National

level with the value of 12.9 while the checks were with low values *i.e.* CSH 40F (12.3), CSH 36F (12.7), CSV 21F (12.4), CSV30F (12.8), CSV 35F (12.5).

Thus, it was concluded from the present study that considering the highest leaf length along with the supporting traits like higher plant height and more number of leaves per plant in the genotype SPV 2805, it need to be exploited in breeding for development of genotypes with better fodder yield contributing character in single cut forage sorghum as the donor parent in the crossing programme.

26. SPV 2596 (SM-2288-3) (IC643980; INGR22026), a sorghum (*Sorghum bicolor*) germplasm for high fresh stalk yield and high biomass

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Introduction

Sorghum breeding programs have accomplished the yield gains through use of germplasm from the primary genetic pool, however, overcoming the yield plateau needs introduction of novel variations into the crop gene pool and development of ready-to-use pre-breeding material. Derivatives from incompatible crosses are reported to produce large de novo variations through genomic shock from the alien. Variations in F₂ are advanced through pedigree method and stable lines are used as pre-breeding material.

Associated Characters and Cultivated Practices

The genotype SPV 2596 is a sweet sorghum line with tall plants and thick juicy stalks. It has high fresh stalk yield and high total fresh biomass. Fresh stalk yield and biomass were significantly more than three checks, CSV 19SS, CSV

24SS and CSH 22SS while they were on par with fourth check, SPH 1880. Total sugars were significantly more than two varietal checks CSV 19SS and CSV 24SS and on par with hybrid checks. Brix percentage is very important factor for use of sweet sorghum in the industry and in the present entry it is significantly superior to all. Data was collected in the Initial and Advanced Sweet Sorghum Varietal and Hybrid Trial (AICRP) during *Kharif* 2018 at 10 locations of All India Coordinated Research Project on Sorghum (Table 1). Fresh stalk, biomass and ethanol yield are the important traits for the improvement of sweet sorghum. In terms of plant height, brix % and computed ethanol yields, it was superior to three checks and holds promise as start material for sweet sorghum breeding among the 15 entries tested in AICRP *Kharif* 2018. This genetic stock is a unique material as the cross between Sorghum and Maize has been accomplished for the first time in sorghum crop improvement program.

Table 1: Initial and Advanced Sweet Sorghum Varietal and Hybrid Trial - *Kharif* 2018 All India data across 10 centres

Entry	Fresh stalk yield (t/ha)	All India ranking	Total fresh biomass (t/ha)	All India ranking	Total Sugars (%)	All India ranking	Brix %	All India ranking	Plant height cm	All India ranking
SPV 2596	47.5	2	62.8	1	14.3	4	16.8	1	300	2
CSV 19SS (Check1)	39.85	16	54.86	10	13.68	13	15.38	14	280.91	5
CSV 24SS (Check 2)	38.43	18	53.28	15	12.55	19	14.17	19	249.55	19
SPH 1880 (Check 3)	46.23	4	61.42	2	13.68	14	15.60	12	326.21	1
CSH 22SS (Check 4)	42.43	11	53.51	12	13.84	11	15.92	11	284.07	4
General Mean	44.3		56.8		13.67		15.7		272	
CV (%)	17.9		19.52		10.23		11.88		10.32	
SE of Difference	3.65		4.35		0.56		0.59		8.9	
P-Value	0.06		0.04		0.01		0.00		0.00	
CD (5%)	7.3		8.6		1.11		1.16		17.54	
CD (1%)	9.7		11.4		1.47		1.53		23.13	

27. IS 1212-4-1-1 (IC643968; INGR22027), a sorghum (*Sorghum bicolor*) germplasm with high oil content (4.49%); hard seed with bigger germ size

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Oil content in sorghum grains is an important component in determining sorghum as a functional food. Sorghum, with its ability to yield high with low inputs, can be used as an alternative oil source with clinical advantages. Sorghum varieties can be additional sources of edible oil due to the presence of clinically important saturated and high concentration of unsaturated fatty acids. Breeding efforts towards identifying end use specific cultivars are required for increased profitability to the farmers. At IIMR, Hyderabad a set of 256 genotypes including the germplasm lines and released cultivars were evaluated for oil content during 2014-15 and 19 genotypes with high oil content were selected. These 19 were evaluated for oil content and hardness during 2015-16 at IIMR, Hyderabad and at three locations (Gulberga, Solapur and Hyderabad) during 2016-17. These genotypes were planted at three locations, viz., Regional Agricultural Research Station, Gulberga; Centre on

Rabi sorghum, Solapur and ICAR-Indian Institute of Millets Research (ICAR- IIMR), Hyderabad during *rabi* season of 2016-17.

Data on oil content was recorded for the samples from IIMR during 2015-16 and all the three locations during 2016-17 to study the stability of oil content (Table 1). Oil content ranged from 3.14% to 4.76%. The germplasm line, IS 1212-4-1-1 was found to have high oil content and also better adapted to all test environments with stable mean oil content. IS 1212-4-1-1 is a pure line selection from germplasm line belonging to Kafir-bicolor (KB) race. It is early flowering (flowering in less than 60 days) with semi loose panicle. The grain color is red and the grains are hard with large germ size. Grains of IS 1212 were harder with large germ size. This genotype is a potential candidate while breeding for high oil content with better storability and could be of more value in breeding as they are suitable across environments.

Table 1: Performance of IS 1212 for oil content and seed traits in sorghum during 2015 and 2016

Genotype	100 grain weight (g)	Grain hardness (N)	Germ size score	Oil Content (%)					
				2015-16		2016-17		Mean 2016	Over all mean
				Hyderabad	Gulberga	Solapur	Hyderabad		
IS 1212-4-1-1	2.06	8.41	7	4.55	4.41	4.35	4.64	4.46	4.49
M 35-1	3.69	7.32	5	2.79	3.11	3.28	3.28	3.22	3.12
CSV 27	1.91	7.50	5	2.80	3.29	3.41	3.66	3.45	3.29
BJV 74	3.06	7.41	5	2.55	3.09	3.35	3.23	3.22	3.06
Mean	2.57	6.32	4.79	2.97	3.82	3.94	4.07	3.94	3.94
C. V.	18.5	20.7	18	4.53	4.14	3.21	3.18	3.51	
CD 0.05%	1.01	1.89	2	0.23	0.26	0.21	0.21	0.23	

Germ size score 1-9 scale where 1= very small; 3=small; 5= medium; 7= large and 9= very large

28. IS 31714-2-1-1 (IC643969; INGR22028), a sorghum (*Sorghum bicolor*) germplasm or high oil content (4.17%); very bold seed with medium hardness and small germ size

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Grain oil content is an important component in determining sorghum as a functional food. Sorghum, with its ability to yield high with low inputs, can be used as an alternative oil source with clinical advantages, and thus sorghum varieties with high oil content can be additional sources of edible oil due to the presence of clinically important saturated and high concentration of unsaturated fatty acids. At IIMR, Hyderabad a set of 256 genotypes including the germplasm lines and released cultivars were evaluated for oil content during 2014-15 and 19 genotypes with high oil content were selected (Table 1). These 19 were evaluated for oil content and hardness during 2015-16 at IIMR, Hyderabad and at three locations (Gulberga, Solapur and Hyderabad) during post rainy season of 2016-17. Mean oil content across environments ranged from 3.14% to 4.76%. The

germplasm line, IS 31714-2-1-1 was found to have oil content significantly more than the check (M 35-1). IS 31714-2-1-1 is a pure line selection from germplasm line belonging to Durra-caudatum (DC) race from Yemen. It has small very compact panicle. The grain color is reddish orange and the grains are medium hard with small germ size. The grains are very bold with 100 grain weight of 4.94 g. It has medium grain hardness and small germ size. This genotype is a potential candidate to breed for high oil content with bold seed. Breeding efforts to identify such end-use specific cultivars are required for increased profitability to the farmers. The information generated and the genotypes identified will help in breeding sorghum with high oil content thus enhancing the demand for sorghum as an industrial crop.

Table 1: Performance of IS 31714 for oil content and seed traits in sorghum during 2015 and 2016

Genotype	100 grain weight (g)	Grain hardness (N)	Germ size score	Oil Content (%)					
				2015-16		2016-17			
				Hyderabad	Gulberga	Solapur	Hyderabad	Mean 2016	Over all mean
IS 31714-2-1-1	4.94	4.47	3	4.38	3.93	4.25	4.12	4.10	4.17
M 35-1	3.69	7.32	5	2.79	3.11	3.28	3.28	3.22	3.12
CSV 27	1.91	7.50	5	2.80	3.29	3.41	3.66	3.45	3.29
BJV 74	3.06	7.41	5	2.55	3.09	3.35	3.23	3.22	3.06
Mean	2.57	6.32	4.79	2.97	3.82	3.94	4.07	3.94	3.94
C. V.	18.5	20.7	18		4.14	3.21	3.18	3.51	
CD 0.05%	1.01	1.89	2		0.26	0.21	0.21	0.23	

Germ size score 1-9 scale where 1= very small; 3=small; 5= medium; 7= large and 9= very large

29. SPV 2017 (IC643970; INGR22029), a sorghum (*Sorghum bicolor*) germplasm for high in-vitro true digestibility of dry matter (TDDM/IVDMD), organic matter digestibility (OMD) and Metabolisable energy content (ME); low Acid Detergent Fibre (ADF) and Acid Detergent Lignin (ADL) content

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Common property resources for grazing and land under fodder cultivation are declining in many countries; hence, dependence on crop residues for animal feeding will increase. In developing countries where livestock are reared under mixed-farming systems, crop residues play a significant role in maintenance and production of ruminants. Several newer cultivars of brown midrib sorghum (containing a mutant *bmr* gene) have been developed through plant breeding and have the potential for early maturity, high grain and forage yields, lower susceptibility to insects and pests and importantly, lower in hydrocyanic acid and lignin contents. Stover cell-wall components such as lignin and structural carbohydrates were shown to be important traits related to stover digestion for quality assessment of stover

samples. In vitro gas production kinetics and degradability along with chemical composition can better predict their potential feeding values.

SPV 2017 was developed from a cross between CSV 15 and the *bmr* germplasm line, IS 21891 during 2007. CSV 15 was released as dual purpose variety for all *kharif* sorghum growing areas in the country and as forage variety for Northern Western zone while IS 21891 is a brown midrib source carrying the gene *bmr* 8. The significance of this brown midrib mutation is that it greatly reduces lignification and cell-wall concentration, increases digestibility and voluntary intake of feed by ruminants. This single-locus mutation signifies the single most rapid and valuable mechanism of genetically modifying nutritional value

of forage and dual-purpose sorghum. The SPV 2017 was evaluated in *kharif* 2010 in AICRP on sorghum trials and in 2018 it is evaluated in four rumen-cannulated Murraha buffalo samples where, four stover samples were collected from two field replicates. The four Murraha buffaloes may be considered as four different environments of the study. The SPV 2017 was found superior in stover digestible factor contents in the present analysis and comparison with forage, sweet and grain sorghum cultivars.

The testing of SPV 2017 in AICRP on sorghum IAVHT trials *kharif* 2010, it has been found that SPV 2017 is superior in IVDMD (42.15%) and ME (6.36 mj/kg) and contains lower quantity of ADF (47.60%) and ADL (5.83%) when compared to CSV 23 variety with lower values of IVDMD (38.70%), ME (5.70 mj/kg) and higher values of ADF (49.85%), ADL (6.62%) and in CSV 21F with lower values of IVDMD (38.45%), ME (5.76%)

and higher values of ADF (49.85%) and ADL (6.59%). In the study conducted with buffaloes, the stover compositional factors like ADF (31.35% of DM) and ADL (1.67% of DM) contents were lower than other cultivars where, lower lignin values are preferable for good stover quality and the higher TDDM (58.35% after 72h of feeding), OMD (52.24% after 72h of feeding), ME (70.75% after 72h of feeding) where, higher values are preferred for good digestion of stover in animals rumen in SPV 2017 line when compared to other check varieties evaluated. These superior stover digestibility related factors in SPV 2017 line makes it most unique derived line among other cultivated lines. This line will serve as an excellent source to identify the genomic regions related to superior stover quality and also as excellent donor for many favourable traits for the development of single cut forage cultivars.

30. VR 1128 (IC644005; INGR22030), a finger millet (*Eleusine coracana*) germplasm with neck blast resistance

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Finger millet is one of the drought resistant small millet valued for its nutrient content in grains. It is a crop of both food and nutritional security. Blast is a major problem in finger millet crop which can hamper the production upto 70% in severe cases. Hence, finger millet improvement is oriented towards development of blast resistant high yielding varieties. Finger millet, VR 1128 is neck blast resistant line developed by crossing Uduru malligae and GPU 48 during 2010 followed by pedigree method of selection upto F₆ generation. It was evaluated with other 23 promising lines along with local and national check for yield; resistant and susceptible check for blast disease resistance in Randomized block design with three replications during consecutive four years (*kharif*, 2017 to 2020) at Agricultural Research Station, Vizianagaram (lat 18°10' N, long 83°39' E and altitude of 74 msl), Andhra Pradesh which is a hot spot for blast disease.

The proposed entry along with other test entries and two checks were tested under high disease pressure under field conditions during consecutive four years from 2017 to 2020.

The entry, VR 1128 is of late maturity (127 days) with medium plant height (123 cm) and with long ear length (9.4 cm). It recorded significantly lower score of neck blast over all the checks during all four years of testing. It recorded 75.75%, 83.33% NS 96.71% less incidence of neck blast over Resistant check (GE 4999), Local check (VR 847) and Susceptible check (VR 708) respectively. It recorded less incidence of neck blast (2.1% pooled data) among all the 30 entries tested for neck blast resistance and it also recorded resistance to finger blast (9.6% pooled data) and banded blight (4.6% pooled data). Hence, VR 1128 is unique in terms of neck blast resistance and moreover it is an advance breeding line which can be directly used for development of high yielding blast resistant varieties.

31. SPV 2595 (SM-2144-8) (IC643981; INGR22031), a sorghum (*Sorghum bicolor*) germplasm with higher brix and total sugars (14.3%), early maturing (116 days) and early flowering (80 days)

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Introduction

Sweet sorghum is a promising energy crop used for ethanol production. This ethanol can be blend upto 20% in India that

demands for higher production and better ethanol yielding genotypes in sweet sorghum. Expansion of genetic base is the key activity for breeding programs to improve the

Table 1: Initial and Advanced Sweet Sorghum Varietal and Hybrid Trial - Kharif 2018 All India data across 10 centres

	All India	All India	Ranking	All India	All India	All India
Entry	Days to flowering (days)	Days to Maturity (days)		Total Sugars (%)	Non Reducing Sugars - Sucrose (%)	Brix %
SPV 2595	80	116	2	14.3	9.99	16.5
CSV 19SS (Check1)	84.76	117.94	7	13.68	9.99	15.38
CSV 24SS (Check 2)	83.73	117.28	5	12.55	9.30	14.17
SPH 1880 (Check 3)	88.42	122.16	17	13.68	9.16	15.6
CSH 22SS (Check 4)	88.06	124.06	19	13.84	9.40	15.92
General Mean	85	119		13.67	9.60	15.7
CV (%)	4.66	4.49		10.23	8.82	11.88
SE of Difference	2	2.04		0.56	0.49	0.59
P-Value	0.00	0.00		0.01	0.87	0.00
CD (5%)	3.94	4.03		1.11	1.03	1.16
CD (1%)	5.2	5.31		1.47	1.41	1.53

traits. Introduction of novel variation is that are not available in the gene pools adds unique opportunity. In these lines we produced and selected one of the de novo variant for sweet sorghum traits. Such agronomically promising novel derivatives from incompatible crosses are reported in rice (Wang *et al.*, 2013, Meng *et al* 2020,). Variations in F₂ are advanced through pedigree method and stable lines are used as pre-breeding material.

Associated Characters and Cultivated Practices

The genotype SPV 2595 is a sweet sorghum line with tall plants and thick juicy stalks. It has high fresh stalk yield and high total fresh biomass. It is an early flowering and early maturing line with more brix than all the checks (Table 1). It has non-reducing sugars 9.99% as high as the best check variety. It showed brix value on par with check, the CSH 22SS (Check 4) and superior significantly compared to all

other three checks. Data was collected in the Initial and Advanced Sweet Sorghum Varietal and Hybrid Trial (AICRP) during Kharif 2018 at 10 locations of All India Coordinated Research Project on Sorghum. Fresh stalk, biomass and ethanol yield are the important traits for the improvement of sweet sorghum. This genetic stock is a unique material as the cross between Sorghum and Maize has been accomplished for the first time in sorghum crop improvement program.

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32. WVN 55 (IC618375; INGR22032), a finger millet (*Eleusine coracana*) germplasm with bold white grains (3.14 g), longer finger length (12.27 cm) and multi-fingers ear head (10.13 cm)

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A total of 36 white finger millet accessions constituted of 27 landraces and nine released varieties were evaluated for 12 morphological characters including grain yield at Hill Millet Research Station, Waghai; Niger Research Station, Vanarasi under Navsari Agricultural University, Navsari and Hill Millet Research Station, Dahod under Anand Agricultural University at Gujarat, during Kharif 2018, 2019 and 2020. The objectives were to assess the genetic potential through

the variability, correlation analysis among the quantitative traits. This research was carried out using randomized block design with three replications at each location. The pooled data of all three locations were used to study the genetic potential of white finger millet genotypes. Moderate genotypic and phenotypic coefficient of variation found for the traits viz, number of fingers per earhead, number of productive tillers per plant, straw yield per plant, grain

yield per plant, finger length, harvest index and main earhead indicating ample scope of variation for these traits, allowing further improvement by selection of these traits. Low value of genotypic coefficient of variation and phenotypic coefficient of variation was found for the traits viz., days to 50% flowering, finger width, days to maturity, plant height and 1000 grain weight indicating low variability for these traits. High heritability estimates were observed for days to 50% flowering, days to maturity, plant height, number of productive tillers per plant, number of fingers per earhead, main earhead length, finger length, 1000 grain weight, grain yield per plant, straw yield per plant and harvest index showing low environmental influence

on these traits and presence of additive gene action for these traits. Hence, priority can be given to these traits during selection to get more genetic gains. Genotypes viz; WVN 55 followed by GN 5 and GNN-7 were high yielding among all thirty six genotypes of white finger millet so they can be considered for varietal development and release for further selection. Thus, WVN 55 is multifinger (10.13), long finger length (12.27 cm), bold grain (3.14 g), multitiller (7.93) white seeded high yielding (11.85 g) promising finger millet genotype with moderately resistance to pest and diseases found during three years of genetic evaluation at three different locations which used as a promising parent in further breeding programme.

33. GPU 28-2081 (IC643979; INGR22033), a finger millet (*Eleusine coracana* ssp. *coracana*) germplasm with longer finger length (10.7 cm)

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Finger millet is largely grown millet next to Pearl millet and Sorghum occupying an area of 1.1 - 1.2 million ha in India. Grains of finger millet are known for its highest level of calcium 300-350 mg/100g which is 20-30 times higher than that of any cereal. GPU28-2081 is a mutant (M_1) line with long fingers derived from induced gamma mutagenesis of GPU 28 variety at ICAR- Indian Institute of Millets Research, Hyderabad. GPU 28 variety were irradiated with gamma rays with doses of 500- 600 Gy. M_1 generation was raised during *kharif*, 2015 and about 2000- 3000 panicles from M_1 populations were harvested separately and were raised in panicle to row progeny in M_2 generation along with parental genotype. Selections were done during M_2 and M_3 generation and 61 selected mutant lines were advanced till M_7 generation. Sixty one promising mutants along their parental genotypes were conducted in replicated field trials in 4 environments: during 2019 and 2020 at Indian Institute of

Millets Research, Hyderabad in Telangana, Latitude 17°23' N, Longitude 78°40' E and Altitude of 505 msl) and during 2020 at Agricultural Research Station, Vizianagaram in Andhra Pradesh, Latitude 18°10' N, Longitude 83°39' E and Altitude of 74 msl), and AICRP Small Millets Project Coordinating Unit, Bengaluru in Karnataka, Latitude 12°97' N, Longitude 77°59' E and Altitude of 920 msl).

The proposed genotype GPU 28-2081 recorded finger length of 10.7 cm (average of four locations) in comparison with its parental variety GPU 28 (7.8 cm) and other check varieties GPU 67 (6.6 cm) and MR 6 (9.9 cm). GPU28-2081 recorded days to 50% flowering of 82.6 days (medium flowering) and recorded 7.4 number of fingers and recorded grain yield of 3.4 t/ha. GPU 28-2081 is unique in terms finger length which is one of the important yield attributing traits and moreover it is an improved breeding line which can be used directly used for grain yield improvement in finger millet.

34. DHBM93-3 (IC643966; INGR22034), a barnyard millet (*Echinochloa frumentacea*) germplasm with glumeless florets and seeds

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Barnyard millet is one of the oldest domesticated crops cultivated in India, Japan, Korea, China and Africa. There are two cultivated species viz., Indian barnyard millet (*Echinochloa frumentacea*) and Japanese barnyard millet (*Echinochloa esculenta*). This crop is mainly cultivated for both grains and fodder in hilly areas like Uttarakhand and Arunachal Pradesh and plain areas like Tamil Nadu, Andhra Pradesh, Karnataka, Telangana. Nutritionally barnyard millet

is rich in dietary fibre content, Iron and Zinc and vitamins.

The recombination breeding is completely lacking in small millets because of the difficulties in doing crosses with tiny flower size and large number of flowers per panicle. The flower opening to remove anthers in emasculation is not a successful way as it leads to drying of the floret. 1000-1200 florets per panicle will open at different time and complete anthesis completes in 7-8 days. The tiny flowers will be

covered with two glumes, two lemma and two palea. Lemma and palea are tightly attached and forceful opening damages the ovary and stigma. Anthesis starts from top to bottom but within each receme of the panicle, florets arranged on side of the rachis will open first and middle florets will open later. All these difficulties make the emasculation and pollination work difficult in barnyard millet. The mutant line is having 90% of the florets not covered with glume and covered only with lemma and palea. Lemma and palea are transparent in nature in barnyard millet which makes to see the anther development without opening of the flower. This glumeless character helps the breeder to select florets which are going to open tomorrow and emasculate today or the fully developed anthers can be seen without opening of florets which helps to identify the florets for emasculation. This definitely helps the breeder to go for hand emasculation and pollination in barnyard millet which is otherwise a difficult

work in the highly self-pollinated crop like this.

The original variety DHBM93-3 (national check variety) was treated with gamma irradiation at 300 Gy. In M5 generation of a population plants with glumeless were identified and advanced to next generations for improving stability. The glumeless mutant DHBM93-3 (DHBM93-3-8-3-32-43) is having normal plant height of 210 cm with erect growth habit. There is no pigmentation on leaf sheath and glumes like original DHBM93-3. The number of productive tillers produced is 2-4 and the panicle length of 20-22 cm and width of 1.8-2.0 cm. The number of recemes produced is ranging from 36-45 per panicle. The days to flowering is between 70-75 days and days to maturity is between 110-115 days. The average fodder yield of two replications sown in 3m row length of two rows is 2.0-2.5 kg and the grain yield is 1.0-1.2 kg. The panicle shape is compact and the florets are arranged on both sides of rachis.

35. 170-SB-19 (J-2642) (IC643982; INGR22035), a pearl millet (*Pennisetum glaucum*) germplasm with high Fe content (84 ppm); high Zn content (50 ppm)

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Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is an important coarse grain cereal and forage crop of the arid and semi-arid region of India and several African regions and is grown on 26 million ha in some of the most marginal arid and semi-arid tropical environments of Asia (11 million ha) and Africa (15 million ha), is a major source of dietary energy and nutritional security for a vast population in these regions (Velu *et al.*, 2008). Micronutrient malnutrition has been recognized as vast health issue, affecting over two billion people, mostly women, children and infants worldwide especially in developing countries (WHO 2001 and Berwal *et al.*, 2019). Several approaches, such as medical supplementation, industrial food fortification, dietary diversification and crop bio-fortification have been suggested to address the problem of micronutrient malnutrition. Biofortification refers to the development of crop cultivars with higher level of micronutrients. Bio-fortification of this crop through conventional plant breeding approaches is the cheapest method for achieving this objective. For this it is first and most important thing to have micronutrient dense parental lines, therefore, the present investigation was carried out to identify the micronutrient dense pearl millet parental line to develop bio-fortified pearl millet hybrids.

The proposed parental line tested across 6 locations viz., Mandor, Jaipur, Hisar, New Delhi, Jamnagar and Dhule in zone A and B during kharif 2019 with view to identify the biofortified lines having high Fe and Zn. The trial comprises 22 entries including two checks having high Fe and Zn

contributed by different centers of ICAR- AICRP on Pearl millet. In this trial, pearl millet research station, Junagadh Agricultural University, Jamnagar was contributed five entries (J-104, J-2615, 170-SB-19, 171-SB-19 and 172-SB-19). The proposed parental line 170-SB-19 (J-2642) is developed at Pearl Millet Research Station, JAU, Jamnagar and its pedigree is (J-2532 × J-2571)-23-1-1-B- B and designated in the year 2021. Among the all parental lines along with two checks viz; PCMHFeB-05 and Dhanshakti analyzed for iron (Fe) content, Zinc (Zn) content, days to flowering, plant height, panicle per plant and agronomic scores. The Fe content ranged from 44 ppm to 136 ppm and Zn content varied from 22 ppm to 63 ppm. On the basis of average value of all the location, six parental lines (170-SB-19, H 19/0002, PCMHFeB-05(C), PCMHFeR17-11, PCMHFeR-17-6 and PCMHFeR-17-7) recorded >85 ppm Fe and >50 ppm Zn in grain. A strong positive correlation was observed between Fe and Zn contents. These results are earlier reported a large variability for grain Fe and Zn content in pearl millet, involving inbred lines, improved open pollinated varieties and germplasm by (Berwal *et al.* 2019 and Velu *et al.*, 2007, 2008). The proposed parental line will be useful for development of biofortified pearl millet hybrids.

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biofortified pearl millet with high grain iron and zinc content. *Crop Improvement*, 126: 182-185.
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36. EC499760 (EC499760; INGR22036), a lentil (*Lens culinaris*) germplasm with bold seed (7.1-7.83 g)

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Lentil (*Lens culinaris* Medik.) is one of the major cool-season pulse crops worldwide. Its increasing demand as a staple pulse has led to the unlocking of diverse germplasm collections conserved in the genebanks to develop its superior varieties. The Indian National Genebank, housed at the ICAR-National Bureau of Plant Genetic Resources, New Delhi, India, currently has 2,324 accessions comprising 1,796 indigenous and 528 exotic collections. Characterization was conducted to unveil the potential of lentil germplasm by assessing its agro-morphological characteristics

and diversity, identifying trait-specific germplasm, and developing a core set. The complete germplasm set was characterized for two years, i.e., 2017–2018 and 2018–2019, and data were recorded on 26 agro-morphological traits. The results revealed significant variations as evident from the mean, range, and coefficient of variations. An unique accession EC499760 was observed with very bold seeds, weighing 7.1–7.83 g of 100 seed weight. The bold seeded expression was validated under field conditions over the years and location.

37. IC241532 (IC241532; INGR22037), a lentil (*Lens culinaris*) germplasm for early flowering (51 days) and maturity (93 days)

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Lentil (*Lens culinaris* Medik.) is one of the major cool-season pulse crops worldwide. Its increasing demand as a staple pulse has led to the unlocking of diverse germplasm collections conserved in the genebanks to develop its superior varieties. The Indian National Genebank, housed at the ICAR-National Bureau of Plant Genetic Resources, New Delhi, India, currently has 2,324 accessions comprising 1,796 indigenous and 528 exotic collections. Characterization was conducted to unveil the potential of lentil germplasm by assessing its agro-morphological characteristics and diversity, identifying trait-specific germplasm, and developing a core set. The complete germplasm set was characterized for two years, i.e., 2017–2018 and 2018–2019,

and data were recorded on 26 agro-morphological traits. The results revealed significant variations as evident from the mean, range, and coefficient of variations. The days to 50% flowering and maturity ranged from 51 (IC241529 and IC241532) to 123 and 93 (IC241529 and IC241532) to 140 days, respectively. A total of 50% of plants with accessions such as IC241532 and IC241529 exhibited very early flowering (51 days) relative to all checks used in the study. For trait stability, These accessions was evaluated under multi-location trial at NBPGR, New Delhi, NBPGR, RS, Ranchi and ICAR-IIPR, RS, Bhopal and ICARDA, Amlaha, MP subsequently and this trait was expressed across the locations. In the rice-fallow areas of South Asia, the top soil layer generally dries at the harvesting

stage, making it impossible to sow the next crop. Under such conditions, extra-early lentil germplasm can be used as donors to breed early maturing varieties to fit into the cropping system and convert these mono-cropped areas into double-cropped areas, thereby increasing the production of lentils under rice-based systems. In a previous study, a lentil accession with 59 days to flower was reported (Kumar and

Solanki, 2014). However, the present experiment discovered accessions, IC241529 and IC241532, flowered in 51 days.

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38. IC259504 (IC259504; INGR22038), a wild bean (*Vigna vexillata*) germplasm highly resistant to bruchid

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Genus *Vigna* although a potential grain leguminous group is greatly affected by bruchid infestation in terms of production and post-harvest storage. Moreover, there is dearth of natural and reliable sources of bruchid resistance in cultivated *Vigna*, possibly because of low seed yield owing to a narrow genetic base. Therefore, a diverse sample of 69 black gram germplasm accessions, representing landraces and crop wild relatives (CWR), were assessed for resistance against *Callosobruchus maculatus*. Considerable variation was observed in physical seed traits. Growth parameters like total oviposition, emergence holes, adult emergence (AE), percent seed weight loss (PSWL) and growth index (GI) varied significantly among

the accessions. Based on three key traits viz. AE, PSWL and GI the accessions were classified into six groups viz., immune (1), resistant (R), moderately resistant (MR), moderately susceptible (MS), susceptible (S) and highly susceptible (HS). Accessions IC259504 (*V. vexillata*) were immune to bruchid infestation, respectively supported by X-ray radiographic evidence. Correlation heat matrix indicated GI was positively correlated with AE ($r = 0.780$) and PSWL ($r = 0.574$). Seed hardness showed a significant negative correlation with AE ($r = -0.379$). The reported immune accessions could be utilized in various breeding programs for the development of bruchid resistant cultivars in *Vigna* species.

39. IC248326 (IC248326; INGR22039), a wild bean (*Vigna vexillata*) germplasm resistant against *Callosobruchus maculatus*

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Bruchids (Coleoptera: Bruchinae) are serious pests of stored pulses causing up to 100% grain losses under unattended conditions. Among several species, three species viz., *Callosobruchus chinensis* L., *C. maculatus* F., and *C. analis* F., are highly destructive and cosmopolitan in nature. Management of bruchids remained challenging over decades as numerous methods adopted were found ineffective across space and time. In this situation, identifying bruchid resistance in germplasm lines, cultivars or landraces is advocated through HPR studies. Simultaneously, introgression of alien genes from crop wild relatives offers a viable option to diversify and widen the genetic base of legume varieties (Pratap et al., 2020). Identification of resistant wild mungbean (TC1966) against *C. chinensis* opened an array of opportunities to explore wild *Vigna* relatives including cultivated ones for bruchid resistance. Several successful hybridization attempts were made to transfer bruchid resistance genes to the

cultivars of interest. Further, studies also identified QTLs responsible for bruchid resistance in wild *Vigna* and their progenies under targeted breeding efforts. However, the studies related to identifying resistance sources from diverse genetic resources in wild *Vigna* species and the attempts to transfer identified genes to cultivated ones still remains limited and therefore warrant additional research.

Considering the above, the study was conducted to screen the wild *Vigna* panel of 42 wild *Vigna* accessions representing thirteen *Vigna* species endemic to India for their relative resistance to three bruchid species (*C. chinensis*, *C. maculatus* and *C. analis*) between 2019-21 at ICAR- IIPR, Kanpur, India. The seeds of *Vigna* accessions were obtained from the Germplasm Bank, Division of Crop Improvement, ICAR- IIPR, Kanpur (India). The qualitative and quantitative seed traits of all the accessions were recorded (following NBPGR minimum descriptors to assess their influence on

bruchid infestation and to identify the trait/s responsible for accessions' resistance reaction to bruchids. The accessions were screened against the three bruchid species following a thorough screening method involving 'Free-choice', 'No-choice' and confirmatory 'no-choice', tests (Revanasidda *et al.*, 2021). The accessions were categorized based on per cent seed damage. Based on seed damage, the accession IC248326 of *V. vexillata* was found resistant against *C. maculatus* with 1.1, 4.4 and 1.11 per cent seed damage in free-choice, no-choice and confirmatory no-choice experiments, respectively even though the seeds received an average of 1, 1.3 and 2.44 eggs per grain, respectively. Advanced correlation studies indicated that the seed morphological parameters did not influence resistance reaction significantly indicating involvement of certain biochemical components

in seed coat and cotyledons. Therefore, this accession can be an invaluable genetic resource for introgression breeding for multiple traits in mungbean and urdbean. Simultaneously, this accession may be deployed in introgression breeding to develop bruchid resistant superior cultivars in *Vigna* crops.

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40. IC331436 (IC331436; INGR22040), a wild bean (*Vigna stipulacea*) germplasm with early flowering (20 days after sowing) and early maturity (49 days after sowing)

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Among various wild and useful *Vigna* species, *Vigna stipulacea* (Lam) Kuntz., is an important one with tremendous potential to be utilized in the *Vigna* improvement programme. This species is a reservoir of various useful traits viz., faster growth, shorter duration and broad resistance to various pests and diseases. The desirable traits can be transferred to the cultivated *Vigna* species. To evaluate and select the best germplasm to be harnessed in the breeding programme, we assessed the genetic diversity of *V. stipulacea* conserved in the Indian National Genebank, based on morphological traits and microsatellite markers. A diverse panel of 94 accessions (acc.) of *V. stipulacea* conserved in the NGB housed at ICAR-NBPGR, New Delhi was grown at ICAR-Indian Institute of Pulses Research (ICAR-IIPR), Kanpur, Uttar Pradesh (26°27'N latitude, 80°14'E longitude, 452.4 meter above mean sea level (AMSL) for two consecutive years, i.e., during the rainy season (*Kharif*) 2018 and 2019. Significant

variation was recorded for the morphological traits studied. Accessions IC331436 was identified for, early flowering. IC331436 was flowered in 20 days after sowing (Gore *et al.*, 2022). Crop duration has a direct impact of fitting a cultivar in a cropping system, and early duration varieties can fit well in short-season windows, thereby offering the opportunities of horizontal increase in area (Pratap *et al.*, 2013).

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41. IC251442 (IC251442; INGR22041), a rice bean (*Vigna umbellata*) germplasm highly resistant to *Callosobruchus maculatus* F

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Among 1700 species of bruchids, nearly 20 species cause economic losses to the stored pulses. Among these, three

species viz., *Callosobruchus chinensis* L., *C. maculatus* F., and *C. analis* F. are considered to be highly destructive and

cosmopolitan in nature (Aidbhavi *et al.* 2023). Management of bruchids remained challenging over decades as innumerable methods adopted have been found ineffective across space and time. Host Plant Resistance (HPR), one of the traditional yet most effective methods being adopted for decades. Efforts were made to find resistant sources in various wild legumes, particularly in *Vigna* subgenus *ceratotropics*. Initial identification of bruchid resistance was done in a wild mungbean accession, TC1966, against *C. chinensis*, followed by identification in both cultivated and wild *Vigna* germplasm lines. Hybridization was also attempted between a bruchid susceptible mungbean cultivar VRM (Gg) 1 and a bruchid resistant wild rice bean genotype TNAU Red, revealing a major QTL (qSD05) on chromosome no. 05 responsible for bruchid resistance.

This study focused on screening 42 diverse and endemic *Vigna* accessions three bruchid species (*C. chinensis*, *C. maculatus* and *C. analis*) using improved screening methodology at ICAR- IIPR, Kanpur. The qualitative and quantitative seed traits of all the accessions were recorded following NBPGR minimum descriptors to assess their influence on bruchid infestation and to identify the trait(s) responsible for accessions' resistance reaction. The

accessions were categorized based on per cent seed damage (Revanasidda *et al.*, 2021b). The accession IC251442 of *V. umbellata* was found highly resistant against *C. maculatus* with 0 per cent seed damage in both first and confirmatory screening experiments. Interestingly, the accession IC251442 of *V. umbellata* has also been reported to be photo- and thermo-period insensitive as well as highly resistant to MYMIV. Therefore, this accession can be an invaluable genetic resource for introgression breeding for multiple traits in mungbean and urdbean. This accession can be characterized by semi-erect growth habit, indeterminate growth pattern and dark green leaves. It has light yellow flowers, smooth, curved and semi flat pods, shiny, beige, oval and large seeds.

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42. IC009634 (IC009634; INGR22042), a rice bean (*Vigna umbellata*) germplasm with bold seeds (100 seed weight 37.44 g)

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Ricebean (*Vigna umbellata* (Thunb.) Ohwi and Ohashi), a multipurpose legume of Fabaceae family mainly grown in *Kharif* season. In the North-eastern hills of India, ricebean is used as a pulse, vegetable, and fodder crop. A total of 2,202 accessions of ricebean collected across the geographical areas of India, which has been conserved in the National Genebank of India at Indian Council of Agricultural Research-National Bureau of Plant Genetic Resources (ICAR- NBPGR), New Delhi. During routine seed monitoring for seed viability in 2020, one unique accession IC009634 (collected from Sikkim) was noticed for the first time with very bold seeds, weighing 37.44 g of 100 seed weight. To date, we have not found any report in literature with such a stable genotype with high average 100 seed weight in any cultivated ricebean genotype. Hence, to validate the bold seededness IC009634 is grown at two

locations viz; ICAR-NBPGR, Regional Station, Shimla (31°5 'N and 77°5'E with 1924 amsl) and ICAR-NBPGR, Regional Station Shillong (25°41'N long 91°55'E with 100 amsl during *Kharif* 2020 and 2021. As ricebean is temperate crop, both these growing stations are well suited for getting the desirable expression. The bold seeded expression was validated under field conditions at both the locations during *Kharif* 2020 and 2021 (Gore *et al.*, 2022). This accession has the potential to be utilized in the *Vigna* improvement programme.

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43. IPF 2014-16 (IC640783; INGR22043), a pea (*Pisum sativum*) germplasm moderately resistant to rust

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Recognition of stable and durable resistance genotypes of field pea against rust, followed by utilization of these genotypes as donors in a resistance breeding program would be a best strategy for disease management in a trustworthy way. During recent past sincere efforts have been made and numbers of genotypes have been developed with good level of resistance to rust. As mentioned above resistance against this pathogen is partial and influence the magnitude of environmental and genotype-by-environment interaction was assessed to understand the dynamism of resistance and identification of durable resistant genotypes through multi-location and multi- year evaluation for rust. A panel of 23 promising field pea genotypes was assessed under inoculated conditions for rust disease for two consecutive years at six locations in India. Genotype namely IPF-2014-16 and IPF- 2014-13 identified as “ideal” genotypes, which can be recommended for release and exploited in a resistance breeding program for the region confronting field pea rust.

Morpho-agronomic characteristics

The genotype IPF 2014-16 is tall type (118-130 cm) and leafy type. It has white colour flowers, white & round seed and yellow cotyledon. It flowered and matured in 55-60 days and 105-110 days respectively with 18.0-20.0 gm 100- seed weight. The average yield of the genotype is more than 2.0t/ha.

Associated characters and cultivated practices

This genotype also has powdery mildew resistance and good yield potential (20-22 qt/ha). It can be grown on different type of soil; however, a well-drained soil is essential to achieve good yield potential of this genotype. The field should be well prepared by two to three ploughings. The seed treatment should be done with fungicide like Thirum/Captan/Carbendazim @ 3.0 g/kg seed + rhizobium culture @ 1 packet/10 kg + Trichoderma @ 4.0 g/kg at 4-5 days before sowing. The optimum sowing time is October 15-November 15 with 75-80 kg/ha seed rate. If available, about 20 tonnes of organic manures should be incorporated in the soil at the time of land preparation. This should be supplemented with 20-30kg/ha nitrogen as starter dose in basal dressing forms at the time of sowing. The phosphorus and potassium should be applied as a basal dose based on soil test. If soil is deficient in these nutrients, apply 40kg/ha P₂O₅ with 20-30 kg K₂O and 20kg Sulphur per hectare. For Zinc deficiency 0.5 per cent Zinc sulphate +0.25 per cent lime should be applied after the appearance of deficiency symptoms. First irrigation should be given at 45 days and second, if needed, at pod filling stage. This genotype has good worth as a donor in future field pea breeding programme towards development of field pea varieties with good level of resistance against rust and in other basic studies.

44. IPFD 18-26 (IC640781; INGR22044), a green seeded pea (*Pisum sativum*) germplasm with extra early maturity and resistance to powdery mildew and rust

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The prolonged *kharif* season enforces farmers to undertake field pea under late planting that eventually exposes the crop to terminal high temperature (> 30°C) resulting into increased incidence of powdery mildew, rust and low

productivity (Mishra *et al.* 2020). Therefore, to avoid or escape the aforementioned constraints, development of extra early maturing varieties (<100 days maturity) has become mandatory to endorse this crop in these circumstances (Dixit

et al. 2014; Parihar *et al.* 2021). Hence, dedicated breeding efforts were made at ICAR-IIPR, Kanpur, using inter-specific hybridization between *Pisum sativum* L. var. *arvense* (DDR 30, DDR 23) with *Pisum sativum* L. var. *hortense* (VRP 22) and the crosses were synthesized. The superior single plants were selected in different generations as per pedigree methods. On the basis of earliness i.e. days to 50% flowering and days to maturity, superior fixed lines were identified and the most promising lines were further evaluated consecutively for four years viz. 2017-18, 2018- 2019, 2019-20 and 2020-21 in post rainy season with available early type released varieties of field pea and vegetable pea. In addition, staggered planting (29/12/18;12/01/19) of the same material was also done during winter 2018-19 to further confirm the earliness. Considering over the years and staggered planting performance, one genotype IPFD 18-26(DDR 23 x VRP 22) was identified as an extra early green seeded genotype, taking < 40 days to achieve 50% flowering and < 100 days to achieve maturity. Hence, IPFD 18-26 can be used as a donor to develop extra early varieties of field pea and also for academic studies related to earliness, seed coat colour etc. Furthermore, it possesses high degree of resistance to powdery mildew and rust.

Morpho-agronomic characteristics

IPFD 18-26 is a dwarf (45-55 cm) and leafy type with white flowers. The dried seed is round and green in colour with 100-seed weight of 16- 22g. It takes 36-44 days to achieve 50% flowering and maturity period varied between 93- 103 days.

45. EC564816 (EC564816; INGR22045), a semi-leafless field pea (*Pisum sativum*) germplasm with intact/extended funiculus

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Introduction

Field pea is a minor winter season pulse crop of India and is being mainly cultivated in Uttar Pradesh, Madhya Pradesh, Bihar, Assam and Odisha (Anonymous 2019). Being an admirable source of quality protein, starch, minerals and vitamins, it is widely used as an important component in many food industries (Parihar *et al.* 2016). The crop wild relatives are reservoir of many traits especially in terms of resistance to biotic & abiotic stresses, reduction or loss of dormancy, hard-seeds, seed shattering, toxic compounds in seed coat etc. These set of traits, which are considered as a defensive traits for plant or seed, have been extensively modified during domestication through conscious or unconscious selection/ breeding to meet the requirement of human kind. Like these traits, the intact/extended funiculus is expected to be a defensive/functional trait developed by field pea seed. Generally, field pea seed remains attached to mother plant through funiculus which is the only means

Associated characters and cultivated practices

IPFD 18-26 also demonstrated resistant against powdery mildew and tolerant to rust. This genotype can be cultivated on well drained soil to achieve good yield potential. The field should be well prepared by two to three ploughings. Before 4 to 5 days of sowing, seeds should be treated with fungicide like Thiram/Captan/Carbendazim @ 2.0 g/kg seed, rhizobium culture @ 1 packet/10 kg seed + Trichoderma @ 4.0 g/kg. The sowing should be done between second fortnight of October to first fortnight of November with 100 kg/ha seed rate. At the time of field preparation 40 kg N, 40-60 kg P₂O₅; 20-30 kg K₂O; 20 kg S; 15 kg ZnSO₄ should be applied. First irrigation should be given at 45 days of sowing and second, if needed, at pod filling stage.

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of communication between seed and mother plant. When the field pea seed matures, the seed detaches itself from the mother plant from the funiculus, and the mature harvested seed normally does not have funiculus attached to them. However, two accessions (EC 564814 and EC 564816) were found to have the funiculus intactly attached with the mature seed even after harvest during 2017-18. The occurrence of intact funiculus in these accessions was further confirmed during *rabi* season of 2018-19, 2019-20 and 2020-21. During seed development, the funiculus provides anchoring and structural support to a developing embryo; also it makes the nutrients available for embryo (Endo 2012; Chan and Belmonte, 2013). However, the role of intact funiculus after maturity and harvesting is not yet known. The hilar-micropyle region is reported to be an initial point of entry for water in legume seeds (Lamichaney *et al.* 2017). The hilar-micropyle region is covered by the funiculus and when the seed matures, it detaches itself from the funiculus,

exposing the hilar– micropyle region. Presence of funiculus in harvested seed as recorded in this investigation may result into slow rate of water imbibitions as intact funiculus could become a physical barrier for entry of water to seed during imbibitions. The investigation suggested that the presence of funiculus in harvested seeds has a significant but negative role in rate of water uptake, as the seeds of both the accessions imbibed more water when funiculus was removed. Such an effect on water uptake, which triggers many physiological metabolisms of dry seed, could have an effect on rate and final germination, dormancy, seed shattering, and cooking time. About 10 million ha area in eastern India is under rice fallow, where initial establishment of the crop is a real challenge due to high initial moisture in the field. Developing field pea varieties with intact funiculus will help in slow uptake of water thus prevents the imbibitional embryo damage due to rapid uptake of water in the standing rice crop and may help in better initial vigour and crop growth. Intact funiculus is not a common trait observed in field pea and may also serve as a morphological marker in seed production programme. Hence, this intact funiculus accessions can be used as donor in field pea breeding program and also for various genetical studies.

Morpho-agronomic characteristics

The genotype (EC564814) is dwarf and leafy type with plant height of about 45-50 cm with purple flower and brown

seed. It flowered and matured in 70-75 days and 105-110 days, respectively with 21-22 g of 100-seed weight. The seed recorded an average length, width and thickness of 7.76, 8.32 and 5.79 mm, respectively. Likewise, the genotype (EC564816) is semi-leafless type with plant stature of about 80-90 cm, purple flower and dark brown seed. It flowered and matured in 80-85 days and 115-120 days, respectively with 17-18 g of 100-seed weight. The seed recorded an average length, width and thickness of 6.42, 7.02 and 5.38 mm, respectively.

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46. IPM 526-11 (IC641993; INGR22046), a mung bean (*Vigna radiata*) germplasm highly resistant to yellow mosaic disease caused by mungbean yellow mosaic India virus.

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Among the biotic stresses, yellow mosaic disease (YMD) is the most important factor negatively affecting the productivity of mungbean (Pratap *et al.*, 2020; Singh *et al.*, 2019; 2020). YMD is caused by at least three begomovirus species (Mungbean yellow mosaic India virus, Mungbean yellow mosaic virus and Horsegram yellow mosaic virus) individually or as mixed infection in the majority of pulse crops including mungbean. The disease is initially characterized by the appearance of yellow and green irregularly shaped areas on the infected leaves of the host plants. The leaf size is gradually reduced, which is followed by complete yellowing and drying. The number of flowers and pods in severely infected plants also become drastically reduced and fewer pods with small and shrivelled seeds are produced in severely affected plants. Furthermore, in severe cases, susceptible mungbean genotypes suffer complete

yield loss, which depends upon the stage at which the crop is infected and may vary from 10-100% (Akram *et al.*, 2021).

Host plant resistance (HPR) is advocated as the best strategy to tackle this issue which relies upon the intrinsic capacity of the plant to resist the disease. The plant materials for the study included a diverse panel of 71 elite mungbean lines including 9 cultivars that are currently in the active seed chain and 62 advanced breeding lines including one susceptible check 'DGGV 2'. All the released varieties and advanced breeding lines were developed at ICAR-IIPR between 2002-2016 at ICAR-IIPR, Kanpur following recombination breeding. For this, a series of crosses were attempted between 2002-16 using identified genotypes with the superior agronomic base as the recipients and some elite donors to transfer the lacking trait. All the donors used were resistant to YMD. Each breeding line was subjected to

single plant selection at F_2 and subsequent stages thereby leading to the identification of high performing stable lines. The identified lines were then subjected to screening against YMD caused by Mungbean yellow mosaic India virus (MYMIV) continuously for 4 years i.e. 2016-2019 during *Kharif* season. The identity of the YMD-causing virus as MYMIV and its prevalence was ascertained through molecular analysis (Akram *et al.*, 2021).

It was observed that the 71 test genotypes recorded the average disease rating score between 1-4 as against 7.75 in YMD susceptible check/infectior row (DGGV2) during all 4 years of testing (2016-2019). DGGV 2 showed high susceptibility to the disease recording a score of 7-9 on a 1-9 scale. This suggested those sufficient sources of inoculums, as well as the vector population, were present during all the crop seasons. Among the test genotypes, 48 exhibited high resistance (1-2 scale) against the disease over the four years of testing. The genotype IPM 526-11 consistently recorded a disease rating score of 1 against YMD and no visible symptoms were observed on the leaves. The plant growth, flowering as well as pod setting were quite normal and the genotype matured in 63-71 days over the years. Therefore, this genotype was identified to be highly promising for resistance against YMD and therefore recognized as a unique material for registration for YMD resistance. This genotype was developed from the cross PDM 139 x SPS 87. Incidentally, both the parents involved in the crossing programme are also resistant to YMD while the genotype PDM 139 is an early

maturing variety recommended for cultivation during the Summer season. Owing to its consistently resistant reaction against YMD during the generation advancement stage as well as extensive screening along with other genotypes and the susceptible check, IPM 526-11 has been identified as having a high level of resistance against YMD caused by MYMIV. Therefore, this genotype can be deployed for introgression breeding for transferring resistance against YMD caused by MYMIV and also tested for its agronomic performance and released as a cultivar, if found promising.

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47. DMR-2-0-7 (IC643987; INGR22047), a cauliflower (*Brassica oleracea* var. *botrytis*) germplasm carrying single dominant gene Ppa207 for downy mildew resistance and highly resistant to downy mildew disease

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Cauliflower is an important vegetable crop grown around the world and downy mildew caused by *Hyaloperonospora parasitica* (Pers.:Fr) Fr. Constantinescu and Fatehi is a most devastating disease causing a heavy toll of the crop during November –January months in north Indian plains. The disease occurs during vegetative to curd development stage in mid group of cauliflower. In the absence of host plant resistance, fungicides are being used to control the disease, which are not only proving to be ineffective in controlling this oomycete pathogen, but are becoming cause of health hazards and environmental pollution. Therefore, this line 'DMR-2-0-7' will have tremendous potential in using downy mildew resistance breeding programmes which can go a long way in saving the input cost of farmers, protecting consumers from health hazards and keeping the environment safe. Disease incidence (DI) of the genotype was observed to be

0 to 0.74% under artificial inoculation condition (Table 1). Genetic studies showed that the resistance is governed by a single dominant gene *Ppa*²⁰⁷. The resistant gene *Ppa*²⁰⁷ was mapped in 4.8 cM linkage interval on linkage group 2 (C02) of cauliflower, flanked by the markers BoGMS0486 and BoGMS0900 at 3.6 and 1.2 cM, respectively (Saha *et al.*, 2020).

Morpho-agronomic characteristics of downy mildew resistance line DMR 2-0-7

The downy mildew resistant line DMR 2-0-7 was derived from homozygous progeny from a cross of 3-5-1-1 (resistant to downy mildew) × DC-466 (susceptible to downy mildew). The plants are vigorous with dark green leaves which are long having prominent mid-rib and strong puckering on margins. Curds are compact, cream white and medium in size, curd weight is in the range of 600 to 700 g. Curds gets

Table 1: Response of DMR-2-0-7 and commercial cauliflower varieties to downy mildew disease in four different season/environment

Genotype/lines	DI in Environment 1/ season 1 (a)	DI in Environment 2/ season 2 (b)	DI in Environment 3/ season 3 (c)	DI in Environment 4/ season 4 (d)	Disease reaction*
DMR-2-0-7	0.0	0.74	0.0	0.0	Resistant
Pusa Sharad	100	96.29	97.04	100	Highly susceptible

Scoring for downy mildew disease and Disease Incidence (DI) calculation according to Mahajan et al. (1991).

a) Published in peer reviewed journal Euphytica (2020) 216:183 <https://doi.org/10.1007/s10681-020-02696-6>, b) Institute annual report of IARI (2020, Page 36), c) Institute annual report of IARI (2019, page no 26)

ready for harvesting in the month of mid- January. Similar to mid-late group genotypes, the 'DMR 2-0-7' line requires 16-20°C temperature for curd initiation and development. The crop becomes ready to harvest in 70-80 days after transplanting in its normal growing season. Desirable horticultural traits along with single dominant gene *Ppa*²⁰⁷ governed downy mildew resistance in mid-group cauliflower genotype DMR 2-0-7 can be explored in downy mildew resistance breeding and hybrid development.

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48. Newar landrace (IC643967; INGR22048), a radish (*Raphanus* sp.) germplasm with high tolerance to irrigation water salinity (EC_{iw} 8-10 dS/m) and soil sodicity (pHs 8.5)

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Introduction

Radish landrace 'Newar' (*Raphanus* sp.), endemic to Jaunpur district of Uttar Pradesh, India, is highly valued for its better keeping long crisp roots, salt tolerance, use in pickling and seed oil extraction. However, changing land use pattern and consumer preferences, and disappearance of the seed network have badly hit its conservation and cultivation (Singh *et al.* 2018).

Morpho-agronomic Characteristics

Based on the DUS descriptors (Srivastava *et al.* 2001), 'Newar' is characterized as a tropical bolting type with mildly pungent, creamy white and crisp roots free from pithiness. It has dark green leaves with purple mid ribs and petioles. It produces light brown bold seeds that maintain viability up to 8 years (Singh *et al.* 2018). The number of leaves (25.0 + 5.0), leaf length (39.5 + 1.0 cm), root length (35.0 + 3.5 cm), root weight (315.0 + 25.0 g) and 1000-seed weight (average 13.5 g) are the distinct morpho-agronomic characteristics (Table 1) of 'Newar' (Singh *et al.* 2020).

Associated Characters and Cultivation Practices

'Newar' displays an exceptionally high salt tolerance in terms of early (4.5 days) and very high (98.5%) seed germination, and high plant vigour at irrigation water salinity (EC_{iw}) of 10.0 dS/m, and maintenance of root fresh weight up to EC_{iw} of

Table 1: Characterization of radish landrace 'Newar' based on DUS descriptors

S. No.	Characteristic	Stage of observation	Remark
1	Early plant vigour	30 DAS	(7) Good
2	Plant growth habit	Flowering	(6) Elongate branching stem supporting leaves and/or heads
3	Leaf colour	30 DAS	(4) Dark green with purple midribs
4	Leaf length (cm)	MRHS	39.5 + 1.0
5	Leaf width (cm)	MRHS	11.5 + 1.0
6	Leaf margin	MRHS	(1) Crenate
7	Leaf apex shape	MRHS	(4) Oval
8	Leaves per plant	MRHS	25.0 + 5.0
9	Leaf pubescence	MRHS	(7) Abundant
10	Petiole length (cm)	MRHS	7.5 + 1.5
11	Petiole colour	MRHS	(7) Purple
12	Days to 50% root harvest	MRHS	50.0+3.0
13	Crown head habit	MRHS	(1) Erect
14	Crown head colour	MRHS	(1) Light green
15	Crown head diameter (cm)	MRHS	5.0 + 1.5
16	Root length (cm)	MRHS	35.0 + 3.5
17	Root diameter (cm)	MRHS	4.7 + 0.5

18	Root Branching	MRHS	(3) Present
19	Root skin colour	MRHS	(2) Creamy white
20	Root shape	MRHS	(2) Triangular
21	Root tail	MRHS	(1) Acute
22	Root weight (g)	MRHS	315.0 ± 25.0
23	Root pithiness	MRHS	(0) Absent
24	Root pungency	MRHS	(3) Mild
25	Root flesh texture	MRHS	(1) Crisp
26	Bolting habit	Flowering	(1) Tropical
27	Inflorescence type	Flowering	(1) Single raceme
28	Days to 50% flowering	Flowering	85.0 ± 3.0
29	Flower head size	Flowering	Average number of effective tillers: 8; with flower c diameter of 2.5 cm
30	Seed coat colour	Harvesting	(3) Light brown
31	Weight of pod per plant (g)	Harvesting	240.62
32	Seed weight per plant (g)	Harvesting	60.90
33	1000 seed weight (g)	Harvesting	13.45
34	Biotic stress susceptibility	Throughout crop season	Very low or no visible sign of susceptibility

8.0 dS/m than commercial radish cultivars like 'Pusa Mridula'. The key traits underpinning salt tolerance in 'Newar' include Na⁺ exclusion, maintenance of shoot and root K⁺ levels and a substantial increase in leaf proline activity. It also maintains root length (35.0 ± 3.5 cm) and fresh root weight (315.0 ± 25.0 g) in sodic soils (pH_s ~8.5, EC_e 1.0 dS/m). It shows very low or no visible signs of susceptibility to insect-pests and diseases. The seeds should be sown in the first fortnight of October on 25 cm high ridges at 2 cm depth, keeping row-to-row and plant-to-plant spacings of 45 cm and 30 cm, respectively.

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49. H-88-78-1 (IC644011; INGR22049), a tomato (*Solanum lycopersicum*) germplasm for multiple resistances (root knot nematode, *Alternaria solani* resistance, ToLCV resistance) and heat tolerance

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Tomato (*Solanum lycopersicum* L.) is a versatile crop cultivated worldwide for its short yield time, culinary importance and health benefits. Biotic stresses caused by pathogens, such as fungi, bacteria, viruses, nematodes etc., cause severe yield penalties leading to significant fluctuations in annual tomato production in India. On the other hand, abiotic stresses especially high temperature limits its production in one or other parts of the country. H-88-78-1 is a unique germplasm with multiple disease resistance and heat tolerance developed and maintained at ICAR-Indian Institute of Vegetable Research Institute, Varanasi. Tomato genotype 'H-88-78-1' was developed through wide hybridization followed by pedigree method

of breeding from a cross between Selection-7 × B6013. B6013 is a *Solanum habrochaites* (*Lycopersicon hirsutum* f. *glabratum*) accession.

Under pot conditions at two inoculation rates viz, 2000 and 4000 J2/Kg soil of *M. incognita*, tomato genotype 'H-88-78-1' has shown highly resistant to immune reaction. With the help of molecular markers (Mi23 & Pmi), it was confirmed that H-88-78-1 has *Mi1* gene in it (Reddy *et al.*, 2018). Under artificial inoculated conditions, tomato genotype 'H-88-78-1' gave immune reaction to *A. solani* (Singh *et al.*, 2017). The genotype has also shown ToLCD resistance under epiphytotic conditions (Singh *et al.* 2015). After 25 days of transplanting H-88-78-1 plants along with heat susceptible

Punjab Chhuhara plants of same age were exposed to high-temperature stress at 42°C by transferring them to a growth chamber. Different molecular, physiological, and biochemical analysis shown that H-88-78-1 has better heat stress profiles in comparison with the susceptible genotype (Karkute *et al.*, 2021).

Tomato line H-88-78-1 is an indeterminate plant with small fruits (average fruit weight 52 g). Fruits have an average locule number of 5 per fruit, pericarp thickness of 0.4 cm and total soluble solids (TSS) of 4.0 Brix. The line H-88-78-1 has slightly flattened shaped fruits with an average fruit length of 3.72 cm and average fruit diameter of 4.62 cm.

Mi1 is a well characterized resistance gene. With gene based markers available for *Mi1* gene, H-88-78-1 can be utilized as a parental material in nematode resistance breeding programmes of tomato. The genotypes can be used in ToLCV and early blight resistant breeding programmes. Heat stress is not only major limiting factor for crop productivity but also highly complex character. With proven heat tolerance tomato genotype H-88-78-1 can be

used in different basic and applied plant breeding programs to transfer these traits to cultivated varieties.

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50. JS2 (IC643977; INGR22050), an Indian mustard (*Brassica juncea*) germplasm resistant against *Sclerotinia sclerotiorum*; with mottle seed colour and high male and female fertility

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Introduction

The inter-generic hybridizations were attempted frequently with the aim of transfer of the genetic resistance/ tolerance to the diseases. However, their success was limited due to pre and post-fertilization barriers within the genera. Therefore, somatic hybridizations were used as an alternative approach. The *Sinapis alba* is a Brassica coenospecies which have potential for biotic and abiotic stress tolerance and is frequently used as a potential donor in somatic hybridization against these stresses (Kumari *et al.*, 2020a, 2020b). However, the stability of these somatic hybrids was limited to the gene introgression probability to the various traits (Gaikwad *et al.*, 1996). Although, very few somatic hybrids of *B. juncea* and *S. alba* were found meiotically stable (Kumari *et al.*, 2020c) so far. We have done the PEG mediated protoplast fusion between *B. juncea* cv. RLM-198 and *S. alba* (Gene Bank Accession No. DRMR-2183) as per protocol (Kirti *et al.*, 1992) with the aim of the introgression of resistance against *Sclerotinia* stem rot (Kumari *et al.*, 2020c). This genetic stock contains various interesting phenotypes that may be useful for resistance and quality breeding.

Morpho- agronomic characters

Somatic hybrid (JS2) shows intermediate morphological characters of their parents and (JS2) attaining an average 184 cm plant height. While the stems were angular in shape, branched, and covered with dense trichomes, where the leaves were considerably larger, thicker, more cuticular, deeper green in color, had more irregular pinnately lobed with serrate margins, and was more covered with dense trichomes on both the surfaces. The plants attained delayed flowering in comparison to *B. juncea* and along with *S. alba*, The inflorescence of the JS2 had an intermediate pattern of their parents (Kumari *et al.*, 2020c). The flower sizes were intermediate relative to the parents' flowers. The stigma was larger than that of the parents with hairy projections on the style, while the stamens of allohexaploids were more prominent than in the parents. The pollen grain viability was found > 91 % with high female fertility remaining as revealed by their seed setting. The siliquae of the allohexaploids were found to be intermediate in size relative to their parents with an increased number of seeds (3–5 seeds/silique) compared to *S. alba*. These allohexaploids

produced viable seeds on selfing and backcrossing with *B. juncea*, which were found to be intermediate in size relative to the parents. However, the 1,000 seeds' weight was recorded as less than their parents.

Associated characteristics and cultivation practices

The allohexaploid *Brassica* JS2 was carried the intermediate seed color of their parents (half yellow and half brown). Interestingly, this is the first instant when half yellow and half brown color seeds were bears after inter-generic protoplast fusion of the two genera which produced different colors seeds. However, these plans were found cytologically stable. The present allohexaploids were found resistant to the *Sclerotinia sclerotiorum* when screened in-vitro and in-vivo for three subsequent years and two locations (Kumari and Singh 2019, Kumari *et al.*, 2020c). The plants also showed siliquae shattering resistance during the preliminary study. However, more investigation will be required in this way.

51. JS1 (IC643976; INGR22051), a yellow seeded Indian mustard (*Brassica juncea*) somatic hybrid resistant against *Sclerotinia sclerotiorum*

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Introduction

A large number of somatic hybridizations have been attempted in oilseed Brassicas with the aim of introgression of genes responsible for many biotic stresses (Henson and Early 1997, Singh *et al.*, 2021). The *S. alba* is a Brassica coenospecies that shows several desirable features such as resistance to biotic diseases, pests and harsh environmental conditions that are unavailable in crop Brassica species (Kumari *et al.* 2020a and 2020b). Therefore, somatic hybridization between *S. alba* (Accession No. DRMR- 2183) and *B. juncea* (RLM-198) through PEG mediated protoplast fusion was attempted. Two symmetric hybrids were recovered from this fusion experiments, out of which one SH (JS₁) had yellow seed colours (Kumari *et al.*, 2020c)

Morpho-agronomic characters

The JS1 plants attained an average plant height of 216 cm. The stems of JS1 were angular, branched, and covered with dense trichomes. The leaf and flower bud margins were similar to *S. alba* with delayed flowering relative to *B. juncea*, where the leaves were considerably larger, thicker, more cuticular, deeper green in color, had more irregular pinnately lobed serrate margins, and was covered with dense trichomes on both surfaces. The inflorescence of JS1 showed an *S. alba*-like condense pattern, the flower sizes were larger in comparison to the parents' flowers. The stigma was larger than that of the

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parents with hairy projections on the style, while the stamens of allohexaploid were more prominent than in the parents. The pollen grain viability of the allohexaploid (JS1) was recorded between 91 and 93% with female fertility remaining high as revealed by their seed setting. These allohexaploids produced viable seeds on selfing and backcrossing with *B. juncea*, which were found to be intermediate in size relative to the parents. However, the weight of 1,000 seeds of JS1 was higher than their parents (Kumari *et al.*, 2020c).

Associated characteristics and cultivation practices

JS1 is carrying yellow seed color along with high degree of resistance against *Sclerotinia sclerotiorum* causing stem rot, high male and female fertility, and stable over the generation.

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52. EC523368-2 (GMU-7399) (IC643960; INGR22052), a safflower (*Carthamus tinctorius*) germplasm tolerant to safflower aphid (*Uroleucon compositae* Theobald)

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Introduction

Safflower (*Carthamus tinctorius* L.) is an important oilseed crop grown in India during the *rabi* season. Aphid, *Uroleucon compositae* (Theobald) is a serious pest on safflower which can cause upto 100% yield loss in susceptible varieties. Development of resistant cultivars assumes importance to enable environmentally sustainable crop cultivation with less input use by saving the cost of application of pesticides. Identification of sources of resistance to aphid is the pre-requisite to meet this target for which perfection of screening techniques and extensive screening has been carried out in safflower (Srinivas and Mukta *et al.*, 2015). The accession EC523368-2 is a selection from EC523368 received from USDA. The accession was selected based on non/few spiny nature of the outer involucre bracts (OIB) during 2005 at the Germplasm Maintenance Unit of Safflower at IIOR, Hyderabad and was multiplied and maintained as GMU-7399.

Morpho-agronomic characteristics

EC523368-2 (GMU-7399) possesses the following morpho-agronomic characteristics: (i) Non/few spiny nature of the outer involucre bracts (OIB), (ii) yellow disc florets turning orange at faded stage, (iii) medium height with 8-9 branches located on the main stem at 2/3rd height from top (iv) 20-25 capitula of medium size (2.3 cm diameter) and (v) white seeds with 100-seed weight of 3.7 g.

Associated characters and utility

The accession EC523368-2 (GMU-7399) has undergone repeated screening for its reaction to safflower aphid, *Uroleucon compositae* (Theobald) at ICAR- IIOR, Hyderabad. It showed tolerant reaction to *U. compositae* based on the screening parameter, aphid infestation index (A.I.I.) (1.5) while the susceptible check CO-1 showed A.I.I. of 4.9 in 2011-12 (A.I.I. grades range from 1 to 5 with 1 indicating highly tolerant reaction and 4.1-5.0 indicating highly susceptible reaction). A scale based on days to wilt was also developed based on the corresponding A.I.I. of entries and days taken to wilt. During 2015-2018, the tolerant reaction of EC523368-2 to aphid was confirmed based on days to wilt after aphid infestation (36-43 days) compared to 19-26 days in the susceptible check CO-1 (Table 1).

Table 1: Tolerance reaction of EC-523368-2 (GMU-7399) against safflower aphid (*Uroleucon compositae* Theobald) at ICAR-IIOR, Hyderabad

Year of screening	Parameter	EC-523368-2 (GMU-7399)	Susceptible check: CO-1
2011-12	Aphid infestation index (A.I.I)	1.5	4.9
2015-16	Days-to-wilt after aphid infestation	36	19
2016-17	Days-to-wilt after aphid infestation	42.1 ±0.8	20.4 ±0.8
2017-18	Days-to-wilt after aphid infestation	43	26

The major mechanism of resistance operating in safflower accession, EC523368-2 (GMU-7399) was tolerance. The accession remained stay green and was found physiologically efficient in tolerating the feeding of aphids (Srinivas *et al.*, 2018). The accession has been utilized in the development of recombinant inbred lines (RILs) through single seed descent method towards mapping of aphid tolerance in safflower based on days to wilt after aphid infestation. A major QTL associated with tolerance to aphid has been detected using SSR markers, which is currently under validation (Jegadeeswaran *et al.*, 2021).

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53. Dedia P-1 (IC643989; INGR22053), a basil (*Ocimum sanctum*) germplasm with unique dark purple leaf colour; maximum essential oil content (0.28 %); maximum eugenol content (60%), β -caryophyllene (14%) and β -elemene content (14%).

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Holy basil (*Ocimum sanctum* L.), a sacred medicinal herb is extensively used in Ayurvedic systems of medicine for various ailments from traditional medicinal systems. Medicinally the herb is useful in a variety of diseases in humans and animals. The plants flourish predominantly as herbs or shrub and survive as annuals or perennial plants in habit. The most common uses are in herbal tea, cosmetics, healing remedies, and as a preservative. From *Rama* and *Krishna* variants of holy basil, in the Indian market, *Krishna* (Shyama) tulsi is more preferred by the pharma industries in the form of dry leaf or powder due to being rich in antioxidants.

The Krishna type holy basil, Dedia P-1 is identified for distinctive dark purple leaf colour (RHS Colour chart: Greyed purple group N186 A) of upper part of leaves with greenish colour at a lower side of leaves (RHS colour chart: Yellow-green group 146 A), dark purple inflorescence and branches (Greyed purple group N186 C) colour, while check "CIM-Angana" having a purple colour leaves and inflorescence (RHS Colour chart: purple group N77A). Additionally, It has better essential oil content (0.28 %), maximum eugenol content (60%), β -caryophyllene (14%) and β -elemene (14%) in fresh herbage suitable for industrial use compared to check "Angana" (0.12 % oil content; 12 % β -caryophyllene and 9 % β -elemene) in Tables 1 & 2. Dedia P-1 is collected

Table 1: Average essential oil content (%) in fresh herbage of Dedia P-1

	2017-18	2018-19	2019-20	2020-21	2021-22	Average (%)
Dedia P-1	0.31	0.22	0.35	0.25	0.28	0.28
Angana	0.13	0.13	0.08	0.11	0.13	0.12

Table 2: Average chemical compound constitute of Dedia P-1 and CIM-Angana.

Dedia P-1	Eugenol content (%)	β -caryophyllen (%)	β -elemene (%)
2021	60.62	14.16	13.91
2020	39.15	-	-
2016	78.78	-	-
Average	60	14	14
Angana	Eugenol content (%)	β -caryophyllen (%)	β -elemene (%)
2021	40.42	11.87	8.96
2020	40.65	-	-
2016	39.94	-	-
Average	40	12	9

from the local area of Dediapada, Gujarat and maintained at ICAR-Directorate of Medicinal and Aromatic Plants Research, Boriavi, Anand, Gujarat.

54. DOB-5 (IC643990; INGR22054), a basil (*Ocimum basilicum*) germplasm with high essential oil content (0.50%) in herbage; linalool (32.13%), β -elemene (2.53%) and germacrene D (3.32%) contents

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Sweet basil (*Ocimum basilicum* L.) is an important culinary herb and source of aromatic essential oils, which gives it a unique flavor. It has been used as a medicinal as well as aromatic plant for centuries and grown in wild as a perennial herb. Basil is cultivated extensively in India, France, Egypt, Iran, Hungary, Indonesia, Morocco, Greece, and Israel, in various regions with temperate and hot climates. Sweet basil tend to have a higher percentage of methyl chavicol or anethole. Basil is commercially grown for its essential oils containing linalool (20–40%), methyl chavicol (20–25%), and eugenol (0–12%). It also contains tannins and flavonoids.

Sweet basil essential oil is extensively used in food, fragrance, and in traditional medicine for treating cough, colds, and chest congestions, and as a mosquito repellent and pesticide. The genus characterized by great variation among plant morphology, growth habit, inflorescence, leaves, stem colour, oil content as well as essential oil components. Wide morphological diversity including light green leaf colour (R.H.S. Yellow green group 146A) with club type purple inflorescence (R.H.S. Greyed purple group 187A) observed in "DOB-5". Additionally, DOB-5 has a medium plant height (70-90 cm), purple leaf colour of

newly developed leaves and mature leaves are light green colour. It has a light green to yellowish stem (R.H.S. Colour chart: Greyed orange group 165A) at full flowering. However, check variety GAB-1 having dark green leaf colour (R.H.S. colour chart: Green group N137A) with club type greenish purple inflorescence (R.H.S. Yellow green group 144A) and it has a dark green grey (R.H.S. Colour chart: Yellow green group 148C) stem colour. Under field condition, DOB-5 had moderate resistance to Anthracnose disease caused by *Colletotrichum* spp. as compared to GAB-1, which is moderately susceptible. The seeds of DOB-5 have a Dark (Jet) black colour, while check having a brownish black colour.

Secondary metabolites characterization is the main criterion for the selection of suitable aromatic varieties for herbal industry. An essential oil-rich chemotype DOB-5 is identified for better essential oil content (0.50 %) in fresh herbage compared to check variety GAB-1/AOB-5 (0.37 %) in Table 1. Additionally, DOB-5 has better Linalool (32.13%), β -elemene (2.53%) and Germacrene D (3.32%) content. However, check variety GAB-1 has a Linalool, β -elemene, and Germacrene D content about 30.65%, 1.18%, and 1.63%, respectively (Table 2).

Table 1: Oil content (%) data of MLT trials

Particulars	Year of testing	No. of trials	Proposed line DOB-05	Check variety GAB-1
Average oil content (%)	2016-17	4	-	0.28
	2017-18	4	0.52	0.42
	2018-19	4	0.55	0.38
	2019-20	4	0.50	0.41
	2020-21	5	0.41	0.36
	Mean		0.50	0.37

Table 2: Chemical profile of DOB-5

Accession	Linalool	β -elemene	Germacrene D
DOB-5 (2017-18)	-	3.12	3.6
DOB-5 (2019-20)	37.44	3.06	2.95
DOB-5 (2020-21)	26.82	1.41	3.41
Average	32.13	2.53	3.32
GAB-1 (2017-18)	-	1.40	1.42
GAB-1 (2019-20)	40.80	1.07	1.31
GAB-1 (2020-21)	20.51	1.09	2.18
Average	30.65	1.18	1.63

55. HD-6 (CIARI-Sampada) (IC590895; INGR22055), a noni (*Morinda citrifolia*) germplasm with big fruit (140-160 g), high pulp recovery; and year round yielder

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Noni (*Morinda citrifolia* L.; Family: Rubiaceae) is a source ingredient in traditional medicine system such as Ayurveda, Chinese, Polynesian, Australian and Nicobari traditional medicinal systems for curing of various ailments including arthritis, colds, diabetes, inflammation, and pain (McClatchey, 2003). It appears to be 'natural reservoir of bioactives' because more than 200 bioactive compounds have been reported including anthraquinones, flavonoids, polysaccharides, glycosides, iridoids, lignins and triterpenoids. Almost all parts of Noni plants are useful for medicinal or industrial purpose but fruits are most commonly used for pulp and juice extraction (Singh *et al.*, 2012). It is well suited potential crop for tropical coastal and island regions and Noni juice has huge potential as health supplement in national and international markets. To explore its commercial potential, it was required to develop genotype(s) which can grow well in open with high yield potential and meet the criteria of processing industry.

The HD-6 is developed by selection from local collection for higher pulp recovery, big size fruits and vigorous tree type plants. It has medium tall and sturdy tree type

genotype of Noni. Plant canopy spread is 2.0 m and height is around 3.5 m. Trunk diameter of 6 year plants is 20-25 cm with rough bark. Internodal length is small (<8 cm). This is well suited for normal distance planting at 4.0 x 4.0 m in open condition. It takes around 110-120 days from bud formation to fruit ripening. It performs well in open condition but can be grown in partial shade condition such as in interspaces in coconut and areacnut plantations without compromising yield levels. The HD-6 bears fruits year round and maintains around higher yield level to sustain industrial requirement.

The big size fruits 'HD-6' are good for fresh juice recovery 60.3 % (fresh weight basis). Total soluble solids content in juice was 9.5°Brix which was significantly higher than CIARI-1 (5.8°Brix). It was found to be rich in polyphenol (365.2 g/100 g fw) with high DPPH antioxidant activity (88.83 %) (Singh *et al.*, 2017). The HD-6 seeds contain 16.60% oil which has industrial value. The GC-MS analysis of revealed that seed oil of HD-6 is rich source of essential fatty acids.

The fruits of Noni genotype 'HD-6' are fits well with criteria of processing industry such fruits of medium to big

size (125-150 g), medium width and length, obovate to round in shape, depressed fruit base, smooth to partial surface at maturity, thin skin and cream pulp.

Hence, the HD-6 has potential for immediate use for (i) commercial cultivation for pulp and juice industry (ii) tap the nutritive and fatty acids potential in developing better health refreshing products and (iii) also in breeding and genetic studies for fruit size, juice recovery level and phytochemicals in noni.

56. IIHR-4 (IC633777; INGR22056), a tuberose (*Polianthes tuberosa*) germplasm for double type flower compactly arranged on short spike; more number of flowers open at a time (7.10); resistant to root knot nematode (*Meloidogyne incognita*)

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Introduction

Tuberose (*Polianthes tuberosa* L.) is an important bulbous flower crop belongs to the family Asparagaceae, native to Mexico. The flowers of tuberose are valued for its long lasting flowers and sweet fragrance. The genetic stock IIHR-4 was developed through hybridization by crossing Mexican Single x Pearl Double, followed by selection. It was developed at ICAR-Indian Institute of Horticultural Research, Bengaluru, Karnataka, India. IIHR-4 is unique for short upright spike, double type flower arranged compactly on spike with shorter internode, more number of flowers open at a time per spike and highly resistant to root knot nematode.

Morpho-agronomic characteristics

IIHR-4 produces shorter spike (62.00 cm) with double florets arranged compactly on spikes with shorter internode and favourable diameter of floret (4.47 cm), number of florets per spike (50.75) and more number of florets (7.10) open at a time (Table 1). The genetic stock IIHR-4 is well suited for cut flower, flower arrangement and garden display.

IIHR-4 was screened for the tolerance/ resistance against root knot nematode *M. incognita* and the results revealed that it was highly resistant under field conditions with least gall index of 1.24. Per cent disease index and host reaction of tuberose genotypes against leaf blight disease caused by *Alternaria* under field conditions were recorded and the results indicated that the breeding line IIHR-4 has better field tolerance to *Alternaria* leaf blight as compared to the other tuberose genotypes evaluated.

Associated characters and cultivation practices

Tuberose prefers tropical climate with the temperature range of 20-35°C. It grows well in loamy to sandy loam soil with the

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Table 1: Morpho-agronomic description of tuberose genetic stock IIHR-4

S. No.	Traits	Two years pooled data
1.	Days to spike appearance	154.15
2.	Spike Length (cm)	62.00
3.	Rachis length (cm)	25.59
4.	Number of florets per spike	50.75
5.	Length of floret (cm)	5.22
6.	Diameter of the floret (cm)	4.47
7.	Bud length (cm)	4.98
8.	Single flower weight (g)	3.16
9.	No. of spikes per clump	4.43
10.	Internodal length (cm)	3.45
11.	No. of florets open at a time on spike	7.10
12.	Flower type	Double
13.	Flower bud tinge	Green tinge
14.	Nature of spike	Straight
15.	Type of flower opening	Wide
16.	Reaction to Root knot nematode	Resistant
17.	Reaction to leaf blight disease	Tolerant

pH of 6.5 to 7.5. Tuberose is sensitive to water logging and hence proper aeration and drainage is necessary. It is a day neutral plant requires vivid sunlight for better flowering. The medium size bulbs of 2.5 cm in diameter are planted on the sides of the ridges with a spacing of 30 x 30 cm at 5.0 cm depth during June-July.

57. QNRCB0045/Borchampa (IC250498; INGR22057), a banana (*Musa spp.*) germplasm resistant to *Fusarium oxysporum* f. sp. cubense (Foc) race 1 (VCG 0124)

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Introduction

NRCB Acc. No. 0045 & IC250498 is a short statured variety among the Mysore sub group of AAB genomic group with 1.8 to 2.2 m height. It is commonly found in North Eastern parts of India.

Morpho-agronomic Characteristics

Pseudostem is green, on which black brown blotches appear and slightly ash coated. Leaves are droopy and appearance is dull in nature. Midrib is green in colour on both upper and lower sides but in younger leaves, midrib is pink on both upper and lower sides. Petiole is green, 30-40 cm long and has wide petiolar canal with erect margins. At the edges of the margins, there is a pink line. Base of the petiole clasp the pseudostem with its margins. There are 4-5 hands of fruits in a bunch. Male bud is small, top shaped and is dark purple in colour. Male bracts are dark purple on outer and crimson

red on inner surface. There are medium grooves on outer surface with moderate wax coating. Bract tip is gradually pointed without yellow tinges. Bract falls after the flowers fell, before falling, they show both reflex and revolute. Male flowers are cream in colour. Fruits are short and very stout, unusually plumpy fruits with a blunt tip. Bunch weight is around 6-7kgs. Fruits weigh 120 – 130 g, 9 to 10 cm long with 16 to 18 cm circumference at the middle. Pedicel is more than 1 cm long and fruits do not easily drop off from the pedicel. Dark green color fruits change into yellow color upon ripening. Pulp is cream colour, juicy, sweet with aromatic flavor.

Associated Characters and cultivated practices

It is the most fragrant among Mysore sub group. Very short duration with 9-10 months compared to other Mysore members (14 to 15 months).

58. NRCB0197/Poovan (IC250650; INGR22058), a banana (*Musa spp.*) germplasm resistant to *Fusarium oxysporum* f. sp. cubense (Foc) race 1 (VCG 0124)

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Introduction

This genotype NRCB Acc. No 0197 & IC250650 is found in throughout the country. Its tender leaf nature makes this genotype amenable for leaf industry. Fruit is slightly acidic, firm and has typical sour-sweet aroma. Fruits turn to attractive golden yellow upon ripening.

Morpho-agronomic characteristics

Plants are tall and robust with the height of 3.0-3.5m and girth of 60-70cm circumference at the base, Pseudostem is dark green with purplish tinge all along the pseudostem, shiny and there are brown black blotches on either sides of the petiolar base. Leaves are dark green, neither drooping nor erect. Upper side of the leaf is dark green but not shiny. The midrib is green on the dorsal side and pink in colour on lower surface, but the midrib of the tender leaf is pink on both upper and lower surfaces. Leaf length ranges from 2.0-2.25m with 70-75cm width. Petiole is 50 to 60cm length, green in colour and heavily wax coated. Peduncle is dark

green in colour and slightly hairy. Bunch is slightly angular in position, cylindrical in shape with 10-12 hands and they are compactly arranged and 15-16 fingers per hand. Medium sized bunch weighing 13-15 kgs, fruit hands are closely packed. Male flower bud is medium sized, purple in colour and ovoid in shape. Male flowers are medium in size and creamish pink in colour. Fruits are dark green in colour, turns towards the peduncle and has bottle necked tip. Fingers are 10-13cm in length and slightly acidic in taste. Fruits turn to attractive golden yellow on ripening. Medium sized bunch, closely packed fruits, good keeping quality and resistant to fruit cracking are the advantages of this variety.

Associated Characters and cultivated practices

Fruits do not easily drop off from the pedicel. Fruit has good keeping quality and resistant to fruit cracking which reduce the post harvest losses. Its tender leaf makes this genotype amenable for leaf industry. Crop duration is 12 to 13 months and suitable for marginal cultivation.

59. NRCB0009/ Borjahaji (IC250462; INGR22059), a banana (*Musa spp.*) germplasm resistant to *Fusarium oxysporum* f.sp. cubense (Foc) race 1 (VCG0124)

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Introduction

This genotype NRCB Acc. No. 0009 & IC250462 belongs to Cavendish sub group. This is semi tall variety with 2.2 to 2.5m height and found in North eastern parts of India. It is highly susceptible to Sigatoka leaf spot diseases. Most of the major banana growing regions of North eastern India are severely infested with Foc race 1.

Morpho-agronomic characteristics

It is a medium statured variety with plant height ranging from 2.0-2.50m and pseudostem girth of 45- 50cm at its base. Pseudostem is green with black brown blotches all along the pseudostem. Leaves are erect, green, dull on both upper and lower surfaces. Peduncle is 30-40cm in length, pubescent in nature and green in colour. Bunch is cylindrical in shape with 10-12 hands of fingers, they are

normally packed and fingers turn towards the stalk. Rachis is 50-60cm in length, completely barren and rarely few hands of persistent neutral flowers just above the male bud. Bract scars are very predominant with maximum interspaces. Pendulous in position but it has slight bend at the distal end. Male bud is brown in colour, lanceolate in shape with pointed tip. Male flowers are whitish in colour. Fruits are green in colour, 15- 20cm in length. It has gradual tip. Pulp is creamy with sweet taste. Pedicel is very short with >1cm length.

Associated Characters and cultivated practices

High yield with 20 to 25 kg/bunch, fruits are completely turned towards the peduncle and bunch is cylindrical in shape. Fruits are long measuring 20 cm and the pulp is sweet with good aroma.

60. NRCB0608/William (IC251061; INGR22060), a banana (*Musa spp.*) germplasm with *Fusarium oxysporum* f. sp. cubense Foc race 1 resistance

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Introduction

This genotype NRCB Acc. No. 0608 & IC251061 belongs to AAA genomic group of Cavendish sub group. It is a dwarf statured variety suitable for high density planting. High yielding with 20 to 25 kgs bunches, fruits are completely turned towards the peduncle.

Morpho-agronomic characteristics

Short statured with about 1.8 to 1.9m height. Pseudostem is dull in appearance and has black blotches all along the pseudostem. Light green and has black brown pigments on either sides of the petiole. Leaves are erect, green in colour, dull on both upper and lower surfaces. Peduncle is 35-40cm

long, pubescent and has black brown blotches spread all along the length with only one empty node. Bunch is huge and hangs vertically down, truncated cone shaped and slightly pendulous in position. Male bud is light brown in colour, smaller in size with less pointed tip and top shaped. Bracts open 2-3 at a time, have no bract shoulder and they remain persistent. Male flowers are small in size and white in colour. Fruits are light green in colour with gradual tip, 4-5 unequal sides with cream coloured flesh which becomes soggy upon ripening.

Associated Characters and cultivated practices

High yielding with 20 to 25 kg bunches. More suitable for high density system of cultivation.

61. NRCB0050/Karthobiumtham (IC250503; INGR22061), a banana (*Musa spp.*) germplasm with *Fusarium oxysporum* f. sp. cubense Foc race 1 resistance

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Introduction

Karthobiumtham (NRCB Acc. No. 0050 & IC250503), an unique type of ABB genomic group, is distributed in north eastern India. Male bud mutations are often observed (bunch without male bud). Fruits are elongated like Horn plantain. Pulp is sugary with high sugar content with 30–32° Brix.

Morpho-agronomic Characteristics

Normal in stature, pseudostem is green and slightly wax coated, with brown blotches. Leaves are dark green and shiny on upper side and slightly wax coated on lower

surface. Petioles are tightly clasped on the pseudostem. Petiole canals are very narrow and the margins are erect. Peduncle is very short, 20–25 cm length and glabrous in nature. Bunch is small, horizontal or slightly angular, 4–5 hands of fruits and loosely packed. Fruits are ashy green, long with 22–24 cm lengths and slightly pointed tip, ashy yellow upon ripening. Male bud is maroon and the male flowers are pink pigmented. Fruit peel is very thick, pulp is cream, very sweet and TSS is up to 32° Brix.

Associated Characters and cultivated practices

Fruit pulp is suitable for making banana figs and wine.

62. MSP/15-26 (IC644002; INGR22062), a potato (*Solanum tuberosum*) clone (*Solanum tuberosum*; 2n=4x=48); possessing yellow flesh colour and high carotenoids in flesh

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Potato has its origin and domestication in Andes Mountains of South America. The introduction of this crop to remaining world was mainly focused on yield and yield attributing traits. Though a potent source of phytonutrients particularly carotenoids coloured flesh potatoes, the commercial exploitation of these health promoting compounds of potatoes have not been emphasized till recently including Indian potato variety breeding mandates. However, increased awareness of health benefits of these plant pigments have interested breeder's to develop coloured varieties including potatoes. Considering this, to get the baseline information of Indian potatoes phytonutrient content status analysis were done and results revealed narrow variations for these compounds (Dalamu *et al.*, 2015). However, nutritional profiling of potato germplasm including indigenous varieties depicted presence of significant variations (Dalamu *et al.*, 2014, Luthra *et al.*, 2018). Thus, development of phytonutrient dense genetic stocks can open new avenues for breeding potatoes with desired functional properties for table purpose as well as for processing.

Carotenoids are fat soluble organic pigments derived from isoprenoids and confer degrees of yellow to orange colour. Cultivated potato (*Solanum tuberosum* L.) contain varying amount of carotenoids in tubers. Potato carotenoids are mainly xanthophylls, and traces of beta-carotene, precursor of vitamin A. Dietary intake of xanthophylls protects from age related macular degeneration while deficiency of vitamin A causes various ailments like xerophthalmia, poor immune system, and infant mortality.

Carotenoids concentrations rather than its composition lead to colour variations in potatoes. Worldwide total carotenoids content in white and yellow fleshed potatoes varied from 50 to more than 2000 ug/100g fresh weight (FW).

MSP/15-26 is a promising clonal selection from the segregating progenies of cross Bareilly Red (an indigenous variety × CP 3770) made in year 2014 (Luthra *et al.*, 2018). It is late maturing have yield productivity of 35–40 t/ha at 90 days crop duration. Plant is tall. Corolla colour is red-violet. It produces attractive, medium sized 18–20 round red tubers with medium eyes, yellow coloured tuber flesh with red colored vascular ring. It is moderately resistant to late blight, possesses extremely long tuber dormancy (>10 weeks) and have excellent tuber appearance after 75 days of storage. Tubers have good mouth feel/ flavor, floury texture and tuber dry matter content of 20%. Based on average of 4 years analysis, MSP/15-26 on an average possessed high carotenoids (823 ug/100g FW) as compared to parents Barrielly Red (460) and CP3770 (422 ug/100g FW) and distinct yellow flesh colour with red vascular ring.

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63. MSP/15-51 (IC644003; INGR22063), a potato (*Solanum tuberosum*) clone with red purple flesh and high ascorbic acid in flesh

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Of late there have been rising trends for natural antioxidants and their health benefits. Epidemiological studies revealed reduction in degenerative ailments with increased consumption of antioxidant rich food. Ascorbic acid is a vitamin that has anti-oxidant properties and enhances mineral absorption particularly iron. Anthocyanins belong to group of naturally occurring pigments that imparts red, blue or purple hues to horticultural and food crops. Anthocyanins have antioxidative properties due to prolific free-radical scavenging activity that helps in mitigating the risk of chronic diseases. Approx. 37 percent of population globally is anaemic due to iron deficiency. The situation is more prevalent in developing nations leading to hidden hunger caused mainly due to insufficient intake of micronutrients like vitamin A, zinc and iron.

Potato (*Solanum tuberosum* L.) holds the position of third most important food crop. This crop has the potential of addressing food security owing to capacity of producing more food per unit area of land. The present potato production scenario depicts more than half of the world's potato crop is produced in developing nations particularly India and China. In addition to good source of energy, potatoes are full of vitamins like ascorbic acid, minerals and proteins of high biological value. Potatoes justifies the concept of biofortified crops owing to good source of ascorbic acid, that itself is antioxidants and also positively correlated with iron bioavailability. In addition potatoes have low levels of anti-nutrients such as phytates and polyphenols that hamper iron availability. Potatoes are in general not considered as good source of anthocyanins however, traditional varieties and /or germplasm with high level of these compounds exists (Dalamu *et al.*, 2014). Traditionally, plant breeders including potato have focused on traits like

processing quality, yield, and resistance and seldom for nutrient content (Dalamu *et al.*, 2015). However, with the projection scenario of rise in per capita demand of potatoes to be reached to 48.50 kg by 2050, there is need of varieties with added advantage of health compounds with yield and other biotic and abiotic stress resistance and/or tolerance.

MSP/15-51 is a clonal selection from the segregating population of cross Bareilly Red × CP 3770 developed in year 2014 (Luthra *et al.* 2018). Plant is tall and corolla colour is white with light purple secondary colour. It is late maturing and have yield productivity of 20-25 t/ha at 90 days crop duration, It produces small (30-32 tubers), purple oblong tubers with medium eyes and red purple flesh. It is moderately resistant to late blight, possesses extremely long tuber dormancy (>10 weeks) and have excellent tuber appearance after 75 days of storage. Tubers have good mouth feel/ flavor, mealy texture and 21 % dry matter content. Based on average of 4 years analysis, MSP/15-51 possessed high ascorbic acid (68 mg/100g FW) in comparison to parents Bareilly Red (56) CP3770 (26) and distinct purple flesh colour and distinct purple flesh.

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CONTENTS

Role of potato in entrepreneurship development <i>Brajesh Singh and Tanuja Buckseth</i>	189
Morphological Characterization and SSR Marker Assay of Mango Landraces of West Coast of Kerala, India <i>Anuradha Sane, Joseph John, C Vasugi, M R Dinesh and K Pradheep</i>	195
Morphological and Molecular Diversity Analysis in Forage Sorghum (<i>Sorghum bicolor</i> (L.) Moench) <i>Arvinth S, RN Patel, RA Gami, Kapil K Tiwari and AH Joshi</i>	206
Variability and Character Association in Different Classes of Rice (<i>Oryza sativa</i> L.) of Assam for Root and Yield Attributing Traits <i>Priyanka Bairagi, Dikshita Gogoi and Kishore Kumar Sharma</i>	215
Identifying Potential Donor Parents for Breeding Against Leaf Curl Virus and Anthracnose Diseases in Chilli <i>Solanki Bal, Arup Chattopadhyay and Asit Kumar Mandal</i>	222
Evaluation of Long-Day Onion (<i>Allium cepa</i> L.) Genotypes for Yield and Quality Traits Under Temperate Conditions of Kashmir Valley in India <i>Geetika Malik, Rafiq A Shah, Alima Shabir, Vishal Dinkar, Javid I Mir and Om C Sharma</i>	232
Seeds of Harmony: Conservation of Landraces by Farmers, Communities and NGOs in the Western Ghats, a Case Study <i>G M Puneeth, Ravi Gowthami, Sanjay Patil, Kailash Chandra Bhatt, Ramesh Vasudeva and Sunil Archak</i>	246
Molecular Characterization of Grapes (<i>Vitis</i> spp.) Using EST-derived Simple Sequence Repeat (SSR) Markers <i>Sharmistha Naik, Jagesh K Tiwari, Rasna Zinta, Narender Negi, Rajnish Sharma, Gopal Singh, and DP Sharma</i>	255
Restoration reaction in the F₁ hybrids derived from F₃ maldandi restorers on different cytoplasm in rabi sorghum [<i>Sorghum bicolor</i> (L.) Moench] <i>Prashant Kariyannanavar*, M. C. Wali, B. D. Biradar, V. S. Kubsad, L. K. Verma, B. N. Prashantha, N. Pavan Kumar and Revanasiddayya</i>	263
Ensemble Habitat Suitability Studies of <i>Vetiveria zizanioides</i> (Linn.): An Important Genetic Resources with Aromatizing, Medicinal and Land Restoration Properties <i>Manish Mathur and Preet Mathur</i>	266
Genetic Diversity Assessment of <i>Cordia ghara</i> (Forsk.) Germplasm for Morpho-Physiological and Fodder Traits in Hot-arid Kachchh Region of India <i>Rahul Dev, Anil Patidar, M Sureshkumar, Sushil Kumar, K Venkatesan, R Saranya, Traloki Singh and Devi Dayal</i>	283
Stem of Woody Pepper (<i>Piper pendulispicum</i> C. DC) as a Source of Phenolic Acids and Piperine <i>Ajit A. Waman, Pooja Bohra, Karthika Devi R., Loksha A.N. and Shivashankara K.S.</i>	294
Collecting Plant Genetic Resources from Remote and Isolated Marwah-Warwan Valley of Jammu and Kashmir, India <i>Sheikh M. Sultan, Susheel Kumar Raina and Praveen Kumar Singh</i>	301
Characterization of Gola Ber Genotypes under Arid Conditions of Western Rajasthan for Qualitative and Quantitative Characters <i>DK Sarolia, Kamlesh Kumar, Lokesh Kumar, D Singh and MK Choudhary</i>	310
Comparative Assessment of Different Earth System Models for Habitat Suitability of <i>Cuminum cyminum</i> (Linn.) Crop: A Machine Learning Evaluation from Arid and Semi-Arid Hot Areas of the India <i>Manish Mathur and Preet Mathur</i>	316
Plant Germplasm Registration Notice	341
Author Guidelines	

