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Indian Journal of Plant Genetic Resources

REVIEW ARTICLE

Developing Leaders and Leadership in Agrobiodiversity Management

Shaun G Coffey*

Abstract

Agrobiodiversity, the variety and variability of animals, plants, and microorganisms used in food and agriculture, is critical for ensuring food security and maintaining ecosystem health. Effective leadership in agrobiodiversity management is crucial for overcoming challenges such as climate change, habitat loss, and unsustainable agricultural practices. This paper examines the key qualities of leadership required for agrobiodiversity management, explores strategies for developing leaders in this field, and discusses the challenges and opportunities for fostering future leadership. The paper concludes by emphasizing the need for ongoing leadership development through education, mentorship, and inclusive practices to ensure the sustainable management of agrobiodiversity.

Keywords: Leadership, Values-based leadership, Improvisational practice, Sustainability, Collaboration, Mentorship, Governance, Innovation, Resilience, Visionary thinking.

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Introduction

Agrobiodiversity, or agricultural biodiversity, encompasses the range and variability of life forms, including plants, animals, and microorganisms, that are essential for food production and ecosystem services. It is central to addressing the challenges of food security, climate change, and sustainable agricultural intensification. However, managing agrobiodiversity effectively is fraught with challenges, such as habitat loss, climate change, and the prevalence of monoculture farming. Effective leadership is key to addressing these challenges, as it drives conservation efforts, promotes sustainable practices, and ensures the long-term viability of agrobiodiversity. This review paper explores the role of leadership in agrobiodiversity management, identifying the key leadership practices required, strategies for developing leaders, and the opportunities and challenges faced in cultivating future leaders.

The Evolving Landscape of Leadership?

Food and nutritional insecurity, ethnic, racial, and religious tensions, geopolitical unrest, trade wars, and wealth and power inequities mean that the context in which leadership is practiced today changes rapidly and unpredictably. The effective practice of leadership, however, remains unchanged and unchanging, albeit largely undiscovered and mostly misunderstood.

Leadership, as an improvisational practice, is not merely about managing resources or dictating outcomes. It is a dynamic, relational practice that thrives in uncertainty and complexity. For too many years, our understanding of leadership has been rooted in the idea of structure, authority, and control-values that

were strongly emphasized in traditional management frameworks. However, as thinking evolves, leadership is increasingly understood to be an improvisational practice that unfolds in real-time, shaped by the unique challenges and needs of the moment (Bateson, 2016; Haslam *et al.,* 2020; Ladkin, 2020).

True leadership involves guiding a group through a shared purpose, influencing individuals not by authority but through inspiration, empathy, and the ability to connect on a human level. It requires vision, the ability to navigate complexity, and the capacity to make decisions that balance short-term needs with long-term sustainability. Leadership is thus seen as a social achievement-emerging not just from one individual's actions, but from the interactions within the team or organization, where each member contributes to the collective direction and purpose.

An essential quality of effective leadership is courage-acting when others hesitate, speaking out when silence is easier, and taking risks despite uncertainty. Leadership in the realm of agrobiodiversity, for instance, requires the courage to champion long-term sustainability goals, even when they challenge short-term market demands. It also requires the ability to inspire trust and engagement, making leaders accountable not just to their teams but to the broader society whose food security and ecosystem services depend on their decisions.

Leadership is a practice of influencing people to change the way they think and act (Jessup, 2024). It is an improvisational, relational, and phenomenological practice, one that requires courage, authenticity, and a deep understanding of the human experience, dependent on making sense of the circumstances you are in at a particular point in time. And effective leadership is highly contextual, requiring leaders to understand what they are leading or managing. Leaders must have sufficient content knowledge that they can use first principles thinking, scepticism of received wisdom, and continuous learning to create understandable change for their teams and organizations.

Over time, our understanding of leadership has moved away from a static, hero-centric view to one that integrates management and leadership seamlessly. This evolution in thought aligns with the insights of thinkers such as Henry Mintzberg (2009, 2023), who emphasized that leadership is "management done well." It is about aligning actions with broader organizational goals and adapting to changing circumstances in a way that maintains momentum toward a collective vision. This review article attempts to capture some of these perspectives.

Values-Based Leadership

As leadership practices shift from authority and control to influence and authenticity, values-based leadership emerges as a critical paradigm. Leadership is often misunderstood, equated with authority, titles, or formal positions of power. But leadership is not about rank-it is about influence, responsibility, and the ability to inspire action. Leadership is a dynamic, ever-evolving practice that requires adaptability, ethical decision-making, and purpose.

In its essence, then, leadership as an improvisational act requires making real-time decisions, responding to unforeseen challenges, and guiding people toward common goals in uncertain environments. The best leaders are not those who rigidly follow a predetermined script but those who adjust, adapt, and navigate complexity with agility. These leaders are guided by their values.

The Nature of Values-Based Leadership

Values-based leadership is predicated on the idea that effective leaders align actions with core ethical principles. Rather than lead by command or compliance, they lead by example, embodying the values they espouse. These values act as an internal compass, guiding decisions even when external conditions are ambiguous or difficult.

This concept contrasts with more transactional approaches, where incentives or penalties drive behaviour. Values-based leadership is relational and reflective, grounded in self-awareness, personal integrity, and mutual respect.

Theoretical Perspectives and Helpful Models

Several leadership models (Greenleaf, 1977; George, 2003; Sanders, 2007; Northouse, 2016) provide foundations for thinking about values-based leadership:

- Transformational leadership focuses on vision, inspiration, and moral purpose.
- Servant leadership promotes humility, empathy, and community.
- Authentic leadership stresses self-awareness and an internalized moral perspective.
- Adaptive leadership demands navigating complexity with values at the core.
- Transcendent leadership argues that self-mastery and spiritual maturity define great leadership.

Each model contributes facets of purpose, ethics, service, and adaptability to a composite image of the values-based leader. Leadership is not about choosing between the models but about using them effectively in context.

Practices of Values-Based Leaders

Self-knowledge and reflection

Values-based leaders engage in continual introspection, refining their awareness of what matters most. They journal, seek feedback, and ask critical questions about their motivations.

Integrity in action

Living one's values means acting consistently. It means standing for principles, even under pressure.

Empowering others

Values-based leaders are committed to developing those around them. They promote inclusivity, listening, and shared success.

Purpose orientation

These leaders often connect their work to a broader societal or ecological mission. Their decisions consider not only shareholders but also stakeholders and future generations.

System awareness

Especially in adaptive and transcendent leadership, values guide leaders in complex systems, where there are no clear-cut answers and multiple truths must coexist.

Sharing and agreeing on values

Getting a team aligned on values is fundamental. By having everyone agree to the values by which the team will operate, there is a solid basis on which success can be discussed, unhelpful behaviours identified and addressed, and professional development coordinated. The process on making values explicit and agreeing as a group to work in honour of them can be the step change often needed for success.

Key Leadership Qualities for Agrobiodiversity Management

Visionary thinking and strategic planning

Effective leaders in agrobiodiversity management must possess a clear vision of a sustainable future where agricultural practices and biodiversity conservation coexist harmoniously. Leaders must be able to anticipate future challenges, such as the impacts of climate change on crop yields and biodiversity, and devise strategies that balance the competing demands of food security and environmental sustainability (Altieri *et al.,* 2017). Visionary leaders inspire others to embrace sustainable practices, integrating ecological, social, and economic perspectives into their long-term goals (Pretty *et al.,* 2018).

Expertise and knowledge

Knowledge of ecological principles, sustainable farming techniques, and biodiversity conservation strategies is essential for leaders in agrobiodiversity management. These leaders must be well-versed in areas such as agroecology, plant and animal breeding, and conservation biology to make informed decisions. Furthermore, they must stay current with the latest research and technological advancements in agricultural sustainability (Brauman *et al.,* 2016; Thrupp, 2016). A well-developed knowledge base is crucial for advocating for appropriate policies and implementing effective conservation strategies (FAO, 2019).

Communication and collaboration

Successful leadership in agrobiodiversity requires strong communication skills and the ability to foster collaboration among various stakeholders, including farmers, policymakers, researchers, and the broader community (De Schutter, 2014). Leaders must be able to articulate the importance of agrobiodiversity, share knowledge effectively, and mobilize support for sustainable practices. Furthermore, collaboration is essential for integrating diverse perspectives and expertise, ensuring that conservation efforts are holistic and inclusive (Altieri and Nicholls, 2020).

Conflict resolution and negotiation

Managing agrobiodiversity often involves navigating conflicting interests among stakeholders, such as balancing conservation goals with the need for increased agricultural productivity. Leaders must possess strong conflict resolution and negotiation skills to mediate between these interests and find solutions that support both biodiversity and food security (Fowler and Hodgkin, 2004). This requires the ability to engage in constructive dialogues, build consensus, and ensure that all voices are heard (Shiva, 2016).

Adaptability and innovation

The dynamic nature of ecosystems and agricultural systems demands that leaders be adaptable and open to innovative solutions. Agrobiodiversity leaders must be able to respond to emerging threats, such as new pest species or changing climatic conditions, and adapt their strategies accordingly. This may involve the development of new technologies, practices, or policies to enhance resilience and sustainability (Pretty *et al.,* 2018). Innovative leadership is crucial for ensuring that agrobiodiversity management strategies remain effective in the face of rapidly changing environmental conditions.

Entrepreneurship and resource mobilization

Effective leaders in agrobiodiversity management must also demonstrate entrepreneurship by identifying funding opportunities and securing the resources necessary to implement conservation initiatives. This requires not only the ability to mobilize financial resources but also the capacity to build partnerships with donors, governments, and the private sector (FAO, 2018). Resource mobilization is essential for sustaining long-term agrobiodiversity management programs and ensuring their scalability (Bennett *et al.,* 2016).

Developing Leaders in Agrobiodiversity Management

Developing effective leaders in agrobiodiversity management requires a multi-faceted approach that includes educational programs, mentorship, and specialized training initiatives. Universities and research institutions play a vital role in equipping future leaders with the scientific and technical expertise necessary for agrobiodiversity management. Programs in agroecology, sustainable agriculture, and environmental science provide students with the foundational knowledge required to address the complex challenges of agrobiodiversity (Altieri *et al.,* 2017).

Mentorship and apprenticeship programs are also essential for developing leadership skills in the field of agrobiodiversity. Experienced leaders can provide guidance and support to emerging leaders, helping them navigate the complexities of the field and develop the critical thinking and decision-making skills necessary for effective leadership (Fowler, 2013). Institutions such as the FAO and IUCN offer mentorship programs that connect young professionals with experienced mentors who can offer practical advice and career development opportunities.

Leadership development workshops and training programs that focus on specific skills such as strategic planning, communication, and conflict resolution are also crucial. These programs provide participants with the tools they need to lead effectively in the context of agrobiodiversity management. For example, the Global Biodiversity Leadership Development Initiative (GBLDI) offers training programs designed to build leadership capacity in the field of biodiversity conservation (FAO, 2018).

The Crawford Fund (2025) has been a leader in practice-based training of research managers. Its highly sought-after Master Classes provide an immersion process where emerging and experienced leaders can have an immediate impact on their management performance. These programs link leadership and management closely, and are developing the value-based approach to both management and, increasingly, to governance. Governance is increasingly important given the collaborative nature of research initiatives, and refers to the arrangements (processes, institutional arrangements, and underpinning compliance requirements) used to make decisions and take actions to ensure project meet their targets.

What Can You Do to Develop as a Leader?

Leadership development is a continuous process that involves both formal and informal learning. It is not merely about gaining technical knowledge but developing a deep understanding of oneself and one's relationship with others in the context of the broader systems in which we operate. Here are several strategies for developing as a leader in agrobiodiversity management:

Self-reflection and self-awareness

Regular self-reflection is essential for leadership growth. It allows individuals to assess their actions, understand their motivations, and identify areas for improvement. Leaders must continuously question their assumptions and be open to feedback from others. This self-awareness is critical for navigating complex challenges and making decisions that align with both personal values and the larger organizational goals.

Seek mentorship and guidance

Mentorship plays a pivotal role in leadership development. Learning from those with more experience helps to develop practical leadership skills and provides valuable insights into navigating the challenges of agrobiodiversity management. Seeking mentors from a variety of disciplines, such as agriculture, environmental science, and policy, helps broaden leadership perspectives.

Pursue formal leadership training

Attending leadership development programs that focus on areas such as strategic thinking, communication, and conflict resolution is essential for building the skills necessary for effective leadership. Engaging in such programs provides opportunities for networking, learning best practices, and gaining new tools that can be applied in real-world settings.

Embrace lifelong learning

Leadership development does not stop after formal education or training programs. The best leaders are those who remain curious and engaged with new developments in their field. Participating in conferences, reading widely, and engaging in continuous learning allow leaders to stay informed and adapt to evolving challenges in agrobiodiversity management.

Foster collaborative relationships

Building strong, collaborative relationships is essential for effective leadership. Leaders must create environments where trust, respect, and open communication can flourish. This involves being receptive to new ideas, supporting others' growth, and facilitating shared decision-making processes.

Case Studies: Successful Leaders in Agrobiodiversity Management

Genetic resources conservation-led global effort

Australia's engagement with plant genetic resources from 1926 to 1980 (Byerlee, 2025) was key in the global movement for conservation and utilization of agricultural genetic diversity. Australian-led plant collection expeditions after World War II, and by the 1950s, Australia had become a global leader in genetic resource conservation, largely due to Sir Otto Frankel's advocacy. Australia's plant exploration efforts were shaped by the country's agricultural dependence on introduced species, with institutions like the Waite Agricultural Research Institute playing a central role. During the 1960s and 1970s, Australia influenced CGIAR centres and global pasture systems but faced challenges in transferring its farming systems to other regions. By the 1980s, the focus shifted from conservation to ownership and access, which has diminished Australia's role as a global leader in genetic resource conservation. The vision, however, has now become embedded more widely throughout the whole genetic resources system.

Dr. Vandana Shiva

Dr. Shiva, a prominent environmental activist and founder of the Research Foundation for Science, Technology, and

Ecology, has been a leader in promoting agrobiodiversity through her Navdanya initiative. Her leadership has focused on preserving seed diversity and promoting organic farming practices. By combining traditional knowledge with modern ecological principles, Dr. Shiva has mobilized communities and influenced policy at both national and international levels (Shiva, 2016).

Dr. Cary Fowler

Dr. Fowler, former executive director of the Global Crop Diversity Trust (Crop Trust), has been instrumental in safeguarding global crop diversity. Under his leadership, the Svalbard Global Seed Vault was established as a secure storage facility for the world's seed collections. His work exemplifies strategic vision, knowledge, and resource mobilization in the field of agrobiodiversity conservation (Fowler and Hodgkin, 2004).

International Rice Research Institute (IRRI)

The International Rice Research Institute (IRRI) is a leader in agrobiodiversity management, particularly in the development of rice varieties that are resilient to climate change and pests. IRRI's collaborative approach, which integrates research, innovation, and partnership-building, has been instrumental in enhancing agrobiodiversity and improving food security in rice-growing regions (IRRI, 2019).

Challenges and Opportunities in Developing Agrobiodiversity Leaders

Challenges

Several challenges hinder the development of leadership in agrobiodiversity management, including limited financial resources, lack of awareness about the importance of agrobiodiversity, and political instability. Additionally, the complexity of agrobiodiversity management requires interdisciplinary approaches, which can be difficult to implement due to institutional silos (Bennett *et al*., 2016). Moreover, the socio-political context in which agrobiodiversity management operates often hinders effective leadership development, as entrenched agricultural practices and policies may prioritize short-term productivity gains over long-term sustainability (De Schutter, 2014).

Opportunities

Despite these challenges, there are significant opportunities for promoting leadership development in agrobiodiversity management. The growing recognition of the importance of sustainable agriculture and biodiversity conservation in global agendas, such as the United Nations Sustainable Development Goals (SDGs), has led to increased funding and policy support for agrobiodiversity initiatives (Global Alliance for the Future of Food, 2019). Furthermore, promoting gender equity and inclusivity in leadership development programs will ensure a diverse pool of leaders with varied perspectives, which is critical for the sustainable management of agrobiodiversity (AWARD, 2020).

Conclusion

Leadership is essential for the sustained management of agrobiodiversity. Leaders who possess a clear vision, strategic thinking, and the ability to collaborate across sectors are crucial for overcoming the challenges facing agrobiodiversity management. By investing in educational programs, mentorship, and leadership development initiatives, we can cultivate the next generation of leaders who will drive the conservation and sustainable use of agrobiodiversity. As global pressures on food systems intensify, the need for strong, visionary and improvisational leadership in agrobiodiversity management will only grow, highlighting the importance of continuous leadership development to ensure the resilience and sustainability of agricultural systems.

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REVIEW ARTICLE

Biofortified Crops for Nutritional Security with Special Reference to the North-Eastern India

Hari Shankar Gupta[1,2*], Firoz Hossain[1] and Devendra Kumar Yadava[1,3]

Abstract

Malnutrition resulting from the consumption of an unbalanced diet has emerged as one of the major health concerns worldwide. Due to its negative impact on growth and development in humans, it adversely affects the livelihood and prosperity of a society too. Among various alternatives, crop biofortification has emerged as the most sustainable and cost-effective approach to alleviate malnutrition. The high-yielding crop cultivars generally lack adequate levels of nutritional quality required for optimum metabolism in humans. Intensive research efforts by the Indian Council of Agricultural Research (ICAR) led National Agricultural Research Education and Extension System (NAREES) involving State Agricultural Universities (SAUs) and Central Universities (CUs), have led to the development of >150 biofortified crop cultivars across cereals, pulses and oilseeds. While nutritional qualities *viz*., protein, iron, zinc, calcium, lysine, tryptophan, provitamin-A, oleic acid and linoleic acid have been enhanced, the concentration of several anti-nutritional factors *viz*., phytate, erucic acid, glucosinolates and trypsin inhibitor have also been significantly reduced through breeding approaches. North-Eastern Region (NER) harbors a rich reservoir of genes spread over various crops like cereals, pseudocereals, pulses, oilseeds, vegetables, fruits, and medicinal and aromatic plants (MAPs). Here, we present a review of the work done by the NAREES on the biofortification of various crops and enlist the biofortified crops developed and released for commercialization by the National system of the country. The presence of a reservoir of genes in NER can help in further biofortifying the wide array of crops which can assist in improving the nutritional security of the people in India in general and the dwellers of the NER of India in particular. In addition, attempts made to popularize these crops by licensing their seed production through private seed companies and farmers' groups like farmers' producer organizations have also been dealt with.

Keywords: Anti-nutritional factors, Biofortification, Hybrid, MAS, Nutrition, Variety.

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Introduction

Consumption of food inadequate in nutritional balance leads to malnutrition in humans (Bouis *et al.,* 2019; Gupta *et al.,* 2019; Yadava *et al.,* 2023). It not only affects growth and development in humans but also has severe socio-economic implications (Vasal *et al.,* 1993; Gupta *et al.,* 2015; Hossain *et al.,* 2024a). Of the 3.1 billion global population that cannot afford healthy food, 735 million people are hungry too (FAO, IFAD, UNICEF, WFP and WHO 2023). Globally, 148.1 million (22.3%) children (<5 years of age) are stunted, while 45 million children (6.87%) face wasting and 37 million (5.6%) children are affected by obesity. In India, 35.5% of the children (<5 years) are stunted, 19.3% wasted and 32.1% are under-weight (NFHS-5 2019-21). Among adults, 57.0% of women (15–49 years) are anemic, while the same was 25.0% among men. Considering the paramount importance of alleviating malnutrition, world leaders at the United Nations framed the 'Sustainable Development Goals' (SDGs) for meeting the current needs (UNDESA 2023). Of the 17 goals, 12 are associated with nutrition. Of these goals, 'Goal-2 (zero hunger)' primarily addresses alleviating malnutrition. In fact,

alleviating malnutrition is the most cost-effective step as every $1 invested in a proven nutrition programme offers benefits worth $16 (Global Food Policy Report 2016). Thus, a balanced and nutritious diet for people assumes great significance in mitigating malnutrition (Bouis and Welch, 2010; Mishra *et al.,* 2025).

Approaches to Alleviate Malnutrition

Four major approaches *viz*., (i) food fortification (ii) medical supplementation (iii) dietary diversification and (iv) crop-biofortification are generally used for alleviating malnutrition (Hossain *et al.,* 2021, 2022; Yadava *et al.,* 2022). 'Food fortification' is a process of physically adding vital nutrients to the food to enrich it. In India, as for example (i) iron, folic acid and vitamin B_{12} fortified wheat and rice flour, (ii) iron and iodine fortified salts, (iii) vitamin-A and vitamin-D fortified oil and milk, have been permitted by Food Safety and Standards Authority of India (FSSAI), an agency of Govt. of India (GoI). 'Medical supplementation' is a process of providing vital nutrients through pills/ tablets. GoI-sponsored programmes, *viz*., (i) Weekly Iron, Folic Acid Supplementation (WIFS) programme for school adolescent boys and girls (10-19 years) and out-of-school girls (10-19 years) in urban and rural areas, and (ii) Vitamin-A Supplementation (VAS) programme for children under five, are some of the successful supplementation programmes in India. 'Dietary diversification' is a process of including diverse cereals, pulses, oilseeds, vegetables and fruits in the diet to enhance the nutritional status. 'POSHAN Abhiyaan' (Nutrition Campaign) by GoI is promoting 'Poshan Thali' (nutritious platter) comprising of cereals, pulses, oilseeds, vegetables, fruits, milk and other components like sugar, jaggery and oils for balanced nutrition to increase low birth weight and reduce the level of stunting, under-nutrition and anemia among young children, women and adolescent girls. However, these programs are not sustainable in the long run. Lack of purchasing power, poor infrastructure, crop seasonality, higher expense, and lower bioavailability are some of the reasons that affect the successful implementation of these progarmmes (van Lieshout and de Pee 2005).

'Crop biofortification' is defined as a process of enhancing the nutritional qualities of edible parts of the plants through genetic approaches such as plant breeding, gene-editing and transgenics (Neeraja *et al.,* 2022). Development of (i) iron, zinc and protein-rich wheat grains, (ii) protein- and zinc-rich rice grains, and (iii) vitamin-E, vitamin-A, lysine and tryptophan-rich maize grains developed through breeding approaches are some of the examples of 'Crop Biofortification'. Besides, provitamin-A-rich 'Golden rice' generated through transgenic approach is another example of a crop biofortification approach. 'Crop biofortification' is regarded as the most sustainable approach, through which nutrients are provided in natural form (Bouis and Welch 2010). Further, people can easily afford the 'biofortified food' as it does not involve any additional cost during cultivation. Besides, biofortified crop varieties are as high-yielding as traditional varieties, thus no yield penalty is suffered by the farmers. More importantly, it does not require elaborate infrastructure facilities as in 'food fortification'. In addition, it also does not need an elaborate distribution system as required in 'medical supplementation' programmes (Yadava *et al.,* 2022).

Globally, the plant breeding-based biofortification program led by HarvestPlus has assisted in developing >293 biofortified varieties, some of which are (i) iron-rich pearl millet and bean, (ii) zinc-rich rice, wheat and maize, and (iii) provitaminA-rich maize, cassava and sweet potato (HarvestPlus Annual Report 2022). More than 1,00,000 tonnes of seeds of biofortified crops were produced and distributed in 2022 by HarvestPlus throughout the globe. An estimated 17.3 million farming households grew biofortified varieties during 2022 thereby enriching the diets of 86.5 million people (www.harvestplus.org). The positive impact of biofortified crop varieties is well documented worldwide. The beneficial effect of iron-rich rice (Haas *et al.,* 2005), zinc-rich wheat (Sazawal *et al.,* 2018), iron-rich pearl millet (Finkelstein *et al.,* 2015), lysine- and tryptophan-rich maize (Gunaratna *et al.,* 2010), provitamin-A rich maize (Sheftel *et al.,* 2017), and provitamin-A rich orange-fleshed sweet potato (Low *et al.,* 2007) have shown great promise in alleviating malnutrition in humans.

Genetic Diversity of Crops for Nutritional Traits in North-Eastern India

North-Eastern Region (NER) of India comprising eight states *viz*., Arunachal Pradesh, Assam, Meghalaya, Manipur, Tripura, Mizoram, Nagaland and Sikkim, is known for its diverse nature of soil, climate, and topography (Asati and Yadav *et al.,* 2004). This region possesses rich reservoir of many crops including cereals, pulses, fruits, vegetables, flowers particularly orchids, spices and medicinal and aromatic plants (MAPs) (Deka *et al.,* 2012; Prakash *et al.,* 2024). Among cereals, rice, barley, coix, finger millet and foxtail millet are native to this region (Upadhaya and Sundriyal 1998). Wide genetic diversity in pulses such as pigeon pea, rice bean, mung bean, winged bean, *Dolichos* bean and sword bean has also been reported (Borthakur 1992). NER also possesses a large diversity of maize (Singh and Rana 1995; Prasanna and Sharma 2005; Sharma and Pradhan 2023). Farmers in the NER still grow traditional landraces of many of these crops for family and community needs (Devi *et al.,* 2023). 'Chakhao' rice landraces of Manipur possess deep-pigmented kernels rich in anthocyanins (Bhuvaneswari *et al.,* 2020). 'Malbhog' landrace of rice is rich in protein and oil. While rice landraces *viz*., 'Bhaja', 'Komal Chaul', 'Komal Ronga', 'Komal Sakowa', 'Sakowa Bora' and 'Sakowa Dhan' are zero cooking soft rice. 'ARC10075', a rice accession collected by Dr. B.C. Patra in 2013

from West Garo Hills, Meghalaya, is rich in protein and has been used as a donor for the development of protein-rich rice varieties *viz.* CR Dhan-310 and CR Dhan-311. Similarly, 'Mimban' landraces of maize are rich in amylopectin (Rathod *et al.,* 2019). Baruah *et al.,* (2023) also reported some of the maize landraces being rich in pigments such as anthocyanins and phlobaphenes. Thus, landraces rich in nutritional qualities such as vitamins, antioxidants and minerals, can serve as suitable donors for the development of high-yielding biofortified crop cultivars.

Crop Biofortification Programs in India

The National Agricultural Research Education and Extension System (NAREES) led by the Indian Council of Agricultural Research (ICAR) in collaboration with State Agricultural Universities (SAUs) and Central Universities (CUs) have contributed immensely to making India self-sufficient in food production (Singh *et al.,* 2022). The food grain production of 50.82 million tons (MMT) in 1950-51 has been increased to 332.22 MMT during 2023-24 (www.indiastat.com). The research efforts on the enhancement of grain yield were initiated during the *'Green Revolution'* in the mid-1960s under the leadership of Prof. MS Swaminathan (Kesavan and Swaminathan 2006). So far NAREES has developed 6,563 high-yielding cultivars of various field crops. In the process of yield enhancement, nutritional quality was not given due importance in the breeding program, and as a result, the majority of these varieties do not possess the desired level of nutritional attributes (Debnath *et al.,* 2023). Realizing the importance of nutritional quality, the research efforts of NAREES have now led to the development and release of a series of biofortified crop varieties through All India Coordinated Research Projects (AICRPs) for different crops through breeding approaches (Yadava *et al.,* 2022). Besides, a special project on the Consortium Research Platform (CRP) on 'Crop Biofortification' was launched in 2014-15, and it is continuing with the development of several biofortified crop cultivars.

Biofortified Crop Cultivars Released for Commercial Cultivation

Research effort at ICAR-led NAREES has led to the release of 152 field crop cultivars across rice, wheat, maize, pearl millet, finger millet, little millet, proso millet, foxtail millet, brown-top millet, lentil, field pea, mung bean, urd bean, chickpea, faba bean, groundnut, linseed, mustard, soybean and grain amaranth **(**Figure 1**)**. These cultivars have been improved for essential nutrients *viz*., iron, zinc, calcium, protein, lysine, tryptophan, provitamin-A, oleic acid and linoleic acid **(**Table 1**).** The concentration of several anti-nutritional factors *viz*., phytate, erucic acid, glucosinolates and trypsin inhibitor has been significantly reduced in some of the cultivars **(**Table 1**)**. Off-flavour of soybean grains has also been reduced.

Some of the important biofortified crop varieties developed across crops are zinc-rich varieties of rice such as 'DRR Dhan-49' (25.2 ppm), 'Zinco Rice MS' (27.4 ppm) and 'CR Dhan-315' (24.9 ppm). Besides, 'CR Dhan-310' with 10.3% protein (in milled rice), and 'CR Dhan-411' with 10.1% protein were also released. In addition, 'CR Dhan 311' possesses both

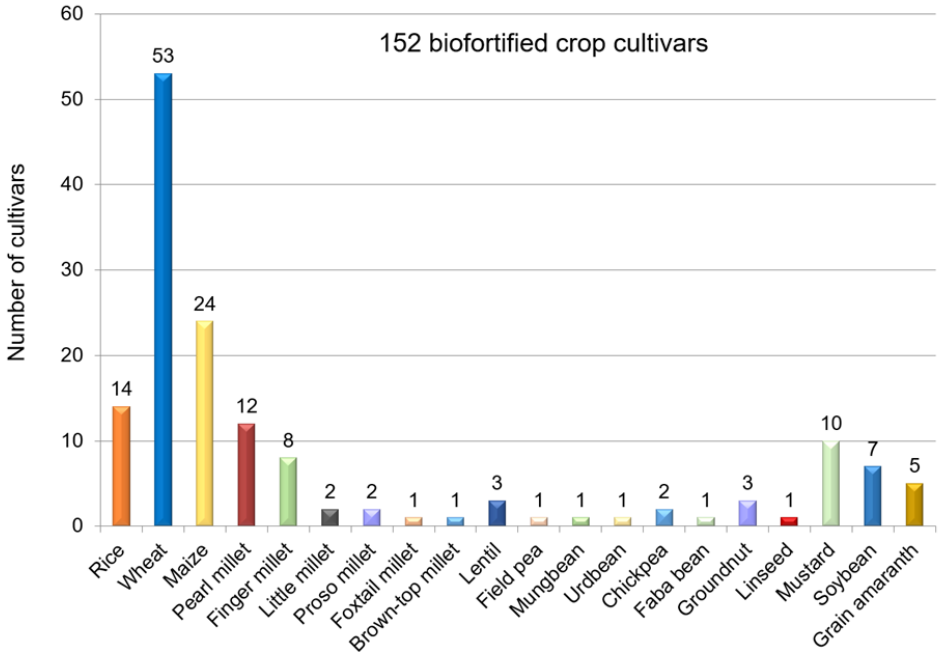

Figure 1: Crop-wise biofortified cultivars developed through breeding

high-grain protein (10.1%) and zinc (20 ppm). In wheat; the concentration of iron, zinc and protein has been improved. Of these, 'HD-3171' (47.1 ppm zinc), 'DBW-187' (43.1 ppm iron) and 'HI-8801' (12.4% protein) are the biofortified wheat varieties released for cultivation. Besides, Pusa Tejas (41.1 ppm iron, 42.8 ppm zinc, 12.0% protein) is a multinutrient-rich wheat cultivar released for commercial cultivation in India (Yadava *et al.,* 2022). In maize, the quality of protein has been enhanced by increasing two essential amino acids, lysine and tryptophan. Though the first quality protein maize (QPM) cultivar (composite), Shakti-1 was released way back in 1997 (Hossain *et al.,* 2019), the first marker-assisted selection (MAS)-derived QPM hybrid, 'Vivek QPM-9' (lysine: 4.19%, tryptophan: 0.83%) was developed by introgressing *opaque2* gene and was released during 2008 (Gupta *et al.,* 2013). Besides, several *opaque2*-based QPM hybrids *viz*., 'Pusa HM4 Improved' (lysine: 3.62%, tryptophan: 0.91%), 'Pusa HM8 Improved' (lysine: 4.18%, tryptophan: 1.01%) and 'Pusa HM9 Improved' (lysine: 2.97%, tryptophan: 0.68%) have been released using MAS (Hossain *et al.,* 2018). Furthermore, maize has also been biofortified with provitamin-A. 'Pusa Vivek Hybrid-27 Improved' with high provitamin-A (5.49 ppm) developed through MAS for *crtRB1* gene has been released for cultivation. Besides, 'Pusa Vivek QPM-9 Improved' with higher provitamin-A (8.15 ppm), lysine (2.67%) and tryptophan (0.74%) were developed through marker-assisted introgression of *opaque2* and *crtRB1* genes (Muthusamy *et al.,* 2014). 'Pusa Vivek QPM-9 Improved' earns the distinction of being the world's first multi-nutrient-rich maize hybrid with higher provitamin-A and protein quality. Maize has also been improved for vitamin-E, and 'Pusa Biofortified Maize Hybrid-5' with enhanced α-tocopherol/vitamin-E (21.60 ppm), provitamin-A (6.22 ppm), lysine (4.93%) and tryptophan (1.01%) has been recently released. This hybrid has been developed by marker-assisted stacking of *vte4*, *crtRB1*, *lcyE* and *opaque2* genes (Das *et al.,* 2021).

Table 1: Important crops and traits targeted for crop biofortification in India

Crop	*Nutrients*	*Baseline levels*	*Levels achieved*
Rice	Protein (%)	7.0–8.0	>10.0
	Zinc (ppm)	12.0–16.0	>20.0
Wheat	Protein (%)	8–10	>12.0
	Iron (ppm)	28.0–32.0	>38.0
	Zinc (ppm)	30.0–32.0	>37.0
Maize	Provitamin-A (ppm)	0.5–1.5	>5.0
	Lysine (%)	1.5–2.0	>2.5
	Tryptophan (%)	0.3–0.4	>0.6
	Vitamin-E (ppm)	6–8	>14
Pearl Millet	Protein (%)	8.0–9.0	>15.0
	Iron (ppm)	45.0–50.0	>70.0
	Zinc (ppm)	30.0–35.0	>39.0
Finger Millet	Iron (ppm)	25.0	>38.0
	Zinc (ppm)	16.0	>24.0
	Calcium (mg/100 g)	200.0	>400.0
Small Millet	Iron (ppm)	25	>55
	Zinc (ppm)	20	>33
Lentil	Iron (ppm)	45.0–50.0	>62.0
	Zinc (ppm)	35.0–40.0	>50.0
Groundnut	Oleic acid (%)	45.0–52.0	>70.0
Linseed	Linoleic acid (%)	20–25	>58
Soybean	Oleic acid (%)	22–25	>40.0
Antinutritional factors			
Maize	Phytate (mg/g)	3–8	<2.5
Mustard	Erucic acid (%)	>40.0	<2.0
	Glucosinolates (ppm)	>120.0	<30.0
Soybean	Kunitz trypsin inhibitor	30–45 mg/g of seed meal	Negligible
	Lipoxygenase	High beany flavor	Low beany flavor

Lentil has been improved for both iron and zinc (Yadava *et al.,* 2022). Biofortified lentils, 'Pusa Ageti Masoor' (65.0 ppm iron) and 'IPL 220' (73.0 ppm iron, 51.0 ppm zinc) have been released. In field pea, 'Arpan' with 91.5 ppm of iron and 50.5 ppm of zinc has been released for cultivation. Besides, 'Shreejan' (33.0 ppm zinc, 215.8 ppm iron) in mungbean, 'TJU-339' (104.6 ppm iron) in urdbean, 'IPC 2005-62' (27.25% protein) in desi chickpea, 'HFB-3' (28.05% protein) in faba bean, and 'RMA 62' (11.83% protein, 5.17% lysine) in grain amaranth have also been released for commercial cultivation.

Groundnut varieties, 'Girnar-4' (78.5 % oleic acid) and 'Girnar-5' (78.4% oleic acid) were developed by introgressing *ahFAD2a* and *ahFAD2b* genes through MAS. In soybean, oleic acid content has also been improved in 'NRC-147' (42%).

In pearl millet, 'AHB-1200Fe' (73.0 ppm iron), 'HHB-299' (73.0 ppm iron, 41.0 ppm zinc), 'AHB-1269Fe' (91.0 ppm iron, 43.0 ppm zinc) have been released. 'HHB-67 Improved2' possessing high protein (15.5%), iron (54.8 ppm) and zinc (39.6 ppm) have also been released for commercial cultivation in India. In addition, biofortified finger millet variety, 'VR-929' (131.8 ppm iron), 'CFMV-1' (428 mg/100g calcium, 58.0 ppm iron, 44.0 ppm zinc) and 'CFMV-2' (454 mg/100g calcium, 39.0 ppm iron, 25.0 ppm zinc) were released. In little millet, 'CLMV-1' with higher iron (59.0 ppm) and zinc (35.0 ppm) was developed and released for commercial cultivation (Yadava *et al.,* 2022). Besides, 'HB-1' (55 ppm iron, 35 ppm zinc) in proso millet, 'Mahanandi' (440 ppm of calcium) in foxtail millet, and 'Hagari Browntop-2' (70 ppm iron, 42 ppm zinc) in brown-top millet, have been released.

In linseed, 'TL-99' possessing 58.8% linoleic acid has been developed.

Reducing the Antinutritional Factors

Antinutritional factors have been significantly reduced through breeding. Phytate, a natural chelator of positively charged ions such as iron and zinc, has been reduced in maize kernel. 'PMH-1-LP' – a single cross maize hybrid developed through marker-assisted introgression of the *lpa2* gene with reduced phytate (1.89 mg/g) has been released for commercial cultivation in India. In the case of mustard, two anti-nutritional factors erucic acid and glucosinolates have been reduced below the threshold level (Yadava *et al.,* 2022). Single zero mustard varieties, 'Pusa Mustard-30' (1.20% erucic acid) and 'Pusa Mustard-32' (1.32% erucic acid) have been developed and released. Double zero varieties, 'PDZM-31' (0.76% erucic acid, 29.41 ppm glucosinolates) and 'PDZM-33' (0.58% erucic acid, 15.17 ppm glucosinolates) were also developed. In soybean, Kunitz trypsin inhibitor (KTI) free varieties were developed through marker-assisted introgression of the null allele of the *KTi3* gene, while lipoxygenase activity was reduced using the null allele of the *Lox2* gene. 'NRC- 127' and 'MACSNRC-1667' are KTI-free varieties. 'NRC-132' is devoid of *Lox2* activity. 'NRC-142' is a double biofortified soybean variety with null alleles of both *KTi3* and *Lox2*.

So far, >37,000 quintals of breeder seeds of various biofortified crop varieties have been produced and distributed. A large number (>90,000) of front-line demonstrations (FLDs) of these biofortified crop cultivars have been undertaken. Currently, >16 mha area is under cultivation of biofortified crops in India. Biofortified cultivars have been licensed to >300 private seed companies through >1,300 memoranda of understanding (MoU) for seed production and marketing leading to faster spread.

Challenges and Way Forward

Despite the great promise, the full potential biofortified crop cultivars are yet to be exploited (Gupta *et al.,* 2015; NAAS 2022; Yadava *et al.,* 2023). Apprehension of the low-yielding potential of biofortified varieties has been the most important factor for slow popularization among farmers. It is now a well-established fact that nutritional traits targeted so far do not pose any penalty on yield. However, some of the nutritional traits (lysine and tryptophan) are governed by recessive genes and contamination by foreign pollen grains from neighboring fields dilutes the quality of the produce in cross-pollinated crops. Adoption of the entire village for cultivation of the same type of biofortified cultivars would solve such a problem. In addition, there is also an urgent need to develop climate-resilient biofortified crop cultivars by stacking various genes and QTLs of biotic- and abiotic stress tolerance with loci governing nutritional qualities. Novel approaches such as gene editing and genomic selection should be an integral part of the crop improvement program (Hossain *et al.,* 2023, 2024b).

Awareness generation on the health benefits of biofortified crops is a key factor for the rapid adoption of biofortified cultivars by farmers (Yadava *et al.,* 2018). Besides, in cases where the enrichment of nutrients such as provitamin-A leads to an altered appearance of the produce (yellow vs. white maize), consumers generally hesitate to accept the newly developed biofortified produce as food (Gupta *et al.,* 2015). Strong promotional extension activities such as field demonstrations, and conveying a message through television talk, radio broadcasts, live-drama and various social media platforms would make the farmers, industry and consumers aware of the existence and benefits of biofortified crops. Further, robust linkages with agri-food-processing and poultry industry would help in the dissemination of biofortified crops.

Strong policy support would help in the rapid dissemination of biofortified varieties and hybrids (Yadava *et al.,* 2018; Hossain *et al.,* 2024b). Strengthening the seed chain to produce and supply good quality seeds is an important step for the popularization of biofortified cultivars. Providing subsidized seeds and other inputs would further contribute to the rapid dissemination of nutritionally improved cultivars among the farmers. Segregation of grains of biofortified crops in the market and assurance of remunerative price through minimum support price and/or premium price for biofortified grains in the market would also encourage the farmers to grow more biofortified crops. Inclusion of these biofortified cereals in different government-sponsored programs such as 'The National Food Security Mission', '*Rashtriya Krishi Vikas Yojna'* as well as nutrition intervention programs such as 'Integrated Child Development Services' scheme, 'Mid-day Meal' and 'Nutrition Education and Training through Community Food and Nutrition Extension Units' would help in providing the much-needed balanced food to poor people. Through these schemes, children, pregnant women, and elderly people would greatly benefit, and would also help in the dissemination of biofortified crop cultivars. 'National Nutrition Strategy – 2017' by NITI Aayog, GoI envisages the alleviation of malnutrition in the country through food-based solutions. Dedication of biofortified crop varieties to the nation by the Hon'ble Prime Minister on October 16, 2020, September 28, 2021, and August 11, 2024, is a testimony of the commitment of GoI to alleviate malnutrition through a food-based system.

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REVIEW ARTICLE

GMOs and Native Crops in Bolivia: Navigating Biotechnology for Food Security and Sustainable Development

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Abstract

Bolivia, a mega-diverse country with significant agricultural potential, faces systemic challenges that hinder the full utilization of its biodiversity for food security and economic development. This paper explores how biotechnology, particularly genetically modified organisms (GMOs), can address Bolivia's agricultural productivity constraints while preserving its native crops. Despite the demonstrated benefits of biotech crops-such as increased yields, reduced pesticide use, and economic gains-Bolivia's outdated regulatory framework and restrictive policies limit the expansion of genetically modified (GM) technology. Additionally, native crops and their wild relatives hold enormous untapped genetic potential for improving food resilience, yet they remain underutilized due to insufficient research and policy support. By modernizing biosafety regulations, strengthening scientific research, and promoting informed public discourse, Bolivia can leverage biotechnology to enhance food sovereignty and agricultural sustainability. The paper highlights the necessity of a balanced approach that integrates scientific innovation with environmental and socio-economic considerations to ensure long-term agricultural growth.

Keywords: Biotechnology, Genetically modified organisms, Biosafety regulation, Native crops, Food security.

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Introduction

The Plurinational State of Bolivia is a part of the eight mega-diverse countries in the world. However, the national strategy does not effectively utilize this biodiversity to achieve a sustainable model. With 11,312,620 inhabitants, its territory is the sixth largest in Latin America. Bolivia has one of the smaller growth domestic products (GDPs) in Latin America, with a 2023 GDP of around USD 45 billion, placing it towards the bottom of the regional ranking. About 36.4% of the population is below the poverty line in 2024, and its per capita income was estimated at around USD 3,919 in 2024(IMF, n.d.). In the last two decades Bolivia has become a major exporter of natural gas (28% of total exports). Other exports include gold (18%), zinc (12%), silver (9%), and soybeans and related products (11%) (Trading Economics, n.d.).

Brief Overview of Bolivia's Agricultural Landscape

Bolivian agriculture, contributing 11-15% to the GDP and employing many, faces significant challenges. Climate change severely impacts this vulnerable sector, demanding investment for adaptation and resilience to ensure food security. Obstacles include fuel shortages, roadblocks, land grabbing, smuggling, and biotechnology restrictions, all hindering productivity and competitiveness. These long-standing issues, exacerbated by inadequate government

support and ineffective policies, threaten the collapse of food production unless immediate action is taken to address the structural problems. A recent report highlights a 17% drop in agricultural production in 2024 (Los Tiempos, 2024), which is a matter of significant concern regarding the long-term sustainability of agricultural productivity in Bolivia.

By 2023, according to Ministry of Rural Development and Lands (MDRyT) data, soybean production reached 3,761,881 tons, with a cultivated area of 1,787,185 hectares, which represents a growth of 8.8% and 17.2% over the previous year (MDRyT, 2024). The main buyers of this production are: Peru (40%), Colombia (39%), Ecuador (12%), and Chile (5%).

Soybeans have become the largest export for both Brazil and its neighbouring country, Argentina. Additionally, Paraguay and Bolivia rank among the top 10 global producers in a market valued at USD 155 billion. In 2022/23 Bolivia cultivated approximately 1.82 million ha of genetically modified (GM) soybean (https://www.statista.com/statistics/957549/bolivia-soy-cultivated-area/) and soybean production reached 3.67 million tonnes (https://www.statista.com/statistics/874054/soybean-production-volume-bolivia/)

Importance of Biotechnology in Addressing Agricultural Challenges

Genetically modified (GM) crops offer several potential benefits for agriculture and food security, but they are not a single solution to all challenges. In Bolivia, where agriculture faces numerous systemic challenges, GM crops could contribute to enhancing productivity and resilience. However, it is crucial to consider both the potential benefits and limitations.

Increased crop yields

Genetic engineering can enhance crop yields, which is particularly important in Bolivia, where agricultural productivity is constrained by various factors. For example, the adoption of biotech soybeans in Bolivia has been associated with a 200,000 ton increase in production per year (National Geographic, n.d.).

Pest and disease resistance

GM crops can be engineered to resist pests and diseases, reducing the need for pesticides. This is significant in Bolivia, where reliance on pesticides can be costly and harmful to the environment. The adoption of insect-resistant biotech soybeans in Bolivia has led to reduced insecticide use (Brookes, 2022a).

Herbicide tolerance

GM crops can be engineered to tolerate herbicides, allowing farmers to control weeds more effectively without harming crops. This can lead to reduced tillage, which helps maintain soil health, reduces fuel use, and saves labour (Brookes, 2022a).

Adaptation to environmental conditions

GM crops can be engineered to better tolerate environmental challenges, such as drought. In the 2023-2024 summer seasons, 1,907,000 tonnes of soybeans, corn, and sorghum were produced in Bolivia. This represents a 34% decrease from the previous summer. In winter, there was a drop of 64% compared to the winter of 2023 (Nueva Economía, 2024). GM crops could provide better resilience to such events.

Economic gains

The adoption of GM crops can lead to economic gains for farmers. In Bolivia, biotech soybeans have saved money on insecticide use and increased production, generating an estimated USD 776 million in economic gains from 2008 to 2016 and USD 54 million in 2016 alone (Brookes, 2022b).

Although these explanations have been presented multiple times to policymakers, the adoption of GM seeds and the development of biotechnology research programs are still lacking, failing to address Bolivia's challenges and demands in a timely manner.

The GMO Landscape in Bolivia

Bolivia's experience with biotech soybeans

Since 2008, Bolivian farmers have cultivated the biotech soybean event GTS 40-3-2, which remained the only approved soybean variety in the country until 2024. Despite a severe drought in 2017, Bolivia's biotech soybean adoption rate reached 100% that year (ISAAA, 2017). The adoption of GM soy in Bolivia has led to several benefits, primarily through increased production, reduced production costs, and enhanced export revenues (IBCE and Guanella, 2016).

Benefits to the agro-industrial sector

The agro-industrial sector sees benefits in the form of increased value added in the oil industry. With increased soy production and milling, the value added to the industry could be around USD 15 million. The transport sector would also see an increase in revenues due to the increased need to transport the additional production; for example, the transport sector could generate an additional USD 3 million in revenues due to the need to transport an additional 10,000 truckloads of product.

Reduced environmental impact

The use of biotech soy has led to a reduction in the use of agrochemicals. This reduction in the use of agrochemicals has led to a reduction in the emission of carbon dioxide and the use of water. It is estimated that this could lead to a reduction of more than 7,000 tonnes of carbon dioxide emissions, equivalent to removing 3,200 cars from the road. Also, there would be a saving of almost 120 million litres of water, equivalent to the water consumption of approximately 1,200 families in Western Bolivia (IBCE and Guanella, 2016).

Benefits due to export revenues

Bolivia has also earned an additional USD 1.7 billion from exports of soy and its derivatives due to the adoption of GM soy between 2005 and 2015 (IBCE and Guanella, 2016). Overall, the adoption of GM soy in Bolivia has had a positive economic impact through increased yields, reduced costs, and enhanced trade opportunities, with additional benefits for the environment. To understand why it has been difficult to go from just one single event to four approved events, a review of the procedures, legislation, and difficulties must be explained.

Timeline of GMO Policy and Legislation

Here is a timeline of key policies and legislation related to GMOs in Bolivia (Moreira-Ascarrunz *et al*., 2019). It has to be mentioned that in February 2009, a new Political Constitution was issued. Take note that the 1997 regulation on GMO Biosafety has never been updated or harmonized with the new Political Constitution.

1992

The Convention on Biological Diversity (CBD) was established, which later influenced biosafety regulations. Bolivia ratified this in 1994.

1997

The Regulation on Biosafety was approved by Supreme Decree (DS) 24676, which primarily regulates the structure and functioning of the National Biosafety Committee (CNB). This regulation is the main legal instrument for regulating biotech crops in Bolivia.

1998

An application for approval of the biotech soybean event GTS 40-3-2 was submitted.

2001

Bolivia ratified the Cartagena Protocol on Biosafety with Law 2274.

2005

The first biotech crop approved for agricultural use in Bolivia, the glyphosate-resistant "RR" soybean, was authorized through Supreme Decree 28225. This occurred after years of a de facto moratorium on biotech crops.

2008

The soybean event GTS 40-3-2 was officially approved. This was about 10 years after the initial application. Since this time, no new events have been approved. The CNB has not functioned and, as a result, no new events have been approved.

2009

The new Political Constitution of the State (CPE) was approved. It includes the following articles regarding GMOs:

- Article 255 prohibits the import, production, and commercialization of GMOs and toxic elements that damage health and the environment. However, this is an exception to Article 409.
- Article 407, numeral 11, gives the State the power to control the entry of GMOs into the country responsibly and does not prohibit the production, import, or commercialization of GMOs.
- Article 409 states that the production, import, and commercialization of GMOs will be regulated by law. It is understood that this article does not prohibit the use of GMOs, but establishes that there will be a special law to regulate all its main aspects.
- Article 410 establishes that international treaties, such as the CBD and the Cartagena Protocol, are laws of a constitutional nature that apply in preference to state laws.

2011

The Law of the Productive, Community Agricultural Revolution (Law 144) was enacted, stipulating that:

- Agricultural technology packages involving GM seeds of species that are native to Bolivia, or that threaten genetic heritage, biodiversity, the health of life systems, and human health, will not be introduced into the country.
- All products for human consumption that contain or are derived from GMOs must be clearly identified.
- There will be measures to control the production, import and commercialisation of genetically modified products.

2012

The Law of Mother Earth (Law 300) was enacted, which prohibits the introduction, production, use, release into the environment, and commercialisation of GM seeds in Bolivia of which Bolivia is a center of origin or diversity. It also prohibits those that threaten genetic heritage, biodiversity, the health of life systems, and human health.

2015

Supreme Decree 2452 was issued to regulate the labeling of products containing or derived from GMOs.

2017

Bolivia cultivated approximately 1.3 million hectares of biotech soybeans, making it the fourth largest producer of biotech crops in Latin America.

2019

The simplified procedure was used to evaluate the HB4 soy, with drought stress tolerance (Decreto Supremo 3874, 2019).

2024

Intacta MON87701 x MON89788 events approved by Administrative Resolution (Resolución Administrativa VMA N° 090/2024, 2024)

2024

HB4 soy is approved by the Administrative Resolution (Resolución Administrativa VMA N° 101/2024, 2024).

It is worth highlighting that despite some restrictions; the existing legal framework does not completely prohibit GMOs, but rather seeks to regulate their use, especially for crops where Bolivia is not a center of origin or diversity. The country also has international commitments to the CBD and the Cartagena Protocol which call for risk assessments before the introduction of GMOs. There has been discussion about updating the legal framework, particularly the 1997 Biosafety Regulations (DS 24676), to address new technologies like gene editing and to ensure that the country's agricultural sector can benefit from innovation while safeguarding its biodiversity.

Current Situation

Bolivia's agricultural sector is navigating a complex landscape shaped by both the adoption of GMOs and the constraints of a regulatory environment struggling to keep pace with scientific advancements. This section examines the interplay of these factors, which have significant implications for agricultural productivity and sustainability in the country.

Economic impacts

Since the approval of the glyphosate-resistant soybean event GTS 40-3-2 in 2008, Bolivia has become a notable adopter of agricultural biotechnology. By 2024, approximately 1.7 million hectares were cultivated with biotech soybeans, positioning Bolivia as the fourth-largest producer of such crops in Latin America (MDRyT, 2024). This adoption has been primarily driven by the economic benefits (Choquehuanca Céspedes *et al*., 2021):

- Increased yields compared to traditional varieties.
- Reduced costs associated with insecticide use.
- Enhanced export revenues, contributing to national economic growth.
- Improved efficiency of agricultural practices.

These factors have led to significant economic gains for Bolivian farmers. However, this success is largely limited to soybean production, whereas other crops are still lagging behind.

Regulatory framework and its challenges

The primary legal framework governing GMOs in Bolivia is the Biosafety Regulation, established by Supreme Decree (DS) 24676 in 1997. This regulation primarily outlines the structure and functions of the CNB. This regulation primarily outlines the structure and functions of the CNB. However, the framework faces several significant challenges (Moreira-Ascarrunz *et al*., 2019):

- *Outdated Legislation*

The 1997 regulation is over two decades old and struggles to address modern biotechnologies like gene editing.

- *Dysfunctional Regulatory Body*

The CNB has not been functioning effectively. Lack of competent members, especially from the governmental side.

- *Inconsistencies in Legal Framework*

The 2009 Political Constitution of the State includes articles that both restrict and permit the use of GMOs. Article 255 prohibits GMOs that damage health and the environment, while Article 409 states that GMOs will be regulated by law, suggesting that their use is not completely banned.

- *Conflicting Legislation*

Law 144 (2011) and Law 300 (2012) impose additional restrictions, particularly concerning GMOs where Bolivia is a center of origin or diversity. This has led to a complex and sometimes contradictory legal landscape that hinders decision-making.

- *Lack of Implementation*

Despite regulations and international commitments to the Cartagena Protocol on Biosafety, Bolivia struggles to implement a functional biosafety system capable of evaluating new applications.

Socio-political and scientific considerations

The situation is further complicated by ongoing debates regarding (IBCE and Guanella, 2016; Choquehuanca Céspedes *et al*., 2021):

- *Expansion of GMO use*

The question remains whether to extend GMO cultivation to crops beyond soybeans, like maize and cotton, which could further enhance economic benefits.

- *Technological sovereignty*

There is growing discussion around the need to foster national scientific capabilities rather than relying solely on imported technologies. This includes developing local expertise in biotechnology to ensure food and technological sovereignty.

- *Environmental concerns*

There are persistent concerns about the potential environmental impact of GMOs and their associated agrochemicals, despite evidence suggesting reduced pesticide use. Concerns also extend to the preservation of native seeds and biodiversity.

- *Public perception*

Public opinion is divided, with some groups opposing GMOs due to concerns about health, corporate control, and environmental risks.

These systemic factors highlight the complex challenges facing the agricultural sector in Bolivia, which extend beyond just the technology of crop production. The need for effective policy, functional administration, clear regulatory

frameworks, and robust communication are critical for improving agricultural productivity and food security in the country.

Limited Focus on Native Crops

Bolivia has a high potential in terms of native crops, stemming from the richness of its agrobiodiversity and status as a center of origin or diversity for many important plant species. This term does not only refer to the variety of plants and animals but also to the variety of agroecosystems that have been developed to grow this variety of products. This potential also covers the traditional knowledge and cultural practices surrounding their cultivation and use (Villegas, 2008). This biodiversity is exemplified by the numerous varieties of crops adapted to different regions, such as over 55 races of maize, each with specific regional uses and deeply rooted in tradition (Rojas Beltrán *et al*., 2022). Additionally, Bolivia boasts a high diversity of potatoes, with many native varieties that are not only culturally significant but also nutritionally valuable.

Many of Bolivia's native crops are considered *superfoods* due to their high nutritional content. For instance, native potatoes from the Altiplano (high altitude flora) contain over 52 antioxidants, while traditional foods like *chuno*, a preparation to preserve dry potato tuber, have modified starch content that makes them more tolerable for diabetics (Rojas Beltran *et al*., 2022). Indigenous communities have preserved and cultivated these crops for millennia, developing unique agricultural practices and adapting their cultures to these foods. Traditional technologies, such as the processing of *chuno*, demonstrate sophisticated ancestral knowledge that has been passed down through generations.

Bolivia is also recognized as a center of origin or diversity for several crops, including beans, peanuts, tomatoes, tree tomatoes, potatoes, corn, quinoa, cañahua, achira (yacon, isano, cassava, blackberry, squash, and various passion fruits. Moreover, it is home to numerous wild relatives of these crops, which serve as a valuable source of genetic diversity for crop improvement. These wild relatives can provide traits such as pest and disease resistance, which are crucial for enhancing crop resilience (Alliance of Bioversity International and CIAT, 2013).

The potential for sustainable agriculture in Bolivia is substantial, given its diverse agroecosystems and traditional farming practices. There is a promotion of agroecology and agroforestry over monocultures, in order to preserve diversity and ensure that traditional crops continue to be available for future generations. According to Cockburn (2014), using native crops can strengthen food sovereignty by reducing reliance on imported foods and supporting local food systems. However, the tools and methodology of this type of production are not efficient and the demand is not covered by local production.

Despite being a producing country, Bolivia imported fresh potatoes worth USD 3.1 million from Peru in 2023, and in the first quarter of 2024, it imported potatoes valued at USD 100,259, according to data from the National Institute of Statistics (INE), compiled by the Bolivian Institute of Foreign Trade (IBCE). Until 2021, it also bought *chuno* and *tunta* (another dry processed potato) from Peru (Belmonte, 2024). Many stakeholders, including activists, foresee that these resources are under threat due to factors such as the expansion of agricultural land to export crops (like soy), the adoption of foreign crop varieties, the impacts of climate change, and a lack of government support for the agricultural sector. Therefore, there is a pressing need for scientific research to explore and understand the properties of native crops and their wild relatives. Conservation efforts, including *in-situ* and *ex-situ* conservation, are also imperative. Additionally, supporting farmers, especially in rural areas, by valuing and promoting their knowledge is crucial. Finally, promoting awareness about the nutritional value of native crops can encourage their consumption and help secure Bolivia's food future.

The Impact of Stakeholders

Whenever the GMO topic sparks a debate in Bolivia, it is usually 3 main stakeholders that are visible: The government, non-governmental organizations (NGOs) against GMOs, and organizations and individuals that are in favour of GMOs.

Government

In 1997, before Bolivia changed its name and constitution, the government passed a regulation establishing the basic steps for GMO risk assessment and approval. The first biosafety committee was formed and though there was the intention to request GEF funding to build the general framework that would cover not only the regulation but also the administrative structure, as well as build capacity, this never happened. Later in 2009, when the country changed its name to Estado Plurinacional de Bolivia, activists inside the key positions, managed to leave unclear articles in different laws or regulations, that still represent an obstacle to developing a coherent biotechnology and biosafety framework that would cover different fields. Furthermore, there is a lack of training among the personnel from the government involved in these topics, which makes it more complicated to deal with these topics.

Unfortunately, the new secondary education curricula approach biotechnology in an inappropriate manner, as they primarily focus on GMOs while emphasizing their risks and potential threats to Bolivia's biodiversity. School teachers, in general, spread the knowledge that *natural and organic* produce is better than anything that sounds complicated like GMOs. This pattern in secondary education leads most students to believe that this technology is harmful to the country.

NGOs against GMOs

Since the late 1980s, Bolivia has witnessed a rise in the number of NGOs and similar institutions that have an environmental protectionist agenda. Some radical changes in the economy led to a mass migration of miners to low lands. By the mid-1990s, the department of Santa Cruz started to copy the leading crop that was used in Brazil and Argentina. Soy was an unknown crop for that region, but since it was already adapted to similar conditions and new workers arrived, the new fields started to use soy as a promising crop. By this time, the first GMOs in soy were obtaining their first approvals in the US. At the same time, international agencies that lobby against GMOs started their activity. Some of them made alliances with Bolivian NGOs and similar organizations and by the 2000s they had moved a long way in creating a loud argument against these biotechnological innovations.

These organizations also were fast to join forces with academic professors. In this sense, even around university classes, you could learn about the terrible GMOs and the threat they were to small farmers and native seeds. Not much has changed since the early 2000s and you can still find scientists in the biological areas expressing their fear of this biotechnology. In recent years, these groups have made less noise about the new events approved. However, significant concerns exist regarding mining activity in Bolivia's Amazon region, which pollutes rivers and causes numerous health problems for local communities. Their actions are focused on addressing this complex issue.

Proponents of biotechnology

Proponents, who tend to be quieter and less visible, often lack the financial resources available to activist groups. This disparity can make it difficult for their voices to be heard. Most of the efforts have been made in the oriental part of the country, the lowlands where producers use GMO soy resistant to glyphosate. In this region, you have small, medium, and large-scale producers. In the beginning, it was mainly the large scale producers who would spread information based on science. But around 2012, small producers demanded to learn the technical part of GMOs as they were using these types of seeds as well. Gradually around 2015-2017, some academicians joined the campaign and also Agroavances.com was launched. This news portal focused on agricultural and biotechnology information and had strong support from producers in Santa Cruz. For 5 years, producers in general, were trained on how GMOs are made, their potential, and about biosafety of GMOs. This portal has limited support and the workshops and other events seldom take place. The big producers decided to work more on the legislation that would allow the opening of new events. The association of agronomists in this region also had some participation during those years but the commitment to keep educating about these topics has been reduced to almost none.

It's worth highlighting that Santa Cruz de la Sierra is where the only university that offers Biotechnology as a career is located. This bachelor's degree has finally graduated the first biotechnology engineers in Bolivia and eventually, these new professionals will face the challenge of not having a solid regulatory framework that allows them to work with biotechnology tools, in general, in different fields. This may lead to a stronger movement that could generate a relevant lobby that eventually could push to generate the conditions needed to work within this field.

Potential of Crop Wild Relatives and Biotechnology

Crop wild relatives (CWRs) are true treasures of nature that have been ignored for a long time. CWRs in Bolivia are an invaluable resource for enhancing food production, particularly when combined with biotechnology. Recognized as a significant center of origin for cultivated plants, Bolivia boasts a rich diversity of wild relatives across its varied ecosystems, which include the Andes and lowland regions. Bolivia hosts 34 wild potato species, 21 of which are endemic. These wild potatoes possess traits that are beneficial for cultivated varieties, such as resistance to diseases like late blight (*Phytophthora infestans*) and tolerance to climatic stresses including extreme heat and drought (Bioversity International, 2023). Additionally, the wild relatives of beans and peppers thrive in this region, contributing further to the genetic diversity available for crop improvement (Lane, 2022).

The application of biotechnology can significantly enhance the potential of these wild relatives in modern breeding programs. By utilizing genetic modification techniques, traits from wild relatives can be introduced into cultivated crops, leading to improved resistance against pests and diseases, as well as enhanced nutritional profiles (Eyzaguirre *et al*., 2022). For instance, successful breeding programs have demonstrated that wild relatives can improve yield and stress tolerance in crops such as wheat and rice (Hajjar and Hodgkin, 2007). Furthermore, incorporating genes from wild relatives can help develop new crop varieties that are more resilient to climate change and capable of higher yields. This approach not only addresses food security challenges but also reduces reliance on chemical inputs by enhancing the natural resilience of crops [International Center for Tropical Agriculture (CIAT), 2023]. The conservation of these wild relatives is essential for ensuring their availability for future agricultural advancements.

Despite the significant potential of CWRs as genetic resources, several challenges impede their full utilization in crop improvement programs. Two primary obstacles are faced by researchers and conservationists in many countries including Bolivia. Firstly, breeding incompatibilities present a substantial hurdle in the integration of beneficial traits from CWRs into cultivated crops. These incompatibilities

can manifest as crossing barriers, hybrid sterility, or reduced fitness in subsequent generations. Such genetic barriers often need advanced breeding techniques, including embryo rescue, chromosome doubling, or bridge crossing, which can be time-consuming and resource-intensive. Secondly, habitat destruction emerges as a critical threat to the *in-situ* conservation of CWRs in Bolivia. The country's rich biodiversity is under increasing pressure from factors such as agricultural expansion, urbanization, mining activity, and climate change. This loss of natural habitats not only reduces the overall population of wild species but also potentially eliminates unique genetic variants adapted to specific environmental conditions.

To understand how helpful these wild plants could be for Bolivia, here are some examples. We are looking at how their good genes could be used to improve commercial crops.

Chili

Bolivia's native chili peppers (ajíes) hold significant agro-industrial potential that could be further enhanced through biotechnology. Reyes Colque *et al.,* (2021) highlight Bolivia's rich diversity of Capsicum species, making it a center of origin and domestication for these crops. The Pairumani Phytotechnical and Seed Center maintains a collection of 731 diverse chili accessions. Biochemical analyses of 96 cultivated and wild chilli peppers revealed valuable nutritional qualities, including capsaicin, colorants, anti-oxidants, flavonoids, quercetin, and vitamin E, which are key components for the food, pharmaceutical, and cosmetic industries.

Biotechnology and chilli peppers could play a crucial role in several areas. Firstly, it could aid in enhancing desirable traits, such as higher capsaicin content for spiciness or increased anti-oxidant levels, appealing to health-conscious consumers. Secondly, it could improve disease resistance in native chili varieties, reducing crop losses and reliance on pesticides. The study also identifies the potential for processing native chilies into pickles, sauces, and jams, maintaining their color, piquancy, and consistency; biotechnology could be employed to optimize these processing characteristics and extend shelf life (Reyes Colque *et al*., 2021). Moreover, the document points out the underexploited wild chili species ("ulupicas" and "aribibis") and their potential for cultivation. Biotechnology could assist in overcoming limitations to their cultivation, such as improving germination, uniformity, and pest resistance, making them more accessible to small farmers. The study also notes that Bolivia consumes a larger diversity of wild Capsicum than probably any other country in the world (Reyes Colque *et al*., 2021).

By combining traditional knowledge with modern biotechnology, Bolivia can create new, high-value chili varieties that are better adapted to local conditions, more nutritious, and more appealing to both domestic and international markets. This approach could empower small farmers, promote sustainable agriculture, and contribute to the country's bioeconomy.

Cacao

The study by Chumacero de Schawe *et al*., (2013) establishes that wild cacao populations in Bolivia are genetically distinct, which is invaluable for targeting conservation efforts and understanding the evolutionary adaptation of the species. That diversity could be harnessed to create new varieties with enhanced flavour, nutritional content, and resilience to environmental stressors. By safeguarding rare and endangered native cacao genotypes, the unique genetic heritage can be preserved for future generations. The Red Book of CWRs (VMABCC-BIOVERSITY, 2009) highlights the species' economic potential as well. Disease resistance is important, and biotechnology offers tools to develop disease-resistant cacao through genetic engineering or gene editing. Identifying and incorporating resistance genes from wild cacao relatives could create more resilient and productive cacao trees. This is supported by Chumacero *et al.,* (2013) data showing low population densities of these populations which suggest that understanding gene flow is elementary for understanding the reproductive success and management of tropical tree species in the area. This approach allows for the development of novel cultivars as well as preserving wild populations through local communities.

By merging traditional farming practices with carefully selected biotechnological interventions, Bolivia has a unique opportunity to unlock the full economic potential of its native cacao. This would contribute to a more sustainable and economically viable agricultural sector while preserving Bolivia's distinctive biodiversity, with careful assessment to the impact on low-density wild populations, and to manage the flow of gene exchange between cultivated and wild cacao in the area.

Quinoa

Wild relatives of quinoa exhibit significant genetic diversity, which is crucial for the improvement of cultivated quinoa. The CWRs Red Book of Bolivia (VMABCC-BIOVERSITY, 2009), identifies several wild quinoa species, subspecies, and varieties that are distributed across the Andean region, including Bolivia's highlands, valleys, and plains. These wild relatives are known for their resistance to various pests and diseases, making them valuable for genetic enhancement. The wild species possess traits such as drought tolerance and adaptability to harsh environmental conditions, which are essential for breeding programs aimed at developing resilient quinoa varieties (Zapata and Apaza, 2007). The genetic variability found in these wild relatives can be harnessed to improve cultivated quinoa's nutritional quality and stress resistance.

Based on the information, the biotechnological potential of utilizing wild relatives of quinoa for breeding encompasses several promising approaches. Marker-Assisted Selection (MAS) offers a means to identify and select for desirable traits linked to specific genes in wild relatives, thereby expediting the breeding process. Advanced methods such as CRISPR/Cas9 could be used to directly edit genes in cultivated quinoa, incorporating beneficial traits from wild relatives. Traditional breeding methods can be enhanced by creating hybrids that combine the hardiness of wild species with the desirable traits of cultivated quinoa. The importance of conserving genetic material from wild relatives is emphasized to ensure that these valuable genes are preserved for future breeding efforts.

Peanut

Peanut production in Bolivia has faced significant challenges, primarily due to low productivity and contamination with aflatoxins, which limits the availability of this important source of protein, vitamins, and minerals. The situation is exacerbated by high poverty rates, affecting 36.4% of the population, particularly in rural and peri-urban areas, leading to nutritional deficiencies (Valles Foundation, 2023).

To address these issues, cultivation has to be standardized, implement quality control, and production of peanuts. According to the "Libro Rojo de Parientes Silvestres de Cultivos de Bolivia" (VMABCC-BIOVERSITY, 2009), there are 79 wild species of peanuts (genus *Arachis*) identified globally, with 20 of these species found in Bolivia. Among these, 12 species are endemic to the country. The wild relatives of peanuts are significant for their genetic diversity and potential for improving cultivated peanuts through traits such as drought resistance and pest resistance (Krapovickas and Gregory, 1994; Atahuachi *et al*., 2009). Wild species, such as *Arachis duranensis*, *Arachis ipaënsis*, and *Arachis krapovickasii*, offer valuable genetic traits that can enhance the performance of cultivated varieties. These wild species possess genes that confer resistance to pests and diseases, making them valuable for breeding programs focused on enhancing the resilience of cultivated peanuts (Atahuachi *et al*., 2009). Additionally, they may have traits that enhance drought tolerance and nutritional quality, which are crucial for food security in changing climatic conditions.

Potato

In the year 2022/23, the production of potatoes in Bolivia amounted to 1.17 million metric tons. This represents a decrease of 5% compared to the previous year (Navarro Villa, 2024). Last couple of years, a good part of the production has been lost due to frost or drought. Bolivia is home to 34 wild potato species, notably 21 of which are endemic. These wild species are of significant interest for the genetic improvement of cultivated potatoes and have been the subject of numerous collection expeditions since the early 19th century.

The wild relatives of potatoes exhibit various traits that are valuable for improving cultivated varieties. For instance, *Solanum achacachense* shows potential resistance to late blight caused by *Phytophthora infestans* and frost, while *Solanum alandiae* has demonstrated resistance to several pests and diseases, including the potato wart pathogen (*Synchytrium endobioticum*) and soft rot (*Erwinia carotovora*). Other species, such as *Solanum arnezii*, have shown varying degrees of resistance to nematodes and other pathogens, making them significant for genetic enhancement efforts (VMABCC-BIOVERSITY, 2009).

The conservation of these wild potato species is critical, as they harbor genetic diversity that can be leveraged to enhance the resilience and productivity of commercial potato crops. The ongoing threats to these species, including habitat destruction and climate change, underscore the urgency of conservation actions. Since 2009, when the Red Book of Crop Wild Relatives for Bolivia was published, few concrete actions have been executed in order to preserve these varieties or to use their genetics to improve commercial crops.

The wild relatives of the above crops contain significant genetic resources that biotechnological advancements can unlock to improve the resilience, nutritional value, and productivity of cultivated crops. Realizing these benefits, however, hinges on establishing clear regulatory frameworks and ensuring that decision-makers are prepared to consult with a scientific committee for evidence-based guidance on employing biotechnology to enhance domestic food production.

Way Forward

Given the potential benefits of biotechnology for native crops in Bolivia, alongside limitations in the current regulatory framework, a clear path forward involves modernizing regulations and building relevant capacities. Drawing from the recommendations already given by Moreira-Ascarrunz *et al.,* (2019), several key actions can be taken to achieve this:

- The current biosafety regulation based on Supreme Decree (DS) 24676 from 1997 is outdated. Therefore, it needs to be updated to incorporate advancements in biosafety concepts and procedures.
- The new regulations should be based on scientific principles to foster confidence and optimize usage of resources. This involves evaluations based on scientific evidence related to environmental and food safety; to avoid legal contradictions, there needs to be a single, coherent legal body to do this.
- To ensure public trust, new regulations should include provisions for publishing applications and decisions on a website to promote transparency. Furthermore, it is essential to account for new innovations in genetic engineering, such as stacked events, gene editing,

and advances in synthetic biology, implementing monitoring to verify risk management activities.
- The reactivation and strengthening of the CNB are crucial to providing technical advice to the National Competent Authority, using scientific criteria.
- A functional administrative system must be established with competent human resources, structure, and tools to effectively evaluate new applications.
- Finally, it is important to develop firm and concrete policies within Bolivian legislation to regulate modern biotechnology in the National Biosafety Framework and ensure norms for OGM regulation align with Bolivia's commitments.

By addressing these aspects, Bolivia can establish a robust regulatory framework that fosters responsible use of biotechnology for improving native crops for food and nutritional security while ensuring environmental protection and public trust.

Challenges and Considerations for GM Crops in Bolivia

- Addressing the complexities of GMO implementation in Bolivia necessitates a holistic approach that navigates concerns surrounding intellectual property rights, public perception, equity, regional adoption, and potential trade-offs. To ensure fair access and benefit-sharing, promoting public-private partnerships, supporting open-source seed initiatives, and bolstering local seed production are crucial steps. These measures can help diversify the seed market, reduce reliance on multinational corporations, and provide farmers with seeds adapted to their specific environmental conditions.
- Building public trust requires transparent communication and informed consumer choice. Investing in science communication initiatives and ensuring regulatory transparency are essential. Such measures empower consumers to make informed decisions while addressing potential misconceptions about the safety and benefits of GM technology. Moreover, targeted subsidies and strengthened social safety nets can level the playing field, ensuring that small farmers can access and benefit from GM technology without exacerbating existing inequalities.
- To fully harness the potential of GMOs in enhancing food security and promoting sustainable agriculture, it's essential to direct research towards developing crops tailored to local conditions while prioritizing public health. This includes increasing research funding for developing GM food staple crops and encouraging practices like integrated pest management, soil health management, and environmental monitoring.

By carefully considering potential trade-offs and prioritizing ecological sustainability, Bolivia can harness the potential of GMOs while minimizing environmental risks.

Conclusion

Bolivia's genetic wealth of native crops and CWRs offers considerable potential for strengthening food security through the strategic use of biotechnology. Native species such as quinoa, potatoes, and chili peppers possess significant economic and nutritional value. These resources can be leveraged through biotechnological applications to enhance the resilience, nutritional quality, and productivity of cultivated varieties. However, this will only be realized with the establishment of a clear normative framework provided decision-makers are fully prepared to rely on a scientific committee for evidence-based recommendations on the application of biotechnology. Public perception and education represent key factors influencing the acceptance and adoption of biotechnology in Bolivia. Negative public sentiment, often fuelled by misinformation and opposition from environmental NGOs and certain segments of the academic community, has contributed to a slower rate of adoption of biotech innovations. Focused and improved educational initiatives, particularly within secondary and tertiary education, are needed to provide balanced, evidence-based perspectives on the risks and benefits of GMOs and biotechnological applications. The prevailing legal framework governing biotechnology in Bolivia requires urgent reform. Current regulations are outdated, inconsistent, and insufficient in addressing modern biotechnological advancements like gene editing. While certain GM crops, like soybeans, have seen approval and widespread adoption, updated policies are essential to facilitate the responsible and sustainable expansion of biotechnology to a wider range of crops. Such policy revisions are critical to ensure both food security and environmental sustainability within the country.

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REVIEW ARTICLE

Fruit Crops and Their Varietal Wealth in the Northeastern Region of India: Current Status and Prospects

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Abstract

The Northeast region comprises eight states: Assam, Arunachal Pradesh, Meghalaya, Manipur, Mizoram, Tripura, Nagaland, and Sikkim. This region spans approximately 25.608 million hectares, accounting for 7.79% of the total geographical area of the country. The area boasts a distinctive array of living organisms, habitats, and ecosystems, collectively contributing to its rich diversity of resources. The region's varied climatic conditions support the cultivation of numerous tropical and temperate fruit crops that are highly nutritious. It serves as one of the most abundant sources of genetic variability and diversity among various horticultural crops, including a wide range of fruits, vegetables, spices, ornamental plants, as well as medicinal and aromatic plants. Here in this review paper, we have attempted to summarize the genetic and varietal wealth of important fruit crops that are being grown in these northeastern states of India. Also, the trend in the area and production of fruit crops in northeastern states in the last 10 years has been discussed. This document will help students, researchers, academicians and policy makers to formulate any future planning related to research and development of fruit crops in these areas.

Keywords: Fruits, Horticulture, North-east, Varieties.

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Introduction

The Northeast region (NE) of India, which includes the states of Arunachal Pradesh, Assam, Meghalaya, Manipur, Tripura, Mizoram, Nagaland, and Sikkim, can be classified physiographically into three main areas: the Eastern Himalayas, the Northeast hills (comprising the *Patkai-Naga* Hills and *Lushai* Hills), and the plains of the Brahmaputra and Barak Valleys situated at the intersection of the Indo-Malayan, Indo-Chinese, and Indian biogeographical realms, the NE region is distinctive for its abundance of habitats, showcasing a wide variety of flora and fauna with a significant degree of endemism. The NE region exhibits significant variations in altitude and topography, which facilitate the presence and distribution of biodiversity within the area (Dutta *et al.,* 2018). Although *jhum* cultivation, a traditional agricultural practice, is frequently cited as a contributing factor to the region's deforestation, it remains a vital economic activity for local tribes, who cultivate 35 different crop varieties (Dutta *et al.,* 2016; Lungmuana *et al.,* 2018). The Indian Council of Agricultural Research (ICAR) has recognized the region as a significant center for rice germplasm, while the National Bureau of Plant Genetic Resources (NBPGR) in India has noted its abundance of wild relatives of crop plants. Additionally, this area is acknowledged as the origin of citrus fruits. The region boasts a wealth of medicinal plants and numerous rare and endangered species. Its significant levels of endemism among higher plants, vertebrates, and bird species have earned it the designation of a biodiversity 'hotspot'. In 1995, the International Union for

Conservation of Nature (IUCN) recognized Namdapha in Arunachal Pradesh as a center of plant diversity (Chatterjee *et al.,* 2006).

Major and underutilized fruit crops represent a significant portion of the biodiversity found in these regions. In the NE region, which is home to the country's most diverse fruit crops, there is a notable variety of citrus, banana, and jackfruit. Additionally, a wide range of other temperate, tropical, and subtropical fruits from genera such as *Pyrus*, *Rubus*, *Ribes*, *Prunus*, *Garcinia*, *Artocarpus*, *Phyllanthus*, *Annona*, *Averrhoa*, *Persia*, *Aegle*, *Passiflora* and *Tamarindus* have also been documented in this area. The local population primarily utilizes these underutilized fruits for medicinal purposes, and they hold significant socioeconomic value in the rural lives of tribal communities, serving as sources for furniture wood, firewood, fodder, dyes, oils, and various other value-added products (Rai *et al.,* 2005; Deka *et al.,* 2012; Singh *et al.,* 2014). The literature has been extensively reviewed by various authors regarding the fruits of NE India, focusing on their ethnomedicinal applications, socioeconomic significance, and strategies for conservation (Dutta *et al.,* 2018). Numerous studies have documented the geographical distribution and ethnomedicinal applications of various local fruits; however, there is a lack of comprehensive reports regarding the diversity of existing fruit crop varieties. For decades, some of the commercial varieties of fruits have occupied significant positions in the socioeconomic growth of these areas. A preliminary investigation into the reporting of the diverse varieties of major and locally cultivated fruit crops was deemed essential to uncover the potential fruit crop varieties found in the remote and difficult-to-access regions of the country. In this review paper, we aim to provide a comprehensive overview of the diverse varieties of significant fruit crops cultivated in the northeastern states of India. This document is intended to assist students, researchers, academics, and policymakers in developing future strategies for the research and advancement of fruit crops in these regions.

Biodiversity of Fruit Crops in the NE Region

The NE region is a significant reservoir of genetic variability and diversity among various crops, particularly different types of fruits and plantation crops (Table 1). This diversity in fruit crops has primarily been preserved and managed by local farmers, with a notable contribution from women. There is substantial variation among the fruit species in this region, encompassing differences in plant types, morphological and physiological traits, responses to diseases and pests, as well as adaptability and distribution patterns. In addition to their nutritional benefits, many local fruit crops are utilized for medicinal purposes and play a crucial role in income generation and poverty alleviation initiatives in rural communities (Rai *et al.,* 2005).

The region is home to a variety of citrus species, showcasing significant genetic diversity. Researchers have identified 17 citrus species along with 52 cultivars and several potential natural hybrids from this area. Notably, there are 32 distinct strains of lemon. The indigenous species include *C. limon, C. medica, C. jambhiri, C. ichangensis, C. latipes, C. macroptera, C. assamensis, C. indica* and *C. aurantium*. Additionally, the Indian wild orange, *C. indica*, is found in the Naga hills and Meghalaya (Bhattacharya and Dutta, 1956). The highest genetic diversity of *Musa acuminata* and *M. balbisiana* is found in NE India. *M. flaviflora* is specifically found in the regions of Manipur and Meghalaya. Additional species are present in Sikkim and the *Khasi* Hills, which require systematic collection and conservation efforts. Several native *Mangifera* species can be located in Tripura, Manipur, Mizoram, and South Assam. The Indo-Burma region is also considered the center of diversity of mango. A cultivar named '*Rangkuai*' is found in the border village of India with Myanmar near the *Koladyne* River. The wild varieties of *M. indica* and its related species, *M. sylvetica*, are found in Arunachal Pradesh, while *M. khasiana* and *M. pentandra* are located in Assam. A significant variety of species is found within the genera *Pyrus, Rubus, Ribes* and *Prunus*. The Shillong plateau, located in the *Khasi* hills of Meghalaya, is home to several *Prunus* species, including *P. napalensis, P. undulata* and *P. cerasoides*. The variety of *Pyrus pyrifolia* var. cubha makai (also known as *P. serotina* Red) is cultivated semi-commercially in Meghalaya, Manipur, and other regions. Additionally, wild kiwi species, such as *Actinidia callosa* and *A. stragosa* thrive in the natural forests of Arunachal Pradesh and Sikkim (Rai *et al.,* 2005). Numerous tropical and subtropical fruits from the genera *Garcinia, Artocarpus, Phyllanthus, Annona, Averrhoa, Persia, Aegle, Passiflora* and others, as shown in Table 1, thrive in the wild within the region. Among the several edible plant species identified in the NE region, several are highly valued by various tribal ethnic groups. Two species of *Elaeagnus*, specifically *E. latifolia* and *E. pyriformis*, are known to thrive in this area (Pandey, 2002). These species are prevalent in *Sibsagar* (*Dikho* Valley), the Naga Hills, as well as the *Khasi* and *Jaintia* Hills. Additionally, *Docynia indica* and *D. hookeriana* are commonly found throughout the region. *Pyrus pashia*, a medium-sized deciduous fruit tree, is also present in the northeastern region (Rai *et al.,* 2005).

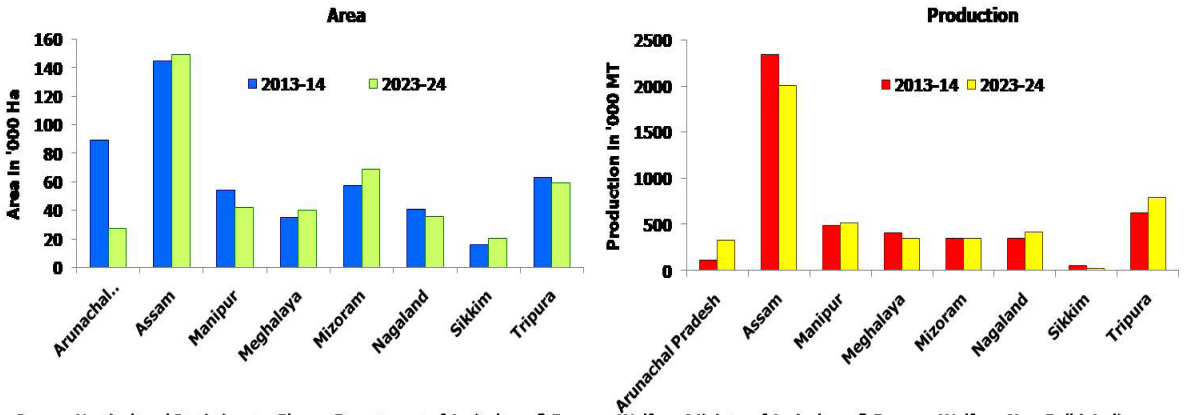
Area and Production of Major Fruit Crops

The whole NE region can be divided into six agroclimatic zones which are the alpine Zone (>3500 m), temperate sub-alpine zone (1500–3500 m), sub-tropical hill zone (1000–1500 m), sub-tropical plain zone (400–1000 m), mild tropical hill zone (200–800 m) and mild tropical plain zone (0–200 m). The alpine zone is found in Arunachal Pradesh and Sikkim and major crops here are wild strawberries, sea buckthorn, blueberries, raspberries and other wild nuts. Temperate sub-alpine zones are found in Arunachal Pradesh,

Meghalaya, Manipur, Sikkim, Mizoram, and Nagaland; here apple, pear, peach, plum, walnut and kiwifruit are mostly grown. Sub-tropical hill zones are found in the states of Arunachal Pradesh, Assam, Meghalaya, Sikkim, Mizoram, and Nagaland; here mango, lemon, guava, banana, strawberry, and passion fruit are mostly grown. Mild tropical hill zones are found in the states of Arunachal Pradesh, Assam, Meghalaya, Sikkim, Mizoram, Tripura and Nagaland; in these areas, pineapple, mandarin, banana, papaya, litchi, areca nut and coconut are the major fruit crops. Mild tropical plain zones are found in almost all the northeastern states; here, pineapple, litchi, mango, banana, jackfruit, citrus and cashew nut are the major fruit crops. The area and production of fruit crops in northeastern states in the last 10 years (2013-14 to 2023-24) are presented in Figure 1. Over the last 10 years, the area of fruit crops has increased in the states of Assam, Meghalaya, Mizoram and Sikkim, while it has decreased in Arunachal Pradesh, Manipur, Nagaland and Tripura. Production of fruit crops has increased in the states of Arunachal Pradesh, Manipur, Mizoram, Nagaland and Tripura, while it has reduced in Assam, Meghalaya and Sikkim. As per the data of Horticultural Statistics at a Glance, Department of Agriculture & Farmers Welfare, Ministry of Agriculture & Farmers Welfare, New Delhi, India, Assam, followed by Mizoram and Tripura, records the maximum area, whereas Assam, followed by Tripura and Manipur, records the maximum in production. In the year 2023-24, the NE region recorded an area of 442.20 thousand Ha and production of 4758.05 thousand MT.

Soil and Climatic Conditions

The NE region of India includes the states of Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim, and Tripura. This region is situated between latitudes 21.5°N and 29.5°N, and longitudes 85.5°E and 97.5°E. The area is classified as a high rainfall zone, with a climate that varies from subtropical to alpine. It encompasses two primary river basins, the Brahmaputra and Barak, and exhibits a significant reliance on natural resources among its population, coupled with underdeveloped infrastructure. The region is marked by a variety of climate patterns that are heavily influenced by the southwest monsoon, which occurs from June to September. Rainfall amounts differ across locations, with *Mawsynram* (Meghalaya) recording a maximum in a single day. The number of rainy days varies significantly, ranging from 80 days in *Jharnapani*, Nagaland, to 181 days in Tadong, Sikkim. Additionally, the region features challenging terrain, considerable differences in slopes and altitudes, diverse land tenure systems, and a variety of agricultural practices. The agricultural system is primarily rainfed, characterized by subsistence-level mono-cropping that is highly susceptible to climate fluctuations. Slash-and-burn agriculture continues to be practiced in nearly all states, except Sikkim, on steep slopes, with a reduced cycle of 2 to 3 years compared to the previous 10 to 15 years, leading to adverse effects on both soil quality and climate (Verma *et al.,* 2015). This region is characterized by monocropping practices that yield low agricultural production, which is insufficient to support the livelihoods of impoverished tribal farmers. Reports indicate that traditional *jhum* farming results in a loss of 76.6 tons of soil per hectare per year. Soil erosion associated with *jhum* cultivation on slopes of 60 to 70% has been documented, showing losses of 147, 170, and 30 tonnes per hectare per year during the first, second, and third years of abandonment, respectively. The area faces significant challenges related to land degradation, soil acidity, and severe erosion due to the current *jhum* farming practices employed by tribal communities. Notably, severe soil acidity (pH <5.5) is widespread in this region, representing approximately 54% of India's acid soils. Soil acidity plays a critical role in influencing nutrient availability for crops and often leads to mineral stress issues. Additionally, the extent of soil erosion in *jhum* cropping varies significantly from year to year, largely influenced by rainfall patterns (Kumar and Meena, 2016).



Source: Horticultural Statistics at a Glance, Department of Agriculture & Farmers Welfare, Ministry of Agriculture & Farmers Welfare, New Delhi, India

Figure 1: Area and production of fruit crops in northeastern states in last ten years (2013-14 to 2023-24)

Table 1: Botanical name, English name, vernacular name, family and parts used of some of the fruit crops in northeastern India

S. No.	*Botanical name*	*English name*	*Vernacular name*	*family*	*Parts used*
1	*Aegle marmelos* (L.) Correa.	Golden apple	*Bael*	Rubiaceae	Fruit and leaves
2	*Aglaia edulis* A. Gray	Aglaia	*Sanulahsune*	Meliaceae	Fruit
3	*Annona squamosal* L.	Custard apple	*Sarifa*	Annonaceae	Fruit, bark, root and leaves
4	*Artocarpus heterophyllus* (*A. integrifolia*)	Jack Fruit	*Rukh katar*	Moraceae	Fruit and leaves
5	*Artocarpus lakoocha* Wall.ex Roxb.	Monkey fruit	*Badar phal*	Moraceae	Fruit, leaves and latex
6	*Baccaurea ramiflora* Lour	Burmese Grape	*Kusum*	Euphorbiaceae	Fruit, wood, seed leaves and bark
7	*Calamus erectus* Roxb.	Rattan fruit	*Fakery*	Arecaceae	Fruit, shoot and seed
8	*Carica papaya* Linn.	Papaya	*Mewa*	Caricaceae	Fruit, leaves and seed
9	*Castanopsis hystrix* Miq.	Chestnut	*Kattus*	Fagaceae	Fruit and leaves
10	*Citrus maxima* (Burm.) Merr.	Pomelo	*Phoksey*	Rutaceae	Fruit, leaves, flower and seed
11	*Citrus medica* L	Citron	*Bimbira*	Rutaceae	Fruit, seed, leaves, flower and shoot
12	*Citrus reticulata* Blanco.	Mandarian orange	*Suntala*	Rutaceae	Fruit and flower
13	*Dillenia indica* L	Elephant apple	*Ramphal/Panchphal*	Dilleniaceae	Fruit, leaves and root
14	*Diospyros virginiana*	Persimmon	*Halle Beth*	Rubiaceae	Fruit and leaves
15	*Diploknema butyraceae* Roxb. Lam.	Indian Butter Tree	*Chuirii*	Sapotaceae	Fruit, seed and leaves
16	*Docynia indica* (Wall.) Decne.	Docynia or Assam apple	*Mehel*	Rosaceae	Fruit
17	*Elaeagnus latifolia* L.	Himalayan Silverberry	*Musleri/Maldhero*	Elaeagnaceae	Fruit
18	*Elaeocarpus sikkimensis* Rox b.	Sikkim Quandong	*Badrasey*	Elaeocarpaceae	Fruit
19	*Ephedra gerardiana* Wall. ex Klotzsch & Garcke	Indian joint-fir	*Somlata*	Ephedraceae	Fruit
20	*Ficus hirta* Vahl.	Hairy fig	*Khasre*	Moraceae	Fruit
21	*Ficus racemosa L.*	Cluster fig	*Dumri*	Moraceae	Bark, root, leaf, fruit and latex
22	*Ficus roxburghii* Wall.	Fig	*Nebaro*	Moraceae	Fruit, leaves and gum
23	*Ficus semicordata Buch-Ham*	Fodder fig	*Khaniu*	Moraceae	Fruit and root
24	*Fragaria nubicola* (Hoof.f) Linn.	Himalayan strawberry	*Bhui aiselu*	Rosaceae	Fruit, rhizome and leaves
25	*Garcinia cowa* Roxb.ex Choisy.	Cowa or Brindal berry	*Kaphal*	Clusiaceae	Fruit, leaves and root
26	*Grewia elastica* Roxb.	Grewia	*Kunsung*	Tiliaceae	Fruit
27	*Helicia nilagirica* Bedd.	Not found	*Bandre*	Proteaceae	Fruit, leaves and bark
28	*Heracleum nepalense* D. Don	Hogweed (cow parsnip)	*Chimphing*	Apiaceae	Fruit, seed and root
29	*Hippophae rhamnoides Linn*	Sea buckthorns	*Chuma/Durchuk*	Elaeagnaceae	Fruit, leaves and seed.
30	*Holboellia latifolia* Wall.	Sausage vine	*Golpha*	Lardizabalaceae	Fruit and leaves
31	*Juglans regia* L.	Wild Walnut	*Okher*	Juglandaceae	Fruit, leaves and wood
32	*Litchi chinensis* Sonn.	Sapindanceae	*Lychee*	Sapindanceae	Fruit
33	*Litsea cubeba* (Lour) Pers.	Mountain Pepper	*Siltimur*	Lauraceae	Fruit and leaves
34	*Machilus edulis* King ex Hook.f.	Wild Avocado or Pumsi	*Pumsi/ Lapche kaulo*	Lauraceae	Fruit and leaves
35	*Malus sikkimensis*	Sikkim crabapple	*Aiphal*	Rosaceae	Fruit
36	*Mangifera sylvatica Roxb.*	Himalayan mango	*Chuche anp*	Anacardiaceae	Fruit

Cont...

S. No.	Botanical name	English name	Vernacular name	family	Parts used
37	*Morus alba* Wall.	Mulberry	*Kimbu*	Moraceae	Fruit, leaves and bark
38	*Musa balbisiana* Colla	Sweet wild banana:	Bankera.	Musaceae	Fruit, root and leaves
39	*Passiflora edulis* Sim.	Passion Fruit	*Grenadelle,*	Passifloracerae	Fruit
40	*Phyllanthus emblica* L.	Indian gooseberry	*Amala*	Phyllanthaceae	Fruit, leaves and root
41	*Prunus cerasoides* D. Don	Wild Himalayan Cherry	*Painyuu*	Rosacea	Fruit, seed, gum, wood, branch, leaves and twig
42	*Prunus domestica* L.	Plum	*Arru bhakara*	Rosaceae	Fruit
43	*Prunus persica* Batsch	Peach	*Arru*	Rosaceae	Fruit
44	*Psidium guajava* linn.	Guava	*Ammuk*	Myrtaceae	Fruit and leaves.
45	*Pyrus pashia* (Buc h.-Hamex Don.	Pear	*Naspati*	Rosaceae	Fruit
46	*Rhus chinensis*	Chinese sumac	*Bhakimlo*	Anacardiaceae	Fruit
47	*Rubus ellipticus* Sm.	Yellow Himalayan raspberry,	*Aiselu*	Rosaceae	Fruit and root
48	*Saurauia napaulensis* DC	Gogan	*Gogun*	Saurauiaceae	Fruit
49	*Spondias axillaris* Roxb.	Hog Plum	*Lapsi*	Anacardiaceae	Fruit and bark
50	*Spondias pinnata* (L.f.) Kurz	Indian hog plum	*Amara*	Anacardiaceae	Fruit
51	*Syzygium kurzii*	Not found	*Ambakey*	Myrtaceae	Fruit and leaves
52	*Tamarindus indica* L.	Tamarind	*Titri*	Fabaceae	Fruit
53	*Terminalia bellirica* (Gaertn.) Roxb.	Bedda nut tree	*Barra*	Combretaceae	Fruit
54	*Terminalia chebula* Retz.	Chebulic Myrobalan	*Harra*	Combretaceae	Fruit, bark and seed
55	*Tetradium fraxinifolium* (Hook.) T.G.Hartley.	Euodia, Evodia, or Bee bee tree	*Khanakpa*	Rutaceae	Fruit, seed and wood
56	*Trichosanthes tricuspidata* Lour.	Redball snake gourd	*Indreni*	Cucurbitaceae	Fruit, seed and leaves
57	*Vaccinium corymbosum* L.	Blueberry	*Chasey/Khekea*	Ericaceae	Fruit
58	*Viburnum erubescens* Wall	Reddish viburnum	*Asaare*	Adoxaceae	Fruit
59	*Zanthoxylum acanthopodium* DC.	Andaliman/lemon pepper	*Bokey timbur*	Rutaceae	Fruit and bark

The NE region of India is experiencing significant challenges due to heavy rainfall during the monsoon season, which leads to soil erosion and flooding in the valleys. The steep terrain exacerbates the issue of drought during the winter months, particularly from November to March. In recent years, there has been a noticeable decline in young Mandarin orchards throughout the entire NE region. Over the past 15 to 20 years, irregular rainfall patterns, rising temperatures, and fluctuations in relative humidity have contributed to the emergence of new biotypes among various insect and pest species affecting many fruits and vegetables. These changes have adversely impacted the flowering, fruiting, production, and overall productivity of these crops (Jha *et al.,* 2015).

Varietal Suitability for Different Areas

Varied agro-ecological conditions characterize NE region; therefore, a variety of fruit crops and varieties are grown in these areas. These improved fruit crop varieties have been introduced over the last few decades and some have been introduced very recently. As these areas were remote, inaccessible and fragile, therefore these areas remained isolated from the mainland for a longer period of time. With the advent of modern infrastructure, many improved fruit crop varieties have been introduced in these areas from the rest of the country. For example, kiwifruits were introduced during the 1960s from the north-western Himalayan region; similarly, avocado was introduced by the kings in Sikkim. Table 2 provides the list of improved varieties of different crops grown in the eight states of northeastern India.

Fruits as Ethnomedicine

Although the NE region is recognized as a global biodiversity hotspot, it has not been extensively studied. The indigenous communities in this area highly value wild edible fruit plants, which also serve as a vital source of ethnomedicine. In a case study conducted in Sikkim (Table 2, data not previously published by the author), 76 individuals from 23 villages

Table 2: Major fruit crop varieties for northeast region

State	*Fruit*	*Varieties*
Arunachal Pradesh	Apple	Black Bendavis, Royal Gala, Jonathan, Red Gold, Gani Gala, Rich-A-Red, Royal Delicious, Red Delicious, Golden Delicious, Cooper- IV, McIntosh, Crofton, Granny Smith, Starkrimson, Fokla, Ruspippin, Rajakori, Ganu and Mutsu
	Orange	Wakro orange, Valencia, Mosambi, Soh-niang Riang and Dambuk
	Kiwifruit	Allison, Bruno, Hayward, Monty, Jintao, Chuhong and Sanuki Gold
	Peach	TA-170, Flordasun, Shan-e-Punjab and Sharbati.
	Pear	High Hills: Bartlett, Red Bartlett, Flemish Beauty, Max Red Bartlett, Starkrimson, Conference' and Clapp's Favourite Mid, Low hills and Valley areas: Patharnakh, Kieffer, William, Lagoon, Fertility, Baggugosha, Punjab Beauty and Punjab Gold
	Plum	Santa Rosa
	Pineapple	Kew, Giant Kew, and Queen (Mauritius)
Assam	Banana	Malbhog, Jahaji, Bar Jahaji, Chenichamma, Kanaibansi, Saapkal, and Garo Moina
	Sweet orange	Valencia, Westin, TM-33, Cutter Valencia, Pera and Natal
	Pineapple	Kew and Queen
	Arecanut	Mohitnagar and Shatamangala
	Coconut	Assam Tall, Kamrupa, West Coast Tall (WCT), inter-varietal hybrids (D × T and T × D) and Chandrasankara (COD × WCT)
	Lemon	Assam lemon
	Guava	Megha Supreme, Megha Magenta, Megha Wonder, Megha Seedless, Allahabad Safeda, Sardar and Red Fleshed
	Jackfruit	Mostly local, Singapore Jack and Rudrakshi
	Mango	Amrapali, Malika, Lengera, Dasahari, Himsagar, Palmar and Red Palmar
	Sapota	Cricket Ball, Kalipatti and Pala
Manipur	Pineapple	Queen and Kew
	Papaya	Red Lady, Ameena, Red Baby, Lunar, Indus Honey Gold, Indus Red Sun Dwarf and Sarpan Solo-109
	Banana	Laphu, Robusta, Rasthali and Red Banana
	Peach	Pratap, Florda Prince, Shan-i-Punjab, Earli Grande, Prabhat, and Punjab Nectarine
	Lemon	Kachai Lemon (Kachai Champra)
	Sweet orange	Tamenglong orange
	Passion fruit	Yellow, Purple and Kaveri
Meghalaya	Pineapple	Kew and Queen
	Guava	Megha Guava-1 (RCGH-1), Megha Saw Priam (RCGH-4), Megha Khongpheram Paudiik (RCGH-7), Megha Priam Thiang (RCG-11), Megha Supreme, Megha Wonder, Megha Magenta and Megha Seedless
	Banana	Dwarf Cavendish, Jahaji, Chenichampa and Malbhog
	Mandarin	Khasi Mandarin
	Sweet orange	Mosambi
	Passion fruit	Purple and yellow type
	Strawberry	Winter Dawn, Festival, Ofra, Camarosa and Sweet Charlie
	Soh-iong (*Prunus nepalensis)*	Local types
	Soh-phie (*Myrica nagi)*	Local types
Mizoram	Mandarin	Khasi Mandarin
		Sweet Charlie, Ofra, Camarosa and Festival
	Banana	G-9 and local types

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State	*Fruit*	*Varieties*
	Papaya	Pusa Nanha, Pusa Delecious and Honey Dew
	Grape	Bangalore Blue and Pusa Navrang
	Passion fruit	Purple, Yellow, Hybrid and Giant
	Hatkora (*Citrus macroptera*)	Local types
	Chenkek (*Garcinia cowa)*	Local types
	Star aonla (*Phylanthus acidus)*	Local types
	Soh-shang (*Elaegnus latifolia)*	Local types
Nagaland	Pineapple	Kew and Queen
	Banana	G-9, Jahaji and Pisang abok
	Sweet orange	Mosambi
	Mandarin	Khasi mandarin
	Passion fruit	Purple
	Jackfruit	Local types
	Litchi	Shahi, Tezpur seedless and China
	Plum	Santa Rosa
	Kiwifruit	Hayward, Monty, Allison and Bruno
Sikkim	Mandarin	Sikkim mandarin
	Passion fruit	Yellow, Purple and Kaveri
	Kiwifruit	Hayward, Allison, Monty, Bruno, Tomuri and local genotypes.
	Avocado	Hass, Furete, Zutano, Pinkerton and Arka Supreme
	Peach	Pratap, Florda Prince, Shan-i-Punjab, Earli Grande, Prabhat and Punjab Nectarine
	Pear	High Hills: Bartlett, Red Bartlett, Flemish Beauty, Max Red Bartlett, Starkrimson, Conference' and Clapp's Favourite Mid, Low hills and Valley areas: Patharnakh, Kieffer, William, Lagoon, Fertility, Baggugosha, Punjab Beauty and Punjab Gold
Tripura	Pineapple	Queen and Kew
	Banana	Shabri Kela, Martaman, Malbhog, Samai/Gopi/Bangla Kela-1, Samai/Gopi/Bangla Kela-2, Champa Kela, Mizo-Cavindish, Katch Kela, Kanai Basi, Red Banana, Attia Kela, AthiaKol and Bhimkol.
	Litchi	Shahi and Bombai
	Jackfruit	Local, Baramasi
	Mango	Amrapali, Himsagar, Fazli and Arunika
	Papaya	Tripra papaya (RCTP1), Honey Dew and Pusa Delecious
	Sapota	Calcuttia and Cricket Ball
	Lemon	Kagzi local, Ellaichi lemon and Gandharaj
	Dragon fruit	Red and white fleshed
	Avocado	Hass and Furete
	Sweet orange	Mosambi
	Mandarin	Jampui Kamala
	Pummelo and Grape fruit	Local types
	Strawaberry	Winter dawn and Festival

were interviewed, encompassing a diverse age range of 45 to 75 years, with both male and female participants. The study identified 59 species of fruit, belonging to 48 genera and 32 families. These local wild edible fruits are known to treat a variety of ailments, including gastrointestinal issues (such as dysentery, diarrhea, ulcers, vomiting, and constipation), fever, bronchitis, diabetes, jaundice, toothaches, anemia, swellings, skin disorders, bone fractures, itching, conjunctivitis, viral infections, urinary conditions, tuberculosis, heart diseases, and more. Similar findings have been reported by numerous researchers globally, particularly in the northeastern Himalayan region.

Among the families studied, Rosaceae was the most prevalent, comprising 14% of the total genera, followed by Moraceae (12%), Rutaceae (8%), Anacardiaceae (7%), Combretaceae (5%), and Elaeagnaceae, Fabaceae, Lauraceae, Myrtaceae, and Rubiaceae, each contributing 3%. In terms of socioeconomic significance, wild fruits are predominantly utilized in rural areas for traditional medicine, firewood, construction, animal feed, and charcoal production. These fruits are typically available in local markets, priced between ₹60 to 300 per kilogram. It is advisable to conserve the diverse range of edible wild fruit plants for future use, as certain species can be cultivated in marginal soils.

Germplasm Conservation and Utilization Efforts

The conservation of germplasm in fruit crops is crucial for ensuring the stability of global food systems and protecting agricultural biodiversity. Maintaining genetic diversity in fruit crops is vital due to the increasing threats from climate change, new diseases, and habitat destruction. The active preservation of diverse varieties through both *in-situ* and *ex-situ* methods is necessary for cultivating resilient and adaptable fruit crops. These innovative plant genetic resources (PGRs) present opportunities for the identification of new genes. Present conservation approaches focus primarily on *in-situ* conservation, which includes participatory plant breeding. There are ongoing collaborative international initiatives aimed at enhancing and regulating the global accessibility of PGRs. Alongside the trade-related intellectual property rights framework, recent endeavours also address issues such as the unfair nature of unrestricted access, plant breeders' rights, the Convention on Biological Diversity, prior informed consent, and global action plans (Gautam *et al.,* 2004). Global partnerships and increased public awareness are essential for addressing financial limitations and ethical issues. The future of germplasm conservation in fruit crops relies on a comprehensive strategy that includes community involvement, advanced technological solutions, and international collaboration to promote sustainable agriculture and enhance the resilience of these crops against evolving environmental challenges.

Future Directions

The selection of high-value, low-volume fruit crops tailored to specific areas, along with the horizontal expansion of improved agricultural practices on cultivable wasteland, aims to enhance productivity at a rate of 7.14 lakh per hectare in the region (De, 2017). This initiative includes replacing low-yielding traditional varieties with high-yielding, disease-resistant varieties, particularly dwarf cultivars suitable for high-density planting. To support this effort, the production of adequate planting materials has to be facilitated through tissue culture techniques and other propagation methods, both in field and protected environments. Nurseries have to be established in each district or block, managed by state horticulture departments, certified growers, farmer-producer organizations, farmers' clubs, or progressive farmers. Additionally, training sessions and workshops have to be organized to disseminate orchard management practices that enhance production and maximize yields. To promote organic nutrient management, production sites for organic compost have to be set up in each identified area. Training on Integrated Nutrient Management (INM), Integrated Pest Management (IPM), Integrated Disease Management (IDM), Integrated Organic Nutrient Management (IONM), Integrated Organic Pest Management (IOPM) and Integrated Organic Disease Management (IODM), will also have to be introduced in these regions. Furthermore, the concept of *Jalkund* (water storage structures), drip irrigation, and other water harvesting techniques will have to be taught to ensure life-saving irrigation. Post-harvest management, processing, and value addition of surplus produce have to be conducted at the block or district level. The adoption of high-tech horticultural practices, including micro-propagation, micro-irrigation, fertigation, protected cultivation, organic farming, and remote sensing, will be crucial for ensuring nutritional security for future generations. Advanced agricultural techniques such as crop diversification, contract farming, precision farming, and fruit-based farming systems have to be implemented to reach underserved areas in real time for high-value fruit crops. Additionally, the development of wholesale and rural markets at the district level, in proximity to urban centers and various agri-export zones has to be established for the North Eastern Region.

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RESEARCH ARTICLE

Investigating the Genetic and Agronomic Factors Influencing Yield in Clustered Spikelets Rice (*Oryza sativa* L.)

Lawangika Tiwari[1], Ravi Raj Singh Patel[1], Antara Thada[1], Samrath Baghel[1], Parmeshwar Kumar Sahu[1], B. K. Das[2] and Deepak Sharma[1*]

Abstract

In the present study, 32 rice genotypes with clustered spikelets, along with 4 checks, were evaluated for a total of 14 yield and yield attributing traits were evaluated at the Research cum Instructional farm IGKV, Raipur (C.G.) in *Kharif* 2023 under RCBD design. The aim was to estimate genetic variability and associations for yield traits across 36 lines. A total of 14 yield and yield-attributing traits were evaluated. Among the traits like filled grains per panicle, total grains per plant, biological yield, harvest index, 100-grain weight, primary and secondary branches per panicle, grains per cluster, and grain yield per plant had high PCV, GCV, h^2, and GAM. Traits like effective tiller, spikelet fertility, biological yield, 100-grain weight, and harvest index positively correlated with grain yield. Selecting these traits and parents from clusters I and III, which had the highest inter-cluster distance, will enhance yield. PCA identified genotypes like *Hathi panjara, Naykain jhaba, Amajhopa, Ganga dhara, Tuphi bora-1, Haruna luchai, Amaruthi, Nariyal phool-1, Chhind guchchhi, Kalinga, Khajur jhopa, Gadakhuta*, and *Nariyal phool-2* as donor parents for specific traits.

Keywords: Cluster, Clustered spikelets rice, Correlation, Heritability, PCA, Variability parameters.

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Introduction

Rice (*Oryza sativa* L.) is a staple crop for more than one-third of the world's population and plays a key role in ensuring food security. Chhattisgarh state of India, popularly known as the "Rice Bowl of India," harbors a rich biodiversity of Rice germplasm. Indira Gandhi Krishi Vishwavidyalaya (IGKV) Raipur, Chhattisgarh, is currently conserving a collection of more than 23250 rice germplasm accessions, thereby playing a pivotal role in preserving the heritage of the State (Sarawgi *et al*., 2017). These rice germplasm accessions have a wide range of unique characteristics, which include clustered spikelets, distinct colors, exceptional taste, and potential medicinal properties (Dwivedi *et al*., 2016).

There are several agronomic traits that directly and indirectly affect rice yield. Among them, seed size, number of grains per panicle and panicle structure play a crucial role. While most of the rice cultivars have panicles with single spikelets (Non-clustered), however, there are some rare rice genotypes that have panicles with clustered spikelets where 2 to 10 spikelets are present in a tight group (Sahu *et al*., 2014; Sarawgi *et al*., 2017). This character of rice can be utilized in rice breeding programmes to improve the yield by increasing the number of grains per panicle. Till now, most of the research for improving grain yield in rice has been focused majorly on positively correlated traits such as tiller numbers, panicle

length, flag leaf length, etc and limited studies have been conducted to evaluate the clustered spikelets rice genotypes towards their impact on grain yield. A clustered spikelet may provide a new epitome for improving yield in rice.

Variability is the most important for selection to be effective in any breeding program. Genetic parameters, such as the genetic coefficient of variation and the phenotypic coefficient of variation, can be used to quantify the degree of diversity present in the population for specific traits. Estimates of heritability help breeders to select parents for crop improvement programs by providing more information about the concerned trait (Kumar *et al*., 2014). The selection procedure will be further aided by knowledge of character associations and the direct and indirect effects of each character on yield. The degree of correlation between grain yield and its contributing traits can be determined using correlation and path analysis. This type of analysis could ultimately assist the breeder in creating his selection techniques to achieve an improvement in grain yield (Singh *et al*., 2018). In contrast, multivariate techniques such as Principal Component Analysis (PCA) and Agglomerative Hierarchical Cluster Analysis help to classify genotypes in various groups and thereby help to identify superior lines for hybridization programs (Dhakal *et al.,* 2020).

Taking the above points into consideration, the present study was undertaken to study grain yield and contributing traits in 36 rice genotypes, which includes clustered spikelet genotypes along with non-clustered check varieties to access the genetic variability correlation patterns between the yield contributing traits.

Materials and Methods

The present experiment was conducted at the Research cum Instructional Farm, Department of Genetics and Plant Breeding, Indira Gandhi Krishi Vishwavidyalaya (IGKV), Raipur -492012 (C.G.).

Experimental Materials

A total of 36 rice genotypes were used, including 32 genotypes with clustered spikelets and 4 non-clustered (single spikelets) check varieties (TCDM-1, Vikram-TCR, CG Devbhog and Rajeshwari). The details of the genotypes, along with the number of spikelets per cluster, are presented in Table 1. All of the genotypes used in the study were obtained from the Department of Genetics and Plant Breeding, Indira Gandhi Krishi Vishwavidyalaya (IGKV), Raipur -492012 (C.G.).

Experimental Details

For the experiment, all of the experimental materials were planted following a randomized complete block design (RCBD) with two replications at the Research cum Instructional Farm, Department of Genetics and Plant Breeding, Indira Gandhi Krishi Vishwavidyalaya (IGKV), Raipur during *Kharif* season 2023. Throughout the experiment, all standard agronomic practices were adopted.

Table 1: List of experimental material

Entry	*Genotype*	*No. of grain per cluster*	*Entry*	*Genotype*	*No. of grain per cluster*
1	KHANDSAR	2	19	HATHI PANJARA	2
2	NARIYAL PHOOL	3	20	KALINGA	3
3	SAARIPHOOL	2	21	GANGA DHARA	3
4	KUSUMBHOG	2	22	TUPAI BORA	3
5	OONI	2	23	TUPAI BORA 1	3
6	GADAKHUTA	2	24	GURMATIYA	2
7	CHANDANI DHAN	3	25	JHILLI MUT DHAN 13-2	2
8	BARKI BARI	2	26	AMAJHOPA	3
9	JHUMMA CHUDI	2	27	KOUDI DHULL	3
10	SABRI KENDUWA	2	28	CHHIND GUCHHI	3
11	RADHE DHAN	2	29	NARIYAL PHOOL SEL-1	2
12	NARIYAL PHOOL -1	3	30	AMARUTHI	2
13	CL-1	3	31	KHAJUR JHOPA	7
14	BHAJNA	2	32	NAYKEIN JHOPA	2
15	LAL DHAN	2	33	TCDM-1 (Check-1)	1
16	HARUNA LUCHAI	2	34	VIKRAM-TCR (Check-2)	1
17	MANKI	2	35	CG-DEVBHOG (Check-3)	1
18	SHANKARJATA	2	36	RAJESHWARI (Check-4)	1

Observations

Observations for a total of 14 traits, which include Days to 50% Flowering (DFF), Plant Height (cm), number of effective tillers per plant, Panicle length (cm), Number of filled grains per panicle, Total number of grains per panicle, Spikelet fertility (%), Biological yield (g/plant), Grain yield per plant (g), Harvest index (%), Hundred seed weight (g), Number of primary branches per panicle, Number of secondary branches per panicle, number of grains per cluster, were recorded on 5 randomly selected plants per replication for each genotype under test.

Statistical analysis

The observations recorded for 14 yield and attributing traits on 36 genotypes under test were subjected to statistical analysis specifically for the analysis of variance (ANOVA), genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability, correlation coefficient analysis, path coefficient analysis, cluster analysis (ward algorithm and Euclidean distance) and principal component analysis (PCA) using R program using a suitable statistical package.

Result and Discussion

Analysis of variance

The replicated data for all 14 traits were subjected to analysis of variance based on randomized complete block design (RCBD) and the results are presented in Table 2. Analysis of variance (ANOVA) indicated that the treatment mean sum of squares for all 14 yield and yield attributing traits were highly significant ($p = 0.01$), indicating the presence of substantial genetic variability among the genotypes under test for all yield and yield attributing traits. Among the yield-contributing traits, the highest variation is exhibited by the number of total grains per panicle and the lowest variation is exhibited by hundred seed weight (g).

Furthermore, while comparing the genotypes based on the CN and GY it was found that the clustered rice genotypes *Khandsar* produced significantly higher grain yield compared to all four of non-clustered check rice varieties used in the study, while rice genotypes *Koudi dhull*, and *Chhindguchhi* produced significantly higher grain yields than the three checks except for the check rice variety *Rajeshwari* (Table 3). The pattern of clustering and panicle of each of the 36 rice genotypes under test is presented in Fig. 1 and Fig. 2(a-b), respectively.

Genetic variability parameters

Parameters of genetic variability such as general mean, range, genotypic and phenotypic coefficient of variance (%), heritability in a broad sense (%) and genetic advance as % of mean were used to decipher the nature and magnitude of variation existing for each of the 14 yield and attributing traits. The results are presented in Table 4.

The phenotypic coefficient of variance (PCV) was, in general, higher than the genotypic coefficient of variance (GCV) for all the yield and attributing traits, showing some influence of environmental factors on these traits. The estimates of GCV and PCV were categorized as low (0–10%), moderate

Table 2: Analysis of variance for 14 yield and attributing traits

SN	*Source of variation*	*Mean sum of square*		
		Replication	*Treatment*	*Error*
1	DFF	6.13	99.709 **	1.21
2	PH	0.18	1890.68 **	2.71
3	ET	7.67	9.41 **	1.75
4	PL	0.12	10.792 **	0.42
5	FGP	0.32	4421.965 **	2.31
6	TGP	0.14	5329.938 **	2.92
7	SF	0.08	195.831 **	0.23
8	BY	1.23	999.896 **	2.60
9	H.I.	14.56	105.756 **	2.28
10	HSW	0.01	0.949 **	0.01
11	PB	0.41	21.836 **	0.80
12	SB	17.01	265.225 **	2.72
13	CN	1.53	2.51 **	0.13
14	GYP	2.15	110.497 **	2.65

* and ** significant at 5% and 1% probability level
DFF = Days to 50% flowering; PH = Plant height (cm); ET = Effective tillers; PL = Panicle length (cm); NFG = Number of filled grains per panicle; TNG = Total number of grains; SF = Spikelet fertility; BY = Biological yield (g); EY= Economic yield (g); HI = harvest index; HSW = Hundred seed weight (g); PN= Panicle number per plant; PB = Primary branch; SB = Secondary branch; CN= Number of grain per cluster; GYP = Grain yield per plant (g)

Table 3: Top-performing rice genotypes based on the number of grains per cluster

S. No.	*Name of Genotypes*	*Number of grains per cluster*	*Grain yield per plant (g)*
1	KHANDSAR	2	43.445
2	KOUDI DHULL	3	32.015
3	CHHIND GUCHHI	3	31.705
4	HATHI PANJARA	2	28.680
5	SHANKARJATA	2	27.940
6	CL-1	3	27.815
	RAJESHWARI (Check-4)	1	38.750
	CG-DEVBHOG (Check-3)	1	27.655
	VIKRAM-TCR (Check-2)	1	23.595
	TCDM-1 (Check-1)	1	18.520
CD at 5%=3.3			

(10–20%) and high (>20%), as proposed by Johnson *et al*., (1955). The traits that exhibited low GCV and PCV are days to 50% flowering. The traits that showed moderate GCV and PCV are plant height (cm), effective tillers per plant, panicle length (cm) and spikelet fertility (%). Traits that exhibited a high range of GCV and PCV are the number of filled grains per panicle, number of grains per panicle, biological yield (g), harvest index, 100-grain weight (g), primary branches per panicle, secondary branches per panicle, number of grains per cluster and grain yield per plant (g). Similar high GCV and PCV were also reported by Prakash *et al*. (2018) for traits grain yield per plant and biological yield (g) and by Saha *et al*. (2019) for traits number of filled grains per panicle and number of grains per panicle. The higher estimates of GCV and PCV indicate towards preponderance of genetic variability among the genotypes for these traits. Therefore, direct selection would be rewarding for achieving improvement in these traits.

Among the 14 yield and attributing traits high heritability coupled with high genetic advance as % of mean was observed for plant height (cm), number of effective tillers, panicle length (cm), number of filled grains per panicle, number of total grains per panicle, spikelet fertility (%), biological yield (g), harvest index, 100-grain weight (g), primary branches per panicle, secondary branches per panicle, number of grains per cluster, grain yield per plant (g). High heritability coupled with high genetic advance as a percentage of the mean indicates the preponderance of additive gene action in these traits. Therefore, selection

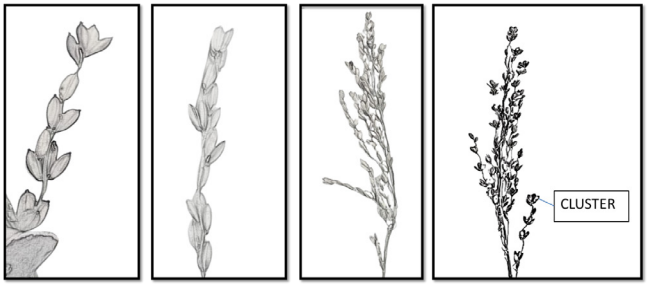

Fig 1: Clustering pattern observed in the clustered spikelets rice genotypes

(mass selection and progeny selection) methods would be effective in the accumulation of additive genes, resulting in the improvement of mean performance for these traits in the populations. Eswaran *et al*., (2016) and Kumar *et al*., (2018) have also reported high heritability coupled with high genetic advance as % of the mean for most of the agro-morphological traits in their experiments, which supports the outcome of the present study.

The trait days to 50% flowering showed high heritability with moderate genetic advance as % of the mean. Heterosis breeding will be successful in enhancing these traits since the combination of high heritability and modest genetic advancement indicates that the character is governed by both additives as well as non-additive gene activity. The results are in line with the findings of Hasan *et al*., (2013) who have also reported high heritability coupled with moderate genetic advance as % of the mean for days to 50 % flowering.

Table 4: Genetic Variability parameters

Traits	*GM*	*Range*		*Variance*		*Heritability (%)*	*GA % mean*
		Min	*Max*	*GCV (%)*	*PCV (%)*		
DFF	111.90	95.00	124.50	6.27	6.35	97.60	12.76
PH	155.05	94.80	197.20	19.82	19.84	99.71	40.76
ET	11.01	6.40	16.10	17.78	21.45	68.66	30.34
PL	22.36	15.10	27.16	10.19	10.59	92.59	20.19
NFG	153.36	87.40	321.20	30.65	30.67	99.90	63.11
TNG	189.27	117.60	345.20	27.27	27.28	99.89	56.14
SF	81.22	46.53	93.04	12.18	12.19	99.76	25.05
BY	77.41	41.00	138.40	28.85	28.92	99.48	59.27
HI	35.53	23.33	56.86	20.25	20.69	95.79	40.82
HSW	2.05	1.22	3.99	33.37	33.76	97.74	67.97
PB	12.91	9.70	28.20	25.11	26.05	92.94	49.87
SB	52.73	28.60	74.60	21.73	21.95	97.97	44.30
CN	2.40	1.00	7.00	45.52	47.96	90.06	88.99
GYP	21.57	8.36	43.45	34.04	34.87	95.31	68.46

DFF = Days to 50% flowering; PH = Plant height (cm); ET = Effective tillers; PL = Panicle length (cm); NFG = Number of filled grains per panicle; TNG = Total number of grains; SF = Spikelet fertility; BY = Biological yield (g); EY= Economic yield (g); HI = harvest index; HSW = Hundred seed weight (g); PN= Panicle number per plant; PB = Primary branch; SB = Secondary branch; CN= Number of grain per cluster; GYP = Grain yield per plant (g)

Fig 2(a): Panicle of all 36 rice genotypes

Fig 2(b): Panicle of all 36 rice genotypes

Correlation analysis

Correlation analysis was carried out to compute the correlation coefficient both at the genotypic and phenotypic levels (Table 5) to understand the relationship between the characters. The results showed a significant and positive correlation of grain yield per plant (g) with the number

of effective tillers, spikelet fertility (%), biological yield (g), 100-grain weight (g) and harvest index. Further grain yield showed a significant negative correlation with secondary branches per panicle. Interestingly, the number of spikelets per cluster showed a non-significant but positive correlation with the grain yield per plant (g).

Additionally, traits such as plant height (cm), panicle length (cm), number of filled grains per panicle and number of primary branches per panicle exhibited non-significant correlation in positive direction. In contrast, traits such as days to 50% flowering and the total number of grains per panicle showed a non-significant negative correlation with the grain yield per plant (g). A similar high correlation of effective tiller, biological yield and 100-grain weight with grain yield was also reported by Surek and Beser (2003).

A positive correlation between characters is advantageous as it facilitates the concurrent development of both attributes through indirect selection. Conversely, a negative correlation will hinder the simultaneous improvement of two traits, necessitating some form of economic trade-off in such an instance.

Path analysis

Path coefficient analysis was performed to get a clearer and more accurate picture of a complex event at the correlation level. The grain yield per plant (g) was used as a dependent

Table 4: Genotypic and Phenotypic coefficient of correlation

Traits	**r**	DFF	PH	ET	PL	NFG	TNG	SF	BY	HI	HSW	PB	SB	CN	GYP
DFF	r_p	1	0.677**	-0.132^{NS}	0.137^{NS}	0.079^{NS}	-0.031^{NS}	0.226^{NS}	0.329**	-0.822**	-0.040^{NS}	0.015^{NS}	-0.296*	0.491**	-0.015^{NS}
	r_g	1	0.685**	-0.173^{NS}	0.161^{NS}	0.079^{NS}	-0.032^{NS}	0.229^{NS}	0.334**	-0.849**	-0.037^{NS}	0.025^{NS}	-0.304**	0.520**	-0.018^{NS}
PH	r_p		1	-0.186^{NS}	0.480**	0.043^{NS}	-0.008^{NS}	0.120^{NS}	0.581**	-0.644**	0.445**	0.251*	-0.211^{NS}	0.452**	0.118^{NS}
	r_g		1	-0.232*	0.496**	0.043^{NS}	-0.009^{NS}	0.121^{NS}	0.583**	-0.654**	0.452**	0.261*	-0.215^{NS}	0.476**	0.121^{NS}
ET	r_p			1	0.065^{NS}	-0.129^{NS}	-0.195^{NS}	0.048^{NS}	0.296*	0.315**	-0.001^{NS}	-0.154^{NS}	-0.302**	-0.101^{NS}	0.493**
	r_g			1	0.074^{NS}	-0.162^{NS}	-0.241*	0.056^{NS}	0.341**	0.398**	-0.014^{NS}	-0.216^{NS}	-0.393**	-0.151^{NS}	0.617**
PL	r_p				1	-0.039^{NS}	-0.046^{NS}	-0.001^{NS}	0.327**	-0.125^{NS}	0.421**	0.273*	-0.104^{NS}	0.129^{NS}	0.183^{NS}
	r_g				1	-0.039^{NS}	-0.047^{NS}	0.001^{NS}	0.336**	-0.115^{NS}	0.434**	0.276*	-0.110^{NS}	0.152^{NS}	0.185^{NS}
NFG	r_p					1	0.877**	0.397**	0.220^{NS}	-0.008^{NS}	-0.313**	0.026^{NS}	0.389**	-0.219^{NS}	0.156^{NS}
	r_g					1	0.877**	0.398**	0.221^{NS}	-0.009^{NS}	-0.316**	0.027^{NS}	0.394**	-0.235*	0.159^{NS}
TNG	r_p						1	-0.075^{NS}	0.036^{NS}	0.054^{NS}	-0.448**	0.085^{NS}	0.552**	-0.264*	-0.025^{NS}
	r_g						1	-0.075^{NS}	0.037^{NS}	0.054^{NS}	-0.451**	0.089^{NS}	0.557**	-0.281*	-0.026^{NS}
SF	r_p							1	0.366**	-0.122^{NS}	0.191^{NS}	-0.077^{NS}	-0.246*	0.016^{NS}	0.336**
	r_g							1	0.368**	-0.124^{NS}	0.191^{NS}	-0.081^{NS}	-0.247*	0.013^{NS}	0.345**
BY	r_p								1	-0.257*	0.499**	0.231^{NS}	-0.291*	0.218^{NS}	0.516**
	r_g								1	-0.258*	0.502**	0.238*	-0.298*	0.230^{NS}	0.530**
HI	r_p									1	-0.027^{NS}	-0.034^{NS}	0.189^{NS}	-0.467**	0.269*
	r_g									1	-0.021^{NS}	-0.037^{NS}	0.193^{NS}	-0.507**	0.281*
HSW	r_p										1	0.318**	-0.207^{NS}	0.185^{NS}	0.338**
	r_g										1	0.328**	-0.208^{NS}	0.193^{NS}	0.349**
PB	r_p											1	0.073^{NS}	0.154^{NS}	0.036^{NS}
	r_g											1	0.071^{NS}	0.154^{NS}	0.046^{NS}
SB	r_p												1	-0.346**	-0.262*
	r_g												1	-0.365**	-0.267*
CN	r_p													1	0.050^{NS}
	r_g													1	0.060^{NS}
GYP	r_p														1
	r_g														1

DFF = Days to 50% flowering; PH = Plant height (cm); ET = Effective tillers; PL = Panicle length (cm); NFG = Number of filled grains per panicle; TNG = Total number of grains; SF = Spikelet fertility; BY = Biological yield (g); EY= Economic yield (g); HI = harvest index; HSW = Hundred seed weight (g); PN= Panicle number per plant; PB = Primary branch; SB = Secondary branch; CN= Number of grain per cluster; GYP = Grain yield per plant (g)

variable on thirteen independent variables mentioned beforehand. The direct and indirect effect of all traits on grain yield is presented in Table 6. The traits that showed the highest positive direct effect on gain yield per plant (g) were total number of grains per panicle (1.555) followed by number of effective tiller (1.115), 100 seed weight (g) (0.939), spikelet fertility % (0.708), number of grains per cluster (0.315), days to 50% flowering (0.281), harvest index (0.148), plant height (0.108), primary branches per panicle (0.039) and secondary branches per panicle (0.003). The trait number of grains per cluster exhibited a moderate positive direct effect on the grain yield per plant (g), suggesting a direct relationship of cause and effect, while its weak negative indirect effects through other traits is the possible reason why it has a non-significant correlation with the grain yield per plant (g) as discussed earlier. The traits that exhibited the highest negative direct effect on grain yield were the number of filled grains per panicle (-0.842) followed by biological yield (-0.581) and panicle length (-0.211). Similar to our findings, Maurya *et al*. (2018) have also reported a direct positive effect of plant height, effective tiller, total number of grains, spikelet fertility and test weight on grain yield.

Table 5: Path analysis

Character	*DTF*	*PH*	*ET*	*PL*	*NFG*	*TNG*	*SF*	*BY*	*HI*	*HSW*	*PB*	*SB*	*CN*	*r*
DTF	**0.281**	0.074	-0.193	-0.034	-0.067	-0.050	0.162	-0.194	-0.126	-0.034	0.001	-0.001	0.164	-0.018^{NS}
PH	0.192	**0.108**	-0.259	-0.105	-0.036	-0.013	0.086	-0.339	-0.097	0.425	0.010	-0.001	0.150	0.121^{NS}
ET	-0.049	-0.025	**1.115**	-0.016	0.136	-0.375	0.040	-0.198	0.059	-0.013	-0.008	-0.001	-0.048	0.617**
PL	0.045	0.053	0.082	**-0.211**	0.033	-0.073	0.001	-0.196	-0.017	0.408	0.011	0.000	0.048	0.185^{NS}
NFG	0.022	0.005	-0.180	0.008	**-0.842**	1.364	0.282	-0.129	-0.001	-0.297	0.001	0.001	-0.074	0.159^{NS}
TNG	-0.009	-0.001	-0.269	0.010	-0.738	**1.555**	-0.053	-0.021	0.008	-0.424	0.003	0.002	-0.089	-0.026^{NS}
SF	0.064	0.013	0.063	0.000	-0.335	-0.116	**0.708**	-0.214	-0.018	0.179	-0.003	-0.001	0.004	0.345**
BY	0.094	0.063	0.380	-0.071	-0.186	0.057	0.260	**-0.581**	-0.038	0.472	0.009	-0.001	0.072	0.530**
HI	-0.238	-0.070	0.444	0.024	0.008	0.083	-0.088	0.150	**0.148**	-0.019	-0.001	0.001	-0.160	0.281*
HSW	-0.010	0.049	-0.016	-0.092	0.266	-0.701	0.135	-0.292	-0.003	**0.939**	0.013	-0.001	0.061	0.349**
PB	0.007	0.028	-0.240	-0.058	-0.023	0.138	-0.057	-0.139	-0.006	0.308	**0.039**	0.000	0.049	0.046^{NS}
SB	-0.085	-0.023	-0.438	0.023	-0.332	0.866	-0.175	0.173	0.029	-0.196	0.003	**0.003**	-0.115	-0.267*
CN	0.146	0.051	-0.168	-0.032	0.198	-0.437	0.009	-0.134	-0.075	0.182	0.006	-0.001	**0.315**	0.060^{NS}

DFF = Days to 50% flowering; PH = Plant height (cm); ET = Effective tillers; PL = Panicle length (cm); NFG = number of filled grains per panicle; TNG = Total number of grains; SF = Spikelet fertility; BY = Biological yield (g); EY= Economic yield (g); HI = harvest index; HSW = Hundred seed weight (g); PN= Panicle number per plant; PB = Primary branch; SB = Secondary branch; CN= Number of grain per cluster.

Table 7: Clustering pattern of genotypes

Cluster no.	*No. of Genotypes*	*Name of Genotypes*
C1	7	Khandsar, Shankarjata, Koudi dhull, CG-Devbhog, Rajeshwari, TCDM-1, Vikram-TCR
C2	21	Chandani dhan, Sabri kenduwa, Saariphool, Kusumbhog, Bhanjna, Haruna luchai, Barki bari, kalinga, Chhind guchchhi, Khajur jhopa, Ganga dhara, Tuphi bora, Tuphi bora-1, Nariyal phool-2, Nariyal phool, CL-1, Nariyal phool-1, Amaruthi, Amajhopa, Hathi panjara, Naykain Jhaba
C3	8	Jhilli mut dhan 13-2, Gadakhuta, Gurmatiya, Radhe dhan, Ooni, Jhumma chudi, Lal dhan, Manki

Cluster Analysis

The cluster analysis was performed using the package NbClust, which, after analyzing 30 indices, provides the best number of clusters following the majority rule, while the distance followed for clustering is Euclidean distance. The clustering method is the ward, which minimizes the within-cluster variance and merges the clusters that have minimum distance at each step. The clustering pattern of genotypes is presented in Table 7. Besides this, various inter and intra-cluster distances were also computed and presented in Table 8 and Fig 3.

In the case of yield characters, the genotypes were grouped into three clusters. The highest number of genotypes appeared in cluster II, which possessed 21 genotypes. The second highest number of genotypes was found in cluster III, which possessed 8 genotypes. The least number of genotypes appeared in cluster I having 7 genotypes.

Out of these three clusters identified, cluster I had the highest intra-cluster distance, followed by Cluster II and Cluster III. The highest inter-cluster distance was found between cluster I and cluster III, followed by cluster I and II.

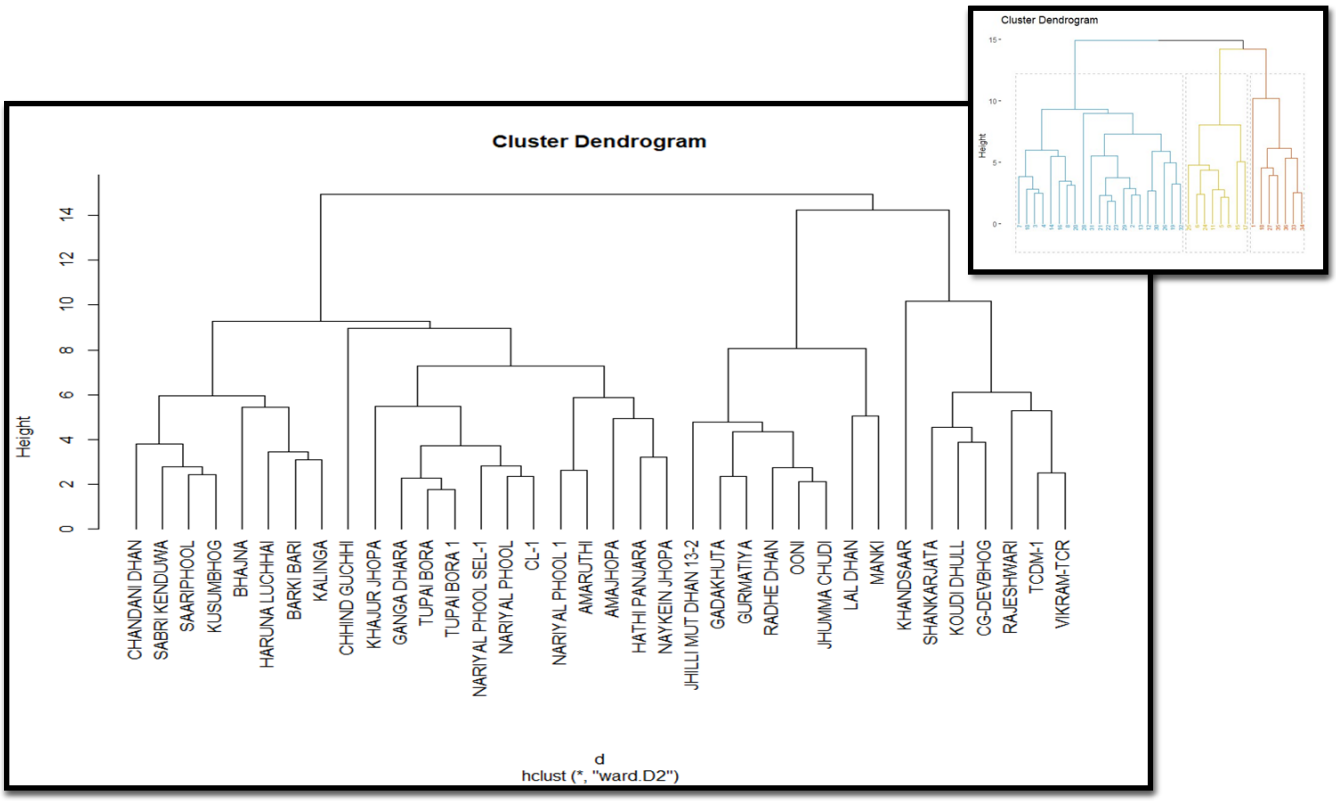

Fig 3: Cluster diagram for yield attributing traits

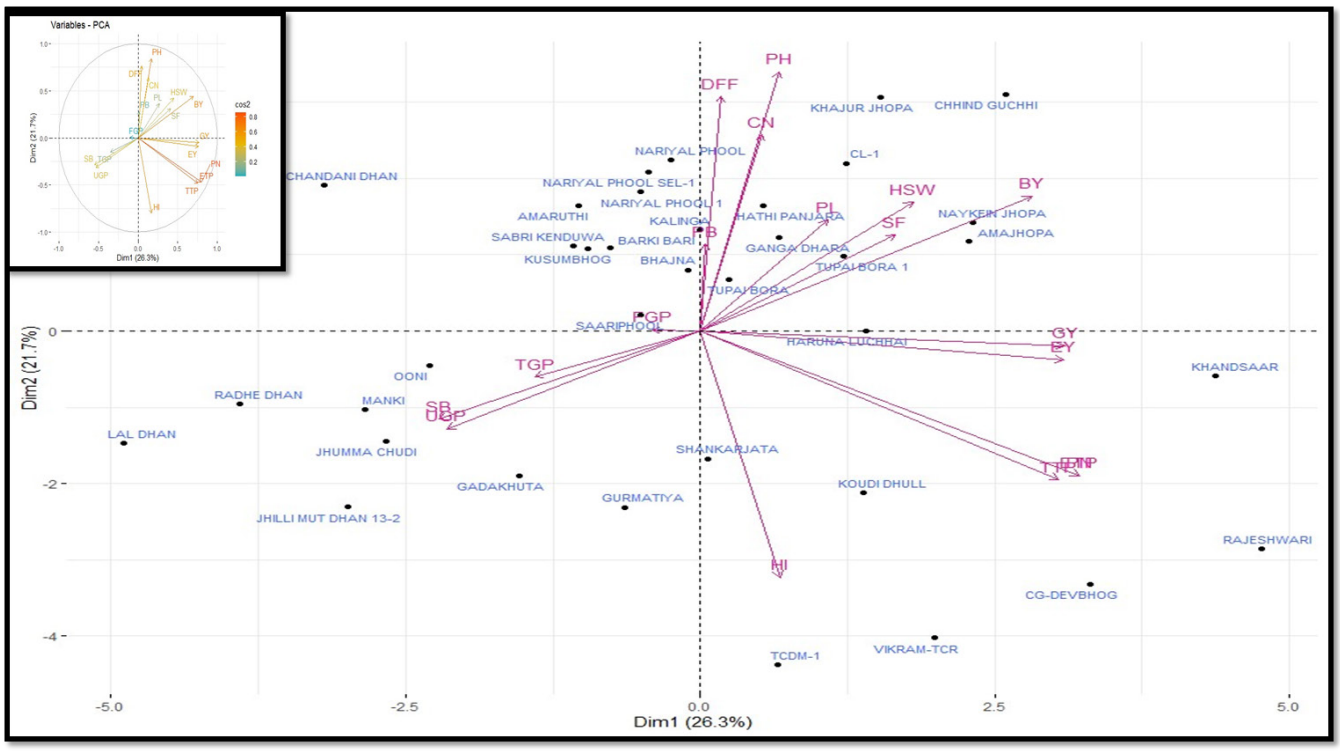

Fig 4: PCA biplot for yield traits

Table 8: Intra and Intercluster distance

Clustering component		*C1*	*C2*	*C3*
Intra cluster distance:				
Intra cluster: complete		9.04	8.63	7.10
Intra cluster: average		5.62	4.68	4.39
Intra cluster: centriod		3.66	3.20	2.88
Inter cluster distance:				
inter cluster: complete	C1	0.00	10.29	11.03
	C2		0.00	10.17
	C3			0.00
inter cluster: average	C1	0.00	6.79	6.89
	C2		0.00	5.98
	C3			0.00
inter cluster: centroid	C1	0.00	4.66	5.20
	C2		0.00	4.18
	C3			0.00

Minimum inter-cluster distance was found between cluster II and cluster III.

Cluster I had the highest cluster mean for harvest index, followed by grain yield per plant and effective tiller. Whereas, Cluster II had the largest mean value for plant height, followed by days to 50% flowering, biological yield (g), spikelet fertility, panicle length (cm), primary branches per panicle, number of grains per cluster and 100 grain weight (g). Cluster III had the largest mean value for the number of total grains per panicle, followed by the number of filled grains per panicle and secondary branches per panicle. The cluster mean is presented in Table 9.

Principal Component Analysis

Five significant principal components were obtained as they had eigenvalue more than 1. The PC1 and PC2 had 48.04% of

Table 9: Cluster mean for yield attributing traits

Group/ Character	*DFF*	*PH*	*ET*	*PL*	*NFG*	*TNG*	*SF*	*BY*	*HI*	*HSW*	*GYP*	*PB*	*SB*	*CN*
C1	105.50	128.48	**14.33**	22.13	151.77	187.57	79.62	80.43	**44.89**	1.94	**30.27**	11.83	52.14	1.57
C2	**115.90**	**174.99**	10.52	**23.11**	151.76	177.90	**84.78**	**84.96**	31.27	**2.32**	21.28	**13.42**	50.00	**2.71**
C3	107.00	125.99	9.39	20.69	**158.95**	**220.60**	73.21	54.94	38.52	1.44	14.73	12.53	**60.41**	1.63

DFF = Days to 50% flowering; PH = Plant height (cm); ET = Effective tillers; PL = Panicle length (cm); NFG = Number of filled grains per panicle; TNG = Total number of grains; SF = Spikelet fertility; BY = Biological yield (g); EY= Economic yield (g); HI = harvest index; HSW = Hundred seed weight (g); PN= Panicle number per plant; PB = Primary branch; SB = Secondary branch; CN= Number of grain per cluster; GYP = Grain yield per plant (g)

Table 10: Percentage of variance contributed by each principal component towards total variance for yield attributing trait

Particulars	*Principal component 1*		*Principal component 2*		*Principal component 3*	
Trait	*Contribution*	*Correlation*	*Contribution*	*Correlation*	*Contribution*	*Correlation*
DFF	0.04%	0.044	14.88%	0.763	0.14%	-0.060
PH	0.58%	0.165	18.16%	0.843	0.91%	0.154
ET	13.43%	0.798	5.65%	-0.470	0.54%	-0.118
PL	1.53%	0.269	3.36%	0.362	1.01%	0.162
NFG	0.22%	-0.102	0.00%	0.005	30.86%	0.893
TNG	2.57%	-0.349	0.56%	-0.148	27.91%	0.849
SF	3.55%	0.410	2.50%	0.312	2.18%	0.237
BY	10.30%	0.698	4.92%	0.438	5.96%	0.392
EY	12.36%	0.765	0.22%	-0.094	9.38%	0.492
HI	0.59%	0.167	16.41%	-0.801	0.61%	0.126
HSW	4.24%	0.448	4.51%	0.420	0.04%	-0.034
GYP	12.42%	0.767	0.06%	-0.048	3.24%	0.289
PB	0.00%	0.011	2.04%	0.282	2.59%	0.259
SB	6.38%	-0.550	2.11%	-0.287	9.76%	0.502
CN	0.36%	0.130	10.43%	0.639	3.18%	-0.287
Eigenvalue	4.74		3.91		2.58	
% Variance	26.33		21.72		14.35	
Cumulative % variance	26.33		48.04		62.40	

DFF = Days to 50% flowering; PH = Plant height (cm); ET = Effective tillers; PL = Panicle length (cm); NFG = Number of filled grains per panicle; TNG = Total number of grains; SF = Spikelet fertility; BY = Biological yield (g); EY= Economic yield (g); HI = harvest index; HSW = Hundred seed weight (g); PN= Panicle number per plant; PB = Primary branch; SB = Secondary branch; CN= Number of grain per cluster; GYP = Grain yield per plant (g)

the variance present in the studied data set (Table 10). From 5 PCs total of 82.34% cumulative variance is explained in the studied data set. For PC1, the most important trait is the number of effective tillers, followed by grain yield per plant, economic yield, total tiller per plant, biological yield, and spikelet fertility. For PC2, plant height is the most significant trait contributing the maximum to PC2, followed by harvest index, days to 50% flowering, number of grains per cluster, 100 grain weight, and panicle length, as revealed by the eigenvalues. For PC3, the number of filled grains per panicle is the most important trait, followed by the total number of grains per panicle, secondary branches per panicle, and primary branches per panicle.

Based on the PCA Biplot analysis (Fig. 4), the rice genotype *Hathi panjara* moderately correlated with the trait plant height (cm) and number of grains per cluster. While the rice genotypes *Naykain Jhaba* and *Amajhopa* were highly correlated with trait biological yield (g). *Ganga dhara* as highly correlated with panicle length (g). *Tuphi bora-1* was highly correlated with spikelet fertility (%). *Haruna luchai* was moderately correlated with grain yield per plant (g).

Conclusion

The present study shows the presence of substantial genetic diversity among the 36 rice genotypes tested. All 14 yield and attributing traits studied had shown significant variation with high heritability coupled with high genetic advance as % of mean for traits such grain yield per plant (g), total number of grains per panicle, number of effective tillers, hundred seed weight (g), and spikelet fertility (%). These traits also exhibited a significant positive correlation along with a strong direct effect on grain yield per plant (g). Although the trait number of grains per cluster exhibited a non-significant correlation with grain yield per plant (g), the path analysis revealed a moderate positive direct effect. This indicates that the number of grains per cluster may contribute to a moderate enhancement in yield when combined with other key yield-attributing traits in the future breeding programs. Further, we found that clustered rice genotypes *Khandsar*, *Koudi dhull*, and *Chhindguchhi* produced significantly higher grain yields compared to non-clustered check rice varieties. The presence of multiple grains per spikelet (clustering) had a moderately positive influence on yield by increasing the number of fertile grains and overall plant productivity. However, the number of grains within each cluster did not directly correlate with yield due to lower grain density. Further research is needed to understand how the trait of clustered grains is inherited. Few mutants and genes have been identified by various researchers, which can be included in the future studies to get better understanding of its inheritance and contribution towards increased grain yield. Further they can be utilized to develop varieties that can break the current yield potentials.

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RESEARCH ARTICLE

Gerechte-Based Mating-Environmental Designs for Varietal Improvement

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Abstract

Diallel crosses are important mating designs used to study and gain useful information to enhance genetic traits in crops. Due to the scarcity of resource availability, one can use a fraction of these crosses, *i.e.*, partial diallel crosses (PDCs), which enlarges the applicability of mating designs. A more suitable design for breeding experiments is mating-environmental design, or ME design, which can be used both as a mating and an environmental design. It is interesting to explore the possibility of obtaining efficient ME designs from Gerechte designs (possessing excellent mathematical properties) as base designs. Gerechte design is an extension of a conventional Latin square design, which has every symbol/treatment in each row, column, and region at most once. In this study, three construction methods for diallel cross experiments have been developed that yield highly efficient designs for a wide range of parametric combinations. An R package is also developed for the generation, categorization, and analysis of proposed designs to enhance the application potential.

Keywords: Breeding experiments, Canonical efficiency factor, Diallel cross, R package (MEDesigns).

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Introduction

In genetic research, breeding experiments are fundamental that aim to improve inherent traits such as productivity, quality, disease resistance, and environmental adaptability. Mating designs like diallel crosses are widely utilized to explore genetic variation among multiple parental lines and the identification of important traits and gene interactions. Through the proper conduct of systematic mating of different lines and analysis of the resulting offspring, researchers can obtain valuable insights into heritability and genetic potential. These methods not only help in developing high-yielding, resilient crops but also make a huge contribution to sustainable agricultural practices and food security, addressing the challenges posed by climate change and a growing global population.

Due to resource limitations, the most commonly used mating designs are partial diallel crosses (PDCs), which are a fraction taken from all possible diallel crosses. Furthermore, another important genre of designs can be obtained through merging these mating designs with environmental designs (block, row-column, *etc.*) for laying out the selected crosses in environmental conditions, which is called mating environmental designs or ME designs. It is always advantageous for a breeder to opt for an ME design that refers to a single design used for the formation of sample crosses and laying them out in environmental conditions. Hinkelmann and Kempthorne (1963) considered the correspondence between PDCs and partially balanced incomplete block (PBIB) designs with *m*-associate classes. These designs are very useful to study the indicators of breeding

experiments, *i.e.*, general combining ability (GCA) and specific combining ability (SCA). Here, GCA is an indicator of how well a particular parent generally combines with other parents to produce a superior offspring. On the other hand, sca refers to the performance of a particular cross between two specific parental lines that is different from what would be expected based on their GCA. For details one can refer to Griffing (1956), Kempthorne and Curnow (1961), Das and Sivaram (1968), Narain *et al.* (1974), Mathur and Narain (1976), Arya and Narain (1977), Narain and Arya (1981), Arya (1983), Agarwal (1985), Kaushik and Puri (1989), Ghosh and Divecha (1997), Gupta and Kageyama (1994), Parsad *et al.* (1999), Bhar and Gupta (2002), Varghese and Varghese (2017) Varghese *et al.* (2005) and Harun *et al.* (2024).

Regions of Gerechte designs can be explored to construct efficient ME designs involving diallel cross experiments.

Gerechte designs are a generalization of popular Sudoku designs where each symbol appears in each row, column, and region only once, and the absence of some of the symbols in rows, columns, or regions leads to an incomplete Gerechte design. For detailed information, one can see Bailey *et al.* (1990, 1991, 2008), Vaughan (2009), Courtiel and Vaughan (2011), Kumar *et al.* (2015a, b). Seeing the application potential of Gerechte designs in forming efficient breeding designs, we have provided a series of Gerechte-based ME-PDCs and ME-CDCs. The obtained ME designs are very efficient, as the underlying Gerechte designs are also of high efficiency. In addition, whenever the proposed Gerechte designs exist, one can easily obtain these ME designs as a by-product.

Materials and Methods

Model and Experimental Setup

The statistical model for diallel crosses, arranged in a block setup, can be expressed as:

$$y_{ij} = \mu + g_i + g_j + s_{ij} + e_{ij} \quad (1)$$

where y_{ij} is the response from the cross between i^{th} line and j^{th} line; μ is the general mean effect; g_i and g_j are the gca effects of i^{th} line and j^{th} line, respectively; s_{ij} is the sca between i^{th} line and j^{th} line and e_{ij} is the error term independently and identically distributed having normal distributed with zero mean and constant variance. It may be noted here that $g_i + g_j + s_{ij}$ constitutes the cross effect.

Degree of Fractionation

Let N_{CDC} be the number of crosses involved in CDC designs, whereas N_{PDC} represents the number of crosses involved in PDC designs for a given number of lines v. The degree of fractionation, DF related to designs involving PDC is calculated as:

$$DF = \frac{N_{PDC}}{N_{CDC}} = \frac{2\,N_{PDC}}{v(v-1)}$$

Canonical Efficiency Factor

If $\mathbf{C}_d$ is the information matrix pertaining to the GCA effects of a complete block (orthogonal) design, d and C_d^* of the proposed incomplete block design, assuming equal estimated error variances, then the canonical efficiency factor (CEF) of the proposed design can be calculated as:

$$CEF = \frac{\text{Harmonic mean of non-zero eigenvalues of } C_d^*}{\text{Harmonic mean of non-zero eigenvalues of } \mathbf{C}_d}.$$

Results and Discussion

Method of Construction

In this section, we describe the construction methods of ME designs based on CDCs and PDCs using the specified Gerechte designs.

Method I

Firstly, an incomplete Gerechte design is constructed for an even number of lines, v (≥ 8) and using that Gerechte design, one can easily obtain ME-PDCs with an excellent efficiency factor as well as a low degree of fractionation.

Construction of Gerechte Design

- Consider the initial row of elements as 1, v, $v-1$, …, 2.
- Expand all odd columns cyclically and even columns reverse cyclically, up to $\left(\frac{v}{2}\right)^{\text{the}}$ element in each column.
- Take two adjacent columns from left to right to form a total of $\left(\frac{v}{2}\right)$ regions (sub-arrays).
- The resultant design will be an incomplete Gerechte design (G) with the number of rows (R) = number of regions (S) = number of replications (r) = $\left(\frac{v}{2}\right)$ and number of columns (C) = v.

Construction of ME-PDC

- Construct two sets from each of the regions of dimension $\left(\frac{v}{2}\right) \times 2$ such that the first set contains all the $[i, j]^{th}$ elements of a region where $i = 1, 2, \ldots, \left(\frac{v}{2}\right)$ and j takes the value 1 (if i is odd) or 2 (if i is even). The second set contains complementary elements of the first set.
- Now construct a block of diallel crosses by making crosses within the sets by following the rule of moving forward, *i.e.*, cross the first and second elements first, for the second cross the second and third elements, and so on.
- Finally, a ME-PDC is obtained with $\left(\frac{v}{2}\right)$ blocks each of size $v-2$.

Example: for v = 10, one can easily construct a Gerechte design using the above-mentioned steps, considering the initial row as 1, 10, 9, 8, 7, 6, 5, 4, 3, 2,1,10,...,3,2 (Table 1).

Table 1: Gerechte design for v = 10 with 5 regions (dim: 5 x 2)

	C1	*C2*	*C3*	*C4*	*C5*	*C6*	*C7*	*C8*	*C9*	*C10*
R1	1	10	9	8	7	6	5	4	3	2
R2	2	9	10	7	8	5	6	3	4	1
R3	3	8	1	6	9	4	7	2	5	10
R4	4	7	2	5	10	3	8	1	6	9
R5	5	6	3	4	1	2	9	10	7	8

Consider the first region

1 10

2 9

3 8

4 7

5 6

First set will contain all the $[i, j]^{th}$ elements in this region where $i = 1, 2, \ldots, 5$. So, the elements of first set are {[1, 1], [2, 2], [3, 1], [4, 2], [5, 1]} $\Rightarrow$ {1, 9, 3, 7, 5} and its complementary elements for second set are {[1, 2], [2, 1], [3, 2], [4, 1], [5, 2]} $\Rightarrow$ {10, 2, 8, 4, 6}.

B1	1 × 9	9 × 3	3 × 7	7 × 5	10 × 2	2 × 8	8 × 4	4 × 6

From first set From second set

Therefore, the final ME-PDC design can be obtained with 5 blocks of size 8 with CEF 0.7801 and DF = 0.4444 as shown in Table 2.

Method II

In this method, firstly, an incomplete Gerechte design is constructed for a composite number, $v = pq$; $p, q \geq 3$ and then taking crosses among inter-region elements, one can obtain ME-PDCs, which are suitable for breeding experiments.

Construction of Gerechte Design

- Write an array of order $p \times q$ such that it is in natural order and consider this as a base region.
- Rotate the columns of the base region, one cyclical shift at a time, and append vertically the base region to form q regions such that each column contains all the v symbols.
- Now, for each of the q regions, rotate the rows and append horizontally to form a complete Gerechte design.
- Delete the first row and the first column of each region.

Resultant arrangement is an incomplete Gerechte design (for $v = pq$) with $R = p(q - 1)$, $C = q(p - 1)$, $S = v = pq$ and $r = (p - 1) \times (q - 1)$.

Construction of ME-PDC

- Form q sets each constituting $\frac{S}{p-1}$ regions sharing the same rows
- Consider all possible unique pairs of regions within a set
- Consider $(q - 1)$ columns of the first region and $(q - 1)$ forward diagonals of the second region for each pair of regions
- Make $(p - 1)$ crosses between corresponding elements of $(q - 1)$ column-diagonal combinations (i^{th} column with i^{th} forward diagonal, $i = 1, 2, \ldots, q - 1$), constituting a block
- Proceeding in a similar manner, we get $\binom{p}{2}$ blocks from each set
- Repeat the process for all other sets, resulting into $q \times \binom{p}{2}$ blocks of size $(p - 1)$ $(q - 1)$ each.

Example: For $v = 4 \times 4$, a Gerechte design of 16 regions of dimension 3×3 using the 2nd associates of treatments constructed as follows as given in Table 3.

Let us consider two consecutive regions of the first row of regions, say,

6	7	8	10	11	12
10	11	12	14	15	16
14	15	16	2	3	4

Now, for i^{th} ($i = 1, 2, 3$) column of the first region, take elements diagonally mod$(v - 1)$ from the i^{th} element of the first sub-row of the second region, which is given as follows:

6	10		7	11		8	12
10	15		11	16		12	14
14	4		15	2		16	3

Table 2: ME - PDC for v = 10

B1	1 × 9	9 × 3	3 × 7	7 × 5	10 × 2	2 × 8	8 × 4	4 × 6
B2	9 × 7	7 × 1	1 × 5	5 × 3	8 × 10	10 × 6	6 × 2	2 × 4
B3	7 × 5	5 × 9	9 × 3	3 × 1	6 × 8	8 × 4	4 × 10	10 × 2
B4	5 × 3	3 × 7	7 × 1	1 × 9	4 × 6	6 × 2	2 × 8	8 × 10
B5	3 × 1	1 × 5	5 × 9	9 × 7	2 × 4	4 × 10	10 × 6	6 × 8

Remark: Two types of replications for the crosses can be observed in the designs developed through Method I.

Table 3: Gerechte design for v = 16 with 16 regions (dim: 3 x 3)

	C1	*C2*	*C3*	*C4*	*C5*	*C6*	*C7*	*C8*	*C9*	*C10*	*C11*	*C12*
R1	6	7	8	10	11	12	14	15	16	2	3	4
R2	10	11	12	14	15	16	2	3	4	6	7	8
R3	14	15	16	2	3	4	6	7	8	10	11	12
R4	7	8	5	11	12	9	15	16	13	3	4	1
R5	11	12	9	15	16	13	3	4	1	7	8	5
R6	15	16	13	3	4	1	7	8	5	11	12	9
R7	8	5	6	12	9	10	16	13	14	4	1	2
R8	12	9	10	16	13	14	4	1	2	8	5	6
R9	16	13	14	4	1	2	8	5	6	12	9	10
R10	5	6	7	9	10	11	13	14	15	1	2	3
R11	9	10	11	13	14	15	1	2	3	5	6	7
R12	13	14	15	1	2	3	5	6	7	9	10	11

Table 4: ME-PDC for v = 16

B1	6 × 15	10 × 15	14 × 4	7 × 11	11 × 16	15 × 2	8 × 12	12 × 14	16 × 3
B2	6 × 14	10 × 3	14 × 8	7 × 15	11 × 4	15 × 6	8 × 16	12 × 2	16 × 7
B3	6 × 2	10 × 7	14 × 12	7 × 3	11 × 8	15 × 10	8 × 4	12 × 6	16 × 11
B4	10 × 14	14 × 3	2 × 8	11 × 15	15 × 4	3 × 6	12 × 16	16 × 2	4 × 7
B5	10 × 2	14 × 7	2 × 12	11 × 3	15 × 8	3 × 10	12 × 4	16 × 6	4 × 11
B6	14 × 2	2 × 7	6 × 12	15 × 3	3 × 8	7 × 10	16 × 4	4 × 6	8 × 11
B7	7 × 11	11 × 16	15 × 1	8 × 12	12 × 13	16 × 3	5 × 9	9 × 15	13 × 4
B8	7 × 15	11 × 4	15 × 5	8 × 16	12 × 1	16 × 7	5 × 13	9 × 3	13 × 8
B9	7 × 3	11 × 8	15 × 9	8 × 4	12 × 5	16 × 11	5 × 1	9 × 7	13 × 12
B10	11 × 15	15 × 4	3 × 5	12 × 16	16 × 1	4 × 7	9 × 13	13 × 3	1 × 8
B11	11 × 3	15 × 8	3 × 9	12 × 4	16 × 5	4 × 11	9 × 1	13 × 7	1 × 12
B12	15 × 3	3 × 8	7 × 9	16 × 4	4 × 5	8 × 11	13 × 1	1 × 7	5 × 12
B13	8 × 12	12 × 13	16 × 2	5 × 9	9 × 14	13 × 4	6 × 10	10 × 16	14 × 1
B14	8 × 16	12 × 1	16 × 6	5 × 13	9 × 2	13 × 8	6 × 14	10 × 4	14 × 5
B15	8 × 4	12 × 5	16 × 10	5 × 1	9 × 6	13 × 12	6 × 2	10 × 8	14 × 9
B16	12 × 16	16 × 1	4 × 6	9 × 13	13 × 2	1 × 8	10 × 14	14 × 4	2 × 5
B17	12 × 4	16 × 5	4 × 10	9 × 1	13 × 6	1 × 12	10 × 2	14 × 8	2 × 9
B18	16 × 4	4 × 5	8 × 10	13 × 1	1 × 6	5 × 12	14 × 2	2 × 8	6 × 9
B19	5 × 9	9 × 14	13 × 3	6 × 10	10 × 15	14 × 1	7 × 11	11 × 13	15 × 2
B20	5 × 13	9 × 2	13 × 7	6 × 14	10 × 3	14 × 5	7 × 15	11 × 1	15 × 6
B21	5 × 1	9 × 6	13 × 11	6 × 2	10 × 7	14 × 9	7 × 3	11 × 5	15 × 10
B22	9 × 13	13 × 2	1 × 7	10 × 14	14 × 3	2 × 5	11 × 15	15 × 1	3 × 6
B23	9 × 1	13 × 6	1 × 11	10 × 2	17 × 7	2 × 9	11 × 3	15 × 5	3 × 10
B24	13 × 1	1 × 6	5 × 11	14 × 2	2 × 7	6 × 9	15 × 3	3 × 5	7 × 10

Remark: Three types of replications for the crosses can be seen in all the designs obtained from Method II.

Table 5: Gerechte design for v = 10 with 5 regions (dim: 5 x 2)

	C1	*C2*	*C3*	*C4*	*C5*	*C6*	*C7*	*C8*	*C9*	*C10*
R1	1	10	9	8	7	6	5	4	3	2
R2	2	9	10	7	8	5	6	3	4	1
R3	3	8	1	6	9	4	7	2	5	10
R4	4	7	2	5	10	3	8	1	6	9
R5	5	6	3	4	1	2	9	10	7	8

These are the 9 crosses obtained for the first block. Finally, the ME-PDC design can be obtained with 24 blocks of size 9 with CEF 0.8228 and DF = 0.7 as shown in Table 4.

Method III

Consider again the Gerechte designs obtained in Method I. Highly efficient ME-CDCs (if $\frac{v}{2}$ is odd) can be obtained using the following simple steps, for the number of lines, *v* ($\geq$ 8).

Construction of ME-CDC

- Take all unique pairs of regions.
- Within a pair of regions, form two blocks such that the first set of *v* crosses are obtained by crossing the two lines appearing on the same position and the second set of crosses are obtained directly by crossing the two lines in the same row within each region.
- Repeat the process for all the $\binom{v/2}{2}$ pairs of regions, giving rise to $\frac{v}{2}(\frac{v}{2}-1)$ blocks of size *v*.

Example: For *v* = 8, a Gerechte design of 4 regions of dimension 4 × 2 constructed as follows as given in Table 5.

Using this Gerechte design, we obtain all the crosses block-wise, as discussed above. The resultant design, ME-PDC with 20 blocks each of size 10, and CEF 0.887, is given in Table 6.

Illustration of Analysis Using Hypothetical Data

A hypothetical data set for ME-PDC design for a number of lines 10 in 5 blocks of size 10 each, is shown in Table 7.

The above dataset has been analyzed using model (1), and the results are consolidated in the following Tables 8 and 9).

R-package

The R package, "MEDesigns", is a specialized tool designed for the generation of proposed ME designs and analysis of ME designs. These designs are very useful in agricultural and biological research, particularly for experiments involving genetic crosses. The package contains functions that incorporate unique algorithms to generate our

Table 6: ME-CDC for v = 10

B1	1 × 10	2 × 9	3 × 8	4 × 7	5 × 6	9 × 8	10 × 7	1 × 6	2 × 5	3 × 4
B2	1 × 10	2 × 9	3 × 8	4 × 7	5 × 6	7 × 6	8 × 5	9 × 4	10 × 3	1 × 2
B3	1 × 10	2 × 9	3 × 8	4 × 7	5 × 6	5 × 4	6 × 3	7 × 2	8 × 1	9 × 10
⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮
B18	7 × 5	8 × 6	9 × 7	10 × 8	1 × 9	6 × 4	5 × 3	4 × 2	3 × 1	2 × 10
B19	7 × 3	8 × 4	9 × 5	10 × 6	1 × 7	6 × 2	5 × 1	4 × 10	3 × 9	2 × 8
B20	5 × 3	6 × 4	7 × 5	8 × 6	9 × 7	4 × 2	3 × 1	2 × 10	1 × 9	10 × 8

Remark: Two types of replications for the crosses can be found in all the proposed designs obtained from Method III.

Table 7: Illustration of analysis using hypothetical data

B1	1 × 9 (129.50)	9 × 3 (60.83)	3 × 7 (120.99)	7 × 5 (68.31)	10 × 2 (69.66)	2 × 8 (75.73)	8 × 4 (78.23)	4 × 6 (80.03)
B2	9 × 7 (116.23)	7 × 1 (119.67)	1 × 5 (127.01)	5 × 3 (98.83)	8 × 10 (117.14)	10 × 6 (114.67)	6 × 2 (109.80)	2 × 4 (103.83)
B3	7 × 5 (100.10)	5 × 9 (94.86)	9 × 3 (86.63)	3 × 1 (131.19)	6 × 8 (88.42)	8 × 4 (108.72)	4 × 10 (94.90)	10 × 2 (95.17)
B4	5 × 3 (83.98)	3 × 7 (122.37)	7 × 1 (122.12)	1 × 9 (134.00)	4 × 6 (85.39)	6 × 2 (97.17)	2 × 8 (101.57)	8 × 10 (93.96)
B5	3 × 1 (110.85)	1 × 5 (127.41)	5 × 9 (114.39)	9 × 7 (106.75)	2 × 4 (91.62)	4 × 10 (84.81)	10 × 6 (90.97)	6 × 8 (82.76)

Response for each cross is given in parentheses.

Table 8: ANOVA

SV	*df*	*SS*	*MSS*	*F-value*	*Pr(>F)*
Block	4	3315	828.70	12.85	<.0001
Cross	19	9746	512.90	7.95	<.0001
Error	16	1032	64.50		

GCA and SCA sum of squares have now been calculated from the cross sum of squares after necessary adjustments.

Table 9: GCA and SCA Analysis

SV	*df*	*SS*	*MSS*	*F-value*	*Pr(>F)*
gca	9	5830.42	647.82	10.04	<.0001
sca	10	3582.73	358.27	5.55	0.001

Remark: It may be noted here that all sca effects are not estimable. However, the estimable sca effects are highly significant.

Table 10: List of ME designs

v	*m*	*n*	*b*	*k*	*CEF*	*DF*	*Method*
8	-	-	4	6	0.683	0.429	I
9	3	3	9	4	0.628	0.750	II
10	-	-	5	8	0.780	0.444	I
	-	-	20	10	0.887	1	III
12	-	-	6	10		0.455	I
	4	3	18	6	0.763	0.818	II
	3	4	12	6	0.696	0.545	II
14	-	-	7	12	0.860	0.462	I
	-	-	42	14	0.923	1	III
15	3	5	15	8	0.721	0.429	II
16	5	3	30	8	0.824	0.857	II
	-	-	8	14	0.882	0.467	I
	4	4	24	9	0.823	0.700	II
18	-	-	9	16	0.898	0.471	I
	3	6	18	10	0.737	0.354	II
	6	3	45	10	0.863	0.882	II
	-	-	72	18	0.941	1	III
20	-	-	10	18	0.910	0.474	I
	4	5	30	12	0.864	0.632	II
	5	4	40	12	0.877	0.758	II
21	3	7	21	12	0.747	0.300	II
	7	3	63	12	0.882	0.900	II
22	-	-	11	20	0.920	0.476	I
	-	-	110	22	0.952	1	III
	-	-	12	22	0.927	0.478	I
24	3	8	24	14	0.754	0.261	II
	8	3	84	14	0.902	0.913	II
	4	6	36	15	0.880	0.587	II
	6	4	60	15	0.907	0.841	II
25	5	5	50	16	0.902	0.733	II
26	-	-	13	24	0.934	0.480	I
	-	-	156	26	0.96	1	III
27	3	9	27	16	0.759	0.231	II
	9	3	108	16	0.911	0.923	II
28	-	-	14	26	0.939	0.481	I
	4	7	42	18	0.885	0.500	II
	7	4	84	18	0.924	0.889	II
30	-	-	15	28	0.944	0.483	I
	3	10	30	18	0.763	0.207	II
	10	3	135	18	0.924	0.931	II
	5	6	60	20	0.918	0.648	II
	6	5	75	20	0.926	0.770	II
	-	-	210	30	0.965	1	III

It can be seen from the above table that the proposed designs are highly efficient with low degree of fractionation in most cases. Obviously, for ME-CDC, degree of fractionation is exactly 1.

proposed designs along with their parameters, eigenvalues, degree of fractionation, and CEF. Also, another function has been developed for studying the properties of a given ME-PDC. In addition, the most important function is developed for data analysis, which may be very useful for the experimenters. "MEDesigns" is particularly suited for researchers in agricultural statistics, plant breeding, and other related fields. Simplifying the complex task of generating mating environmental designs enhances the applicability of these designs. The package is freely available on the Comprehensive R Archive Network (CRAN) (https://cran.r-project.org/package=MEDesigns).

Using this R package, CEFs of the designs are computed and listed along with the parameters of ME designs obtained using Method I, II and III for $v \leq 30$ (see Annexure) (Table 10).

Discussion

Considering a part of or a full Gerechte design as a base design and moving towards ME designs for a diallel cross experiment is a unique approach. One obvious result is that whenever these Gerechte designs exist, one can easily obtain ME-PDCs and ME-CDCs through our proposed methods. These Gerechte designs exist for a wide range of parameters, and the developed designs are highly efficient. Furthermore, Table 1 contains a list of designs to provide a comprehensive overview of the proposed designs. To increase the utility of this research work, an R package has also been developed to provide easy accessibility to the designs along with the analysis of the provided data.

Conflict of interest

The authors declare that there is no conflict of interest.

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RESEARCH ARTICLE

Evaluation of Soybean [*Glycine max* (L.) Merrill] Germplasm for Yield and Attributing Traits for Rabi Season in the Southern Indian Zone

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Abstract

The present study was undertaken to assess the genetic variability and character associations for seed yield and component characters in 481soybean (*Glycine max*, L.) germplasm accessions and identification of lines suitable for Rabi season cultivation. These lines were raised in an augmented block design in four blocks with five replicated checks during 2018. The analysis of variance revealed the presence of sufficient genetic variability in the gene pool. High PCV and high GCV were recorded for grain yield plot per plant, number of pods per plant and plant height. High heritability coupled with high genetic advance were observed for grain yield, followed by pods per plant, 100-seed weight and plant height, indicating the predominance of additive gene action in controlling the trait. Correlation and regression analysis indicated a significant positive effect of pods per plant, branches per plant and plant height on grain yield. Genetic diversity analysis grouped all germplasm accessions into 4 clusters. PCA revealed the first two principal components to explain 62.84% of total variation, mostly contributed by grain yield, seed size and number of pods. The agronomical variability and its genetic nature revealed from this study may be useful in present and future soybean breeding programmes. The promising accessions identified from the present study will help in strengthening the breeding program.

Keywords: Correlation, Genetic variability, Germplasm diversity, Off-season, Soybean.

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Introduction

Soybean (*Glycine max* L. Merril) is the world's most important seed legume, which contributes to about one-fourth of the global edible oil and about two-thirds of the world's protein concentrate for livestock feeding (Agarwal *et al.*, 2013). It is considered to be nature's highest yielding usable protein source of plant origin, as three times more usable protein per unit area than other important food grains, i.e., rice, wheat, or maize (Kumar, 2005). Soybean is widely used in the preparation of diversified food and food ingredients, including full-fat soy flour, milk, cheese, curd, ice cream, sprouted and roasted snacks, soy fortified bakery, soy protein concentrate, dietary fiber, single-cell protein, citric acid, margarine, etc. (Kumar, 2005). It also has a spectrum of industrial and pharmaceutical applications in the manufacturing of soap, cosmetics, crayons, resins, plastics, inks, solvents and clothing. The crop further improves soil fertility by fixing atmospheric nitrogen through symbiotic association with soil bacteria *Bradyrhizobium japonicum*. Owing to its diverse and quintessential utility, the soybean has been christened as 'miracle bean' (Orf, 2010).

Soybean is a leading oilseed crop, accounting for the production of 358.77 million metric tons in 2018–19 (USDA, 2019). In India, it grows annually as rain rain-fed crop with a production

of 11.50 million metric tons in 2018–19 (USDA, 2019). In India, soybean growing regions have been divided into six zones, i.e., Central, Northern plain, Northern Hills, Eastern Zone, North eastern Hill Zone and Southern Zones. However, it is mainly grown in the Central Zone and the Southern Zone (AICRP 2019). Bangalore location in the Southern Zone is chosen to carry out the present experiment because of its congenial environment throughout the year for growing soybean germplasm multiplication and characterization. The Bangalore location is also being utilized in offseason generation advancement of segregation breeding material.

Remarkable progress made in plant genetic resource management in recent years has resulted in the collection of a huge set of plant germplasm that hinders the very purpose for which they exist (Odong *et al.,* 2013*).* In India, ICAR- Indian Institute of Soybean Research, made an effort to bring core collection from USDA and more than 2500 accessions are being brought to Indian through ICAR-NBPGR, New Delhi (ICAR-IISR Annual Report 2019. Germplasm accessions collected from different states of India and introduced from overseas have been conserved in the national gene bank under long-term storage at NBPGR, New Delhi and medium-term storage (MTS) at ICAR-IISR, Indore. Soybean germplasm has been evaluated and characterized for different traits by in large experiments at Indore location (Central Zone) (Agarwal and Bhawsar 2013; Gireesh *et al.,* 2015; Nataraj *et al.,* 2020). However, we need to test the germplasm expression for various traits at Bengaluru station (Southern Zone), as no single report about germplasm evaluation.

Evaluation of germplasm at a particular location helps to identify the adapted genotypes with desired traits. The abundance of genetic diversity present in any genetic material indicates the scope for further improvement of the particular crop (Baig *et al.*, 2018). The knowledge of genetic variability existing within the different parameters associated with yield is an important criterion for yield enhancement, but in highly self-pollinated crops like soybean, where natural variation results limited scope for selection opportunity (Jain *et al.*, 2015). Similarly, understanding the nature of associations between yield and its attributes and their direct and indirect contributions to seed yield is necessary for efficient selection (Kumar *et al.*, 2018). Therefore, the present study was carried out to evaluate the nature and magnitude of genetic diversity available in the soybean gene pool and find out associations between seed yield and its component characters in soybean germplasm accessions, which would help in the selection of efficient genotypes for further utilization in the breeding programme.

Materials and Methods

Plant material

The germplasm material used in this study comprised 481 Soybean (*Glycine max* (L.) Merrill) accessions collected and conserved between 1986-2018 at an active germplasm site (ICAR-IISR, Indore) from different soybean growing states of India and different countries of the world, which were imported between 1970-2018 (Supplementary Table S1). Five morphological data on the germplasm accessions were recorded in the experimental fields at AICRP center of Soybean, UAS, GKVK, Bengaluru, during December 2018-March 2019. This experimental site is located at 12° 51′ 17.75′′ N and 77° 35′ 40.42′′ E and an elevation of 926.59 m above sea level. The soil of the experimental farm is sandy loam inclined more towards a skeletal nature, dry, shallow and moderately rich in organic matter. Average rainfall is 32.1 mm during the months from December to March. Therefore, the plants were given drip irrigation as and when required. Mean minimum and maximum temperature ranges between 13.1- and 33.7°C. The soybean crop was sown in one row of 4 m length with 45 cm row-to-row spacing and harvested. Standard cultivation practices recommended for the Southern zone were followed (ICAR-IISR, 2019). The trial was conducted in an Augmented Block Design (ABD) along with five checks, *viz*. DSb 21, JS 335, JS 93-05, KBS 23 and RKS 18 to estimate phenotype variation attributing to genetic factors with respect to the germplasm panel studied and five checks were repeated once in each block.

Plant characters and data observations

The data on six quantitative traits on these 476 germplasm accessions and five checks were recorded. Days to 50% flowering, plant height (cm), number of branches, number of pods, grain yield (g)/plot and 100 seed weight (g) were recorded using standard methods (IBPGR, 1984). Each genotype, at its R8 stage, was harvested and grain yield per plot was recorded. Three random samples of a hundred seeds of respective accession were taken for weighing to record the 100 seed weight. Plot yield (kg) was converted into yield per hectare (q/ha) using the formula (plot yield/1.8) ×10,000.

Statistical analysis

Adjusted means for all six traits under consideration of 476 accessions were estimated by using the R package 'augmented RCBD' (Aravind *et al.,* 2018). Before undertaking statistical analysis on the basis of adjusted mean values, homogeneity of variance was tested as per Levene (1960). Frequency distribution graphs were obtained for the traits and the traits were further analysed for various descriptive statistical parameters, viz., mean, range, variance, etc., by using the R package 'augmentedRCBD'. Correlation analysis was performed by using the R function cor(), and linear regression analysis was performed using the R function lm(). Correlation plots were obtained by using the R packages 'corrplot' (Wei and Simko, 2017) and PCA was performed by using the R packages 'corrplot' (Wei and Simko, 2017), 'factoextra' (Kassambara and Mundt, 2017) and 'FactoMineR'

(Lê *et al*., 2008). The K-means cluster analysis was carried out R package 'factoextra' (Kassambara and Mundt, 2017).

Results

Variability and genetic parameters

The analysis of variance illustrated the existence of a remarkable amount of genetic variability among the soybean germplasm accessions for yield and yield-related parameters under study (Table 1). Analysis of variance for an augmented randomized block design revealed that mean squares of all six traits were significant for different sources of variations except mean squares due to blocks, ignoring the treatments & due to genotypes vs. checks for the trait of number of branches per plant. The mean, range, variance, coefficient of variance, heritability and genetic advance for all six agro-morphological characters are presented in Table 2. Among the traits, grain yield, 100-seed weight and number of pods per plant were found to have more variations than other traits. The mean value of grain yield per plot was recorded as 419.18 g with a range of 47.1 g (EC 100804) - 951.5 g (Shilajeet). The 100 seed weight ranged from 6.7 g (EC 172577) to 27.69 g (AGS 174) with an average value of 13.67 g. Grain yield per plot (41.03/39.38), plant height (37.82/36.28), number of pods per plant (32.28/29.81) and number of branches per plant (31.18/27.41) were found to have high PCV and GCV (Table 1). The heritability was highest for seed index (98.63%), days to 50% flowering (98.62%), grain yield (92.09%), and plant height (92.02%), and the lowest heritability was recorded for the number of branches (77.26%). Genetic advance as percent mean was recorded high for the traits *viz*., grain yield (77.95%), plant height (71.8%) and number of pods (56.81%), and it was lowest for days to 50% flowering (20.45%).

Frequency distribution of quantitative traits

Frequency distribution showed that around 10% of the accessions produced grain yield per plot of more than 650 g and around 20% of lines produced grain yield of 500 to 650 g/plot (data not shown). The number of pods also showed a large variation and 25% of the accessions were found to have pods per plant in the range of 60 to 111. The trait 100-seed weight also showed a good amount of variability, where 30% of the lines produced 15 to 27 g seed. The frequency distribution of six quantitative traits is shown in Fig. 1.

Identification of promising accessions

Promising germplasm accessions were identified based on the best check values and critical difference (CD) value at the 5% level of significance. Accessions such as Shilajeet (951.5g) EC 481571(860.2 g), EC 550828 (852.8 g), EC 333924 (847.4 g), M 1059 (845.2 g), AGS 170 (829.6 g), MAUS-142 (825.2 g), EC 241650 (803.8 g), EC 251416 (792.6 g), EC 467282 (791.8 g) were found promising for grain yield per plot. Germplasm lines i.e.EC 291400 (111.1), EC 241778 (110.6), EC 550828 (106.1), EC 481571 (105.1) and EC 389164 (101.3) were found superior for number of pods per plant while genotypes EC 100804 (113.2 cm), NRC 2396 (113.1 cm), EC 390981 (113.0 cm), Bragg (111.7 cm) and EC 389164 (111.2 cm) found promising for good plant height. For high seed weight, accessions such as AGS 174, MACS 1034, EC 251432, EC 15553 and JSM245 were found promising among the germplasm accessions.

Correlation and regression

The correlation matrix showed significant positive correlation of grain yield with pods per plant (0.72), plant height (0.42), branches per plant (0.27) and days to 50% flowering (0.27); however, 100-seed weight showed a negative correlation (-0.09). 100-seed weight was found to be negatively correlated with all other yield-attributing traits. All traits except 100-seed weight were found to be positively correlated with each other. (Fig. 2). Simple linear regression analysis performed for grain yield versus other traits showed that pods per plant had the highest direct effect on 100-seed weight, followed by plant height and days to 50% flowering and branches per plant (data not presented).

Genetic diversity and principal component analysis

Multivariate analysis among the germplasm lines was performed using genetic divergence and principal component analyses. All the 481 germplasm accessions,

Table 1: Statistical parameters of genetic variability in gene pool under study

Trait	*Mean + SE*	*Variance*	*Variance*	*Range*	*PCV (%)*	*GCV (%)*	*Heritability*	*Genetic advance as*
		(P)	*(G)*					*% of mean*
Days to flowering	54.03 ± 0.26	29.51	29.1	33.25–64.35	10.05	9.98	98.62	20.45
No. of branches per plant	4.08 ± 0.06	1.62	1.25	0.83–12.88	31.18	27.41	77.26	49.71
Plant height (cm)	55.5 ± 0.96	440.54	405.37	13–113.2	37.82	36.28	92.02	71.8
No of pods per plot	51.29 ± 0.75	274.04	233.8	10.42–111.1	32.28	29.81	85.31	56.81
100-seed weight (g)	13.67 ± 0.14	9.42	9.29	6.7–27.6	22.45	22.29	98.63	45.68
Grain yield per plot (g)	419.18 ± 7.8	29583.2	27242.5	47.1–951.5	41.03	39.38	92.09	77.95

Table 2: Analysis of variance of six quantitative traits

Source	*DF*	*Grain yield per plot (g)*	*100 seed weight (g)*	*No. of pods per plant*	*No. of branches per plant*	*Days to flowering*	*Plant height (cm)*
Block (ignoring Treatments)	3	38788.7***	38.96***	692.63***	0.99^{ns}	27.68***	426.55***
Treatment (eliminating Blocks)	480	29528.48***	9.24***	270.06***	1.6**	40.4***	446.03***
Treatment: Check	4	15286.3**	12.01***	367.65**	0.55^{ns}	98.18***	288.73**
Treatment: Test and Test vs. Check	476	29648.16***	9.22***	269.24***	1.61**	39.92***	447.35***
Residuals	12	2340.67	0.13	40.24	0.37	0.41	35.17

ns-P > 0.05; * - P <= 0.05; **-P <= 0.01; **- P <= 0.001

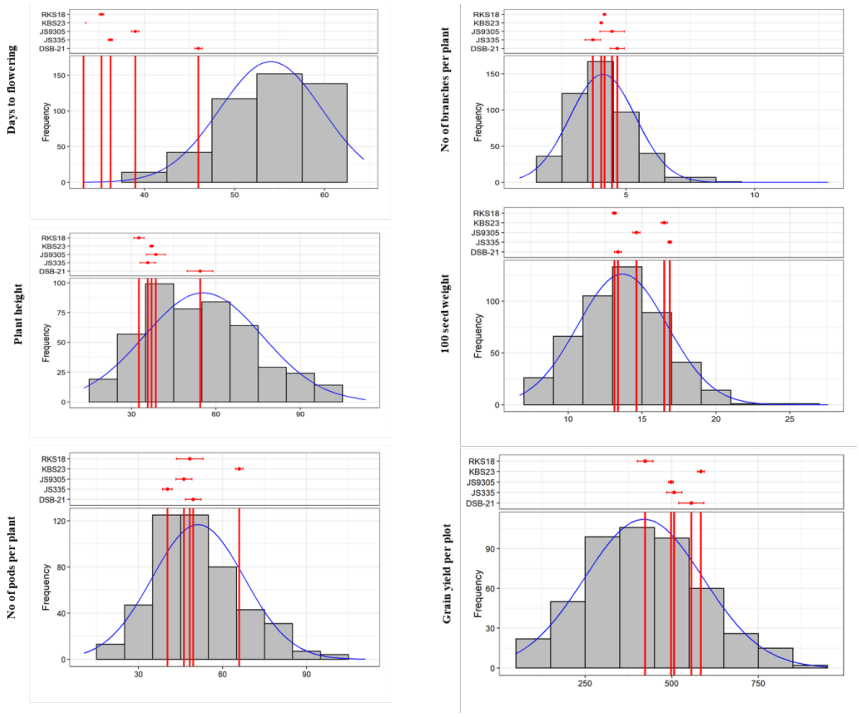

Fig. 1: Frequency distribution of traits under study

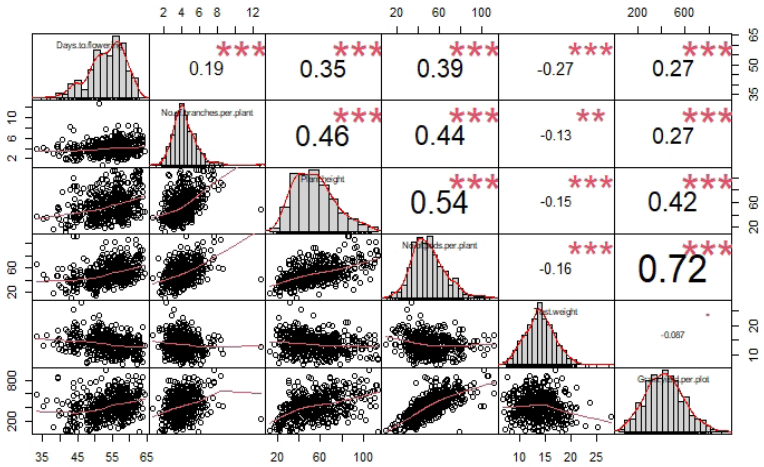

Fig. 2: Graphical depiction of correlation among the quantitative traits in germplasm accessions

Table 3: K-means clustering of genotypes under study

Cluster	*No of genotypes*	*Genotype code*
Cluster 1	79	3, 14, 18, 26, 27, 32, 45, 52, 59, 65, 67, 70, 75, 77, 85, 86, 88, 89, 102, 118, 122, 126, 130, 131, 145, 148, 149, 151, 156, 157, 158, 169, 176, 181, 182, 183, 185, 197, 199, 205, 207, 219, 220, 238, 242, 250, 251, 259, 264, 266, 267, 272, 274, 284, 291, 295, 296, 303, 328, 329, 357, 358, 381, 388, 392, 412, 416, 424, 444, 445, 446, 448, 450, 456, 464, 467, 473, 475 and 476
Cluster 2	165	2, 4, 5, 10, 11, 12, 20, 28, 29, 34, 36, 39, 48, 49, 55, 56, 61, 62, 64, 68, 71, 7, 81, 83, 87, 98, 99, 101, 103, 107, 110, 112, 113, 116, 120, 134, 137, 138, 140, 154, 155, 159, 160, 162, 164, 165, 168, 172, 174, 177, 178, 180, 184, 187, 189, 196, 201, 202, 203, 206, 209, 212, 217, 221, 224, 227, 229, 232, 233, 236, 239, 240, 247, 249, 254, 255, 256, 257, 263, 268, 277, 280, 281, 288, 290, 299, 302, 304, 308, 314, 315, 316, 317, 318, 319, 320, 323, 326, 330, 331, 332, 333, 336, 337, 338, 340, 341, 343, 348, 353, 354, 355, 359, 360, 361, 362, 369, 373, 374, 375, 376, 383, 389, 391, 398, 399, 400, 402, 404, 408, 411, 413, 414, 418, 419, 423, 428, 429, 430, 431, 434, 436, 437, 439, 440, 441, 442, 449, 451, 452, 453, 454, 458, 460, 462, 463, 466, 470, C1, C2. C3. C4 and C5
Cluster 3	73	8, 19, 21, 22, 23, 24, 31, 42, 46, 47, 51, 53, 57, 60, 74, 92, 93, 95, 100, 105, 106, 121, 135, 139, 142, 143, 163, 167, 179, 194, 195, 204, 211, 214, 215, 223, 228, 230, 243, 244, 245, 252, 265, 270, 271, 278, 289, 293, 294, 298, 312, 321, 335, 345, 347, 349, 386, 387, 390, 396, 405, 415, 421, 425, 426, 427, 455, 465 and 472
Cluster 4	164	6, 7, 9, 13, 15, 16, 17, 30, 33, 35, 37, 38, 40, 41, 43, 44, 50, 54, 58, 63, 66, 69, 73, 76, 78, 79, 80, 82, 84, 91, 90, 96, 104, 108, 109, 111, 114, 115, 117, 119, 123, 124, 125, 127, 128, 129, 132, 133, 136, 141, 144, 146, 147, 152, 170, 171, 173, 175, 186, 190, 191, 192, 193, 198, 200, 210, 216, 218, 222, 225, 226, 234, 235, 237, 241, 246, 253, 258, 260, 261, 262, 269, 273, 276, 279, 282, 283, 285, 286, 287, 292, 297, 300, 301, 305, 306, 307, 310, 311, 313, 322, 324, 325, 327, 334, 342, 344, 346, 350, 351, 352, 356, 363, 364, 365, 366, 367, 368, 370, 371, 372, 377, 378, 379, 380, 382, 384, 385, 393, 394, 395, 397, 401, 406, 407, 409, 410, 417, 420, 422, 432, 433, 435, 443, 447, 457, 459, 461, 471 and 474

including checks, were grouped into 4 clusters using k-means clustering. Cluster 1 (C1), cluster 2 (C2), cluster 3 (C3) and cluster 4 (C4) were found to have 79, 165, 73 and 164 accessions respectively (Table 3 and Fig. 3). The mean value of accessions grouped into each cluster (Supplementary Table S2) showed that accessions in C1 and C2 produced high grain yield per plot and pods per plant whereas cluster 3 was found to have the highest 100-seed weight. Plant height, number of branches and days to 50% flowering reported maximum mean in cluster 1, whereas minimum mean in cluster 3 (Table 4).

Table 4: Cluster means for different traits under study

Cluster variables	*C1*	*C2*	*C3*	*C4*
Days to flowering	56.25	54.79	50.44	53.79
Plant height	72.7	56.07	43.31	52.05
No. of branches	4.639	4.195	3.695	3.87
No. of pods	69.97	56.99	34.05	44.21
Seed index	13.62	13.41	14.45	13.62
Grain yield	686.62	491.17	163.37	331.79

PCA revealed that all six quantitatively measured traits have been loaded on six components; however, a major portion of variance (~63%) in germplasm accessions is explained by the first two components with eigenvalue>1 (Table 5). The first component (PC1) accounted for 45.54% of variation largely through grain yield, number of pods and plant height, while PC2 contributed 17.30% through 100-seed weight and days to 50% flowering. PC3, PC4, PC5 and PC 6 contributed 13.56, 11.36, 8.10 and 4.14% of total variation, respectively. Biplotting of PC1 and PC2 indicated 100-seed weight (44.73%) contributed the highest to the first two principal components, followed by number of pods (19.35%) and grain yield (18.14%) (Figure 4).

Discussion

Soybeans are one of the most important oilseeds in the international market, being the fourth most produced and consumed crop worldwide (Gesteira *et al.*, 2018). It has been studied and enhanced for most of its economically important traits, i.e., yield and attributing traits. Yield is considered a complex trait, governed by many genes and the particular environment and their interaction. Development of adapted varieties for a particular location necessitates the deployment of well-adapted high-yielding genotypes in hybridization programmes. Characterization and evaluation of germplasm accessions is the most important activity of any plant gene bank. This is also a bottleneck between the genetic diversity conserved in a gene bank and its utilization in breeding programmes of a particular area. Therefore, most of the soybean breeding programmes are based on very few trait-specific parental lines, which have resulted in poor genetic gains and cause for the creation of a narrow genetic base in the released cultivar. Modern soybean varieties, which account for the major share of total soybean production in India, have been developed exploiting only a small spectrum of soybean variability. Therefore, the limited variability within the modern cultivars has become a restrictive factor for yield increase and increased incidence of many biotic and abiotic stresses. However, this study shows that the soybean (*Glycine max*) gene pool has a great amount

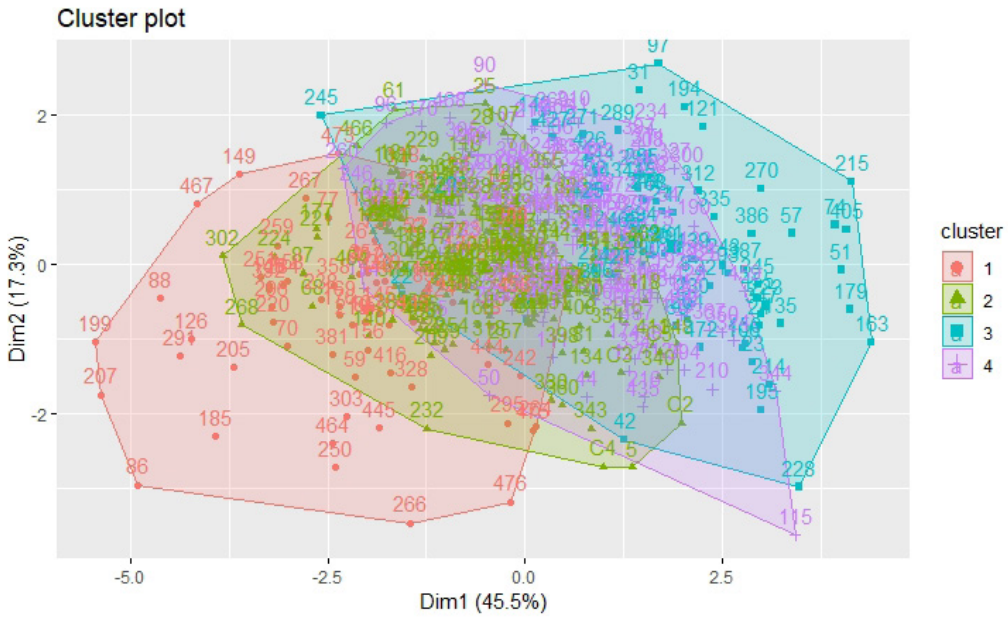

Fig. 3: Biplot of germplasm accessions into different clusters

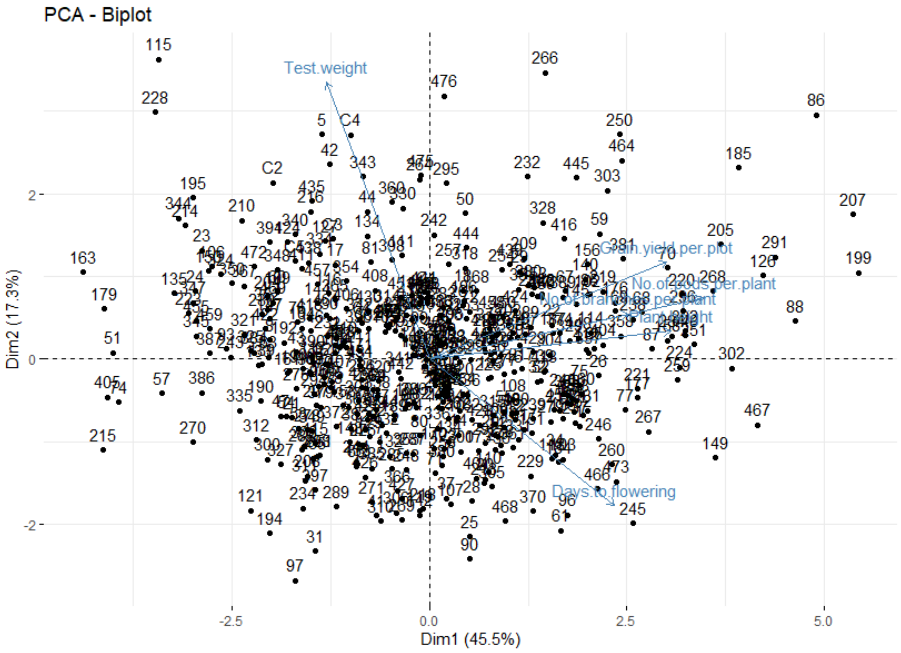

Fig. 4: Principal Component Analysis of different traits under study

of useful variability for yield-attributing traits.

In this study, ANOVA represented variability for all traits except for the number of branches. Similar results were also reported by Kumar *et al*. (2018) for the number of branches and Bhartiya and Aditya (2016) for the number of pods per plant, indicating sufficient genetic diversity for all traits except the number of branches. High PCV and GCV were recorded for grain yield, plant height and number of pods/plant. High heritability coupled with high genetic advance were observed for plant height, 100-seed weight, number of pods per plant and seed yield per plot, indicating governance of additive gene action for these traits. Similar results have been reported by Kumar *et al*. (2015) for plant height and 100-seed weight, for seed yield per plant, Ekka and Gabrial (2016),) for plant height Kumar *et al*. (2018), for 100-seed weight and seed yield per plant Shivakumar *et al*. (2019) for number of nodes and plant height in soybean nested association mapping population.

Table 5: Eigen values, variance contribution (%), variables coordinates and percent contribution of each variable on the individual five principal components of germplasm accessions

		Days to flowering	*Plant height*	*100 seed weight*	*No. of branches*	*No. of pods*	*Grain yield (g)*	*Eigen values*	*Variance (%)*
PC1	Coordinates	0.58	0.76	-0.32	0.63	0.87	0.75	2.73	45.54
	Contribution (%)	12.49	21.22	3.86	14.46	27.43	20.54		
PC2	Coordinates	-0.44	0.08	0.83	0.14	0.18	0.29	1.04	17.3
	Contribution (%)	18.83	0.68	67.15	1.79	3.29	8.24		
PC3	Coordinates	0.3	-0.23	0.11	-0.68	0.19	0.4	0.81	13.56
	Contribution (%)	11.37	6.56	1.59	56.47	4.24	19.77		
PC4	Coordinates	0.57	0.22	0.42	0.02	-0.16	-0.31	0.68	11.36
	Contribution (%)	48.29	6.8	26.41	0.09	3.88	14.53		
PC5	Coordinates	0.2	-0.56	0.07	0.35	0.08	0.05	0.49	8.1
	Contribution (%)	8.12	64.23	0.93	24.64	1.47	0.6		

Correlation studies revealed that traits such as number of pods and plant height had the highest significant positive effect on grain yield; similar kinds of trends were also observed by Gireesh *et al.* (2015), Yashpal *et al.* (2019) and Shivakumar *et al.* (2019). Grain yield was positively correlated with the number of pods in other legumes like Mung Bean [*Vigna radiata* (L.) Wilezek] (Dar *et al.*, 2025). In the current study, 100-seed weight was found to be negatively correlated with other yield attributes, and has also been reported by Iqbal *et al.* (2010) and Kumar *et al.* (2018). PCA can evaluate the relative contribution of different elements to the total divergence together with the nature of forces operating at intra- and inter-cluster levels (Sharma *et al.*, 2009). In the current study, the first three components (PCs) explained >75% of the total variation. In the first two PCs, the maximum percent of variation is contributed by grain yield, 100-seed weight and number of pods. Therefore, these traits are important in explaining the genetic variation in the germplasm accessions. The results are in agreement with various other studies that reported the maximum contribution of grain yield in soybean (Dubey *et al.*, 2018; Maranna *et al.*, 2019). The PCA was also employed in studying variability in other legumes like Horse Gram [*Macrotyloma uniflorum* (Lam.) Verdc.] (Kumari *et al.*, 2025). Cluster analysis grouped the 481 accessions into 4 clusters, with cluster C4 comprising the maximum number of germplasm lines, indicating close relatedness of these genotypes. A similar way of grouping for yield traits of 900 NAM RILs of soybean into 10 clusters was reported by Shivakumar *et al.* (2019). Genotypes having the highest grain yield per plant, plant height and number of pods fall under cluster C1, whereas the lowest days to 50% flowering and the largest seed-sized germplasm accessions fall under cluster C3. This study identified a number of promising accessions that can be further utilized in soybean hybridization programmes for improving the yield potential of new varieties.

Conclusion

Progress in breeding high-yielding varieties of soybean has been reserved and one of the constraints shown by many soybean breeders is the narrow genetic base present in the released varieties. Given this limitation, plant breeders require genetically diverse germplasm not only for yield but also for its contributing traits. In this direction, this study generated information on a large number of soybean germplasm accessions evaluated in the southern region of India. From this study, it is evident that a wide range of genetic variability for different yield attributes and several diverse and promising germplasm accessions were identified for future use in the hybridization program to create new breeding material with a broad genetic base.

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Supplementary Tables

Table S1: Details of the genotypes evaluated in the current study

Code	*Name*	*Code*	*Name*	*Code*	*Name*	*Code*	*Name*
C1	DSb-21	117	EC 274683	238	EC 589400	359	JS 80-21
C2	JS335	118	EC 457464	239	EC 572019	360	JS 93-05
C3	JS9305	119	EC 467282	240	EC 391172	361	SQL 3
C4	KBS23	120	EC 615160	241	EC 309537	362	SQL-40
C5	RKS18	121	EC 550828	242	EC 389399	363	SQL -12
1	AGS 218	122	EC 251817	243	EC 456556	364	SQL-98
2	AGS 166	123	EC 251378	244	EC 538828	365	SQL 11
3	AGS 108	124	EC 572086	245	TGX 802-150D	366	SQL-5
4	AGS 128	125	EC 175321	246	TGX 86-34	367	SQL-81
5	AGS 174	126	EC 93601	247	TGX 702-4-8	368	SQL 93
6	AGS 156	127	EC 287754	248	TGX 298-7D	369	SQL-106
7	AGS 116	128	EC 481571	249	TGX 899-285D	370	SQL 83
8	ABL-1	129	EC 250591	250	ABL-14	371	SQL -31
9	AGS 143	130	EC 34060	251	TGX 722-110E	372	PK 722
10	AGS 190	131	EC 095297	252	TGX 825-1E	373	SQL 97
11	AGS 230	132	EC 39511	253	TGX 824-35	374	PK 474
12	AGS 38	133	EC 113770	254	TGX 814-148D	375	PK 1284
13	AGS 205	134	EC 175329	255	TGX 863-26F	376	P 318
14	AGS 83	135	EC 250588	256	TGX 849-309A	377	SQL 18
15	AGS110	136	EC 241650	257	TGX 849-47F	378	PK 566
16	AGS 158	137	EC 274701	258	TGX 803-99E	379	PK1225
17	AGS 02	138	EC 100778	259	TGX 854-42D	380	SPL-4
18	AGS 105	139	EC 615187	260	TGX 863-2C	381	PLSO-79
19	ABL-2	140	EC 251498	261	TGX 722-155F	382	P-318
20	AGS102	141	EC 232275	262	TGX 814-78D	383	PS 1029
21	AGS 193	142	EC 41319	263	TGX 539-2D-7	384	SL 142
22	AGS 166	143	EC 18672	264	TGX 822--10E	385	K 53
23	AGS 157	144	EC 602288	265	TGX 984-18A	386	L 129
24	AGS 42	145	EC 309522	266	TGX 849-309F	387	KB 249
25	AGS 153	146	EC 325102	267	TGX 849-294D	388	B-160-3
26	AGS 186	147	EC 103334	268	TGX 885-44E	389	T-49
27	AGS 170	148	EC 291399	269	TGX 1070-F6	390	VLS-63
28	AGS 150	149	EC 250577	270	TGX 802-150E	391	WT 169
29	AGS 41	150	EC 241756	271	TGX 849-207 D	392	GP 493
30	AGS 142	151	EC 274712	272	TGX 854-71E	393	VLS 75
31	AMSS34	152	EC 241995	273	TGX 1016-5F	394	V 55
32	AKSS 65	153	EC 251402	274	TGX 854-60A	395	EC 538814
33	AK 887	154	EC 171536	275	TGX 860-11D	396	HAL
34	AKSS 143	155	EC 25082	276	TGX 855-53D	397	WT 150

35	ACC 1026	156	EC 95815	277	TGX 814-148F	398	HARDEE
36	AKSS 63	157	EC 251446	278	TGX 996-5F	399	LESOY 273
37	AGS 104	158	EC 39177	279	TGX 984-18E	400	GC 12
38	B 1666	159	EC 45732	280	TGX 855-44G	401	ELGIN(S)
39	B 471	160	EC 172663	281	TGX 803-99D	402	HIMSO-175
40	B 252	161	EC 100772	282	TGX 1016-19F	403	NANKING
41	BR 10	162	EC 18673	283	TGX 85B-48	404	SEHORE-1
42	B 1665	163	EC 100804	284	TGX854-429	405	INDORE-2
43	BRG 1	164	EC 457052	285	TGX 702-4-2	406	NRC -1
44	DS 366	165	EC 39376	286	TGX 844-313-B	407	NRC 37
45	BRG 12	166	EC 24200	287	TGX 713-1F	408	NRC 57
46	DS 321	167	EC 241310	288	TGX 293-65E	409	NRC 2396
47	BR 15	168	EC 172577	289	TGX 854-77D	410	NRC 105
48	DE 201	169	EC 241778	290	TGX -813-14D	411	NRC 2346
49	B 1667	170	EC 18593	291	TGX -855-32E	412	NRC 2007-1-3
50	DS 205	171	EC 251886	292	ABL-15	413	NRC 86
51	EC 333908	172	EC 325113	293	TGX B-1435E	414	NRC 2320
52	AGS 74	173	EC 291453	294	TGX -312-012F	415	N 928
53	AGS 74A	174	EC 242091	295	TGX -8116-21D	416	M-1085
54	EC 341822	175	EC 30968	296	TGX -825-3D	417	M-1052
55	EC 343310	176	EC 528620	297	TGX 802-100F	418	M-1059
56	EC 333924	177	EC 76759	298	TGX 317-37E	419	MAUS-142
57	EC 343312	178	EC 589409	299	TGX 298-7E	420	MACS-150B(VG)
58	EC 242108	179	EC 287469	300	TGX 573-209	421	MAUS 47
59	EC 114527	180	EC 393231	301	TGX 239-43D	422	MACS 1034
60	EC 333940	181	EC 389173	302	TGX 849-D 13-4	423	MAUS 41
61	EC 481571	182	EC 289161	303	TGX 573-219D	424	MACS 227
62	EC 308328	183	EC 538801	304	TGX560-20D	425	MACS 250
63	EC 241712	184	EC 396067	305	TGX 416-06C	426	MACS 708
64	EC 251529	185	EC 393224	306	TGX 297-16F	427	MACS 1028
65	EC 333859	186	EC 15553	307	TGX 849-14-3D	428	MACS 58(S)
66	EC 241761	187	EC 572108	308	TGX 330-325D	429	MACS 330
67	EC 251405	188	EC 396065	309	TGX 854-420-1	430	IC 24532A
68	EC 251388	189	EC 93413	310	ABL-16	431	IC 0596790
69	EC 313974	190	EC 572130	311	TGX 302A-68D	432	IC 16821
70	EC 245988	191	EC 391181	312	ABL-17	433	IC 13051
71	EC 528623	192	EC 391346	313	TGX 780-5A	434	PI 283327
72	EC 287464	193	EC 572187	314	ABL-18	435	PI 340900
73	EC 2206 B	194	EC 251456	315	TGX 849-813	436	PI 200465
74	EC 309513	195	EC389156	316	TGX 825-17E	437	IC 4882
75	EC 287460	196	ABL-8	317	TGX 854-4F	438	ICS84/86-858-41
76	EC 251413	197	EC 13054	318	TGX 576-21-9	439	ICAL 122
77	ABL-3	198	EC 389167	319	TGX 814-13E	440	EC 287457

78	EC 581521	199	EC 389148	320	TGX 802-100D	441	EC 333870
79	EC 34115	200	EC 389166	321	TGX 442-20D	442	EC 280129
80	EC 343311	201	EC 538802	322	TGX 311-59E	443	EC 389153
81	EC 34079 B	202	EC 572160	323	TGX 573-97D	444	EC 457161
82	EC 389152	203	EC 380522	324	TGX 342-376	445	RAUS -5
83	EC 37104	204	ABL-9	325	TGX 297	446	ALANKAR
84	EC 241715	205	EC 457280	326	TGX 86-24-10	447	MONETTA
85	EC 100027	206	EC 289099	327	TGX 293-47E	448	PALAM SOYA
86	EC 251514	207	EC 572035	328	TGX 86-24-6E	449	IMPROVED PLICAN
87	EC 274713	208	EC 389165	329	TGX 293-46E	450	INDRA SOYA9
88	EC 389154	209	EC 644409	330	TGX 996-4F6	451	KHSb2
89	EC 325104	210	EC 572071	331	JSM 288	452	PS 1347
90	EC 357998	211	EC 389179	332	JSM 258	453	PUSA 22
91	EC 341115	212	EC 592195	333	JSM 302	454	ADT-1
92	EC 389164	213	EC 390979	334	JS 82	455	PK 308
93	EC 333880	214	EC 390981	335	JS 355	456	ANKUR
94	EC 274711	215	EC 456574	336	JS (SH) 2001-64	457	CO-SOYA-2
95	EC 250601	216	ABL-10	337	JS 94-52	458	GUJARAT SOYBEEN-1
96	EC 291400	217	EC 309529	338	JS 20-49	459	CO-1
97	EC 289091	218	EC 251416	339	JS 95-52	460	KALILAR
98	EC 333876	219	EC 333899	340	JS 20-37	461	SL-295
99	EC 309541	220	EC 357990	341	ABL-21	462	SHIAJEET
100	EC 329157	221	EC 389391	342	JS 20-76	463	LEE
101	EC 309543	222	EC 333872	343	JS 20-38	464	BNAGG
102	EC 251401	223	EC 589407	344	JS 98-02	465	SHIVATIK
103	ABL-4	224	EC 528622	345	JS 94-67	466	DURGA
104	EC 309511	225	EC 572127	346	JS 79-82	467	PUNJAB
105	EC 389160	226	EC 546881	347	JS 20-86	468	PUSA 16
106	EC 242003	227	ABL-11	348	JS 97-52	469	PS 1024
107	EC 251432	228	ABL-12	349	JS(SH)99-02	470	PK 472
108	ABL-5	229	EC 18673	350	JS 20-73	471	PK 262
109	EC 2579	230	EC 606917	351	TGX 802-265D	472	MACS -13
110	ABL-6	231	EC 538807	352	TGX 311-101F	473	MACS 450
111	ABL-7	232	EC 389178	353	TGX 239-6D	474	MACS 58
112	EC 547464	233	EC 39573	354	TGX 573-209-23	475	ABL-19
113	EC 23003	234	ABL-13	355	TGX 328-049	476	ABL-20
114	EC 245986	235	EC 389163	356	TGX 536-1009		
115	EC 291397	236	EC 528666	357	JSM 245		
116	EC467282	237	EC 542431	358	JS 20-72		

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RESEARCH ARTICLE

Development of Morphological Descriptors of Some Orchid Species

LC De*, SS Biswas, Suman Natta, Bidyarani Senjam and SP Das

Abstract

Amongst different morphological descriptors of commercially grown orchid genera, diversity in pseudobulb shape, leaf shape, inflorescence variation, and floral characteristics was studied in detail. Morphological descriptors of 42 orchid species namely *Acampe rigida, Acampe papillosa*, *Aerides rosea, Arachnis labrosa, Arundina graminifolia*, *Ascocentrum ampullaceum, Calanthe masuca, Cattleya maxima,* *Coelogyne elata, Coelogyne flaccida, Coelogyne fuscescens, Coelogyne nitida, Coelogyne ovalis, Coelogyne suaveolens, Coelogyne orchracea, Coelogyne graminifolia, Coelogyne cristata, Coelogyne barbadense, Cleisocentron trichonum, Cottonia peduncularis, Cymbidium cyperifolium, Dendrobium nobile alba, Dendrobium farmerii, Diplomeris hirsuta, Epidendrum radicans, Epidendrum secundatum, Epidendrum xanthium, Eria bambusifolia, Eria coronaria, Eria flava, Eria suaveolens, Gastrochilus bellinis*, *Lycaste cruentus, Lycaste macrophylla Paphilionanthe vandarum, Paphiopedilum spicerianum, Phaius flavus, Phaius wallichii, Phalaenopsis mannii, Renanthera imschootiana, Thunia marshalliana* and *Vanda motesiana* developed which could be useful for identification of unique germplasm for pot plants, medicinal orchids, breeding materials and preparation of value added products.

Keywords: Descriptors, Hybrids, Orchid, Species.

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Introduction

Orchids are the second-largest family of flowering plants (Willis, 2017) and are distributed throughout the world. The family Orchidaceae is divided into five subfamilies (*Apostasioideae, Cypripedioideae, Vanilloideae, Orchidoideae, Epidendroideae*). Orchids account for approximately 8% of angiosperm species diversity (*Chase et al*., 2015). To date, 29,199 species have been identified and accepted (Govaerts *et al*., 2017), although several hundred new species are added each year. By the end of 2017, the IUCN Global Red List included assessments for 948 orchid species, of which 56.5% are reported to be threatened (IUCN, 2017). Orchids are monocot plants. They may be epiphytic, terrestrial, and lithophytic. About 70% of the world's orchids are epiphytic and/or lithophytic, 25% are terrestrial, and 5% of the world's orchids grow in mixed substrates (both lithophytic, epiphytic, and terrestrial) (Arditti, 1992). In international trade, among the top ten cut flowers, orchids rank the sixth position and among orchids, Cymbidium ranks first in floricultural crops (De *et al*., 2014).

In addition to their geographical and taxonomic diversity, orchids are also widely used for a variety of reasons, both legally and illegally, sustainably and unsustainably (Fay, 2015). One of the best-known plant groups in the global horticultural and cut flower trades (De, 2015; FloraHolland, 2015), orchids are also harvested, grown, and traded for a variety of purposes, including as ornamental plants, medicinal products, and food. Orchid species are also admired for their unusual growth habits (e.g., leafless orchids, such as species

of *Dendrophylax* Rchb.f. and *Chiloschista* Lindl.), miniature size (e.g., species of *Platystele* Schltr. and *Bulbophyllum moniliforme* F. Muell.), scent (e.g., species of *Cattleya* Lindl. and *Dendrochilum glumaceum* Lindl.), patterned leaves (e.g., jewel orchids in the genera *Anoectochilus* Blume, *Goodyera* R.Br., *Ludisia* A.Rich. and *Macodes* Lindl.) and as cut flowers (*Renanthera imschootiana*, *Vanda coerulea*). Several local species of *Ascocentrum, Calanthe, Cymbidium, Dendrobium, Paphiopedilum,* and *Vanda,* etc., are in great demand in the international market for breeding materials (Kumar *et al*., 2007).

In monopodial orchids, the stem emerges from a single bud, elongates, and produces leaves from the apex each year. The flower stem emerges from the base of the uppermost leaves, e.g., *Phalaenopsis*, *Vanda,* and *Vanilla*. The base of the stem of sympodial epiphytes, or in some species essentially the entire stem, may be thickened to form what is called a pseudobulb that contains nutrients and water for drier periods, e.g., *Cymbidium, Cattleya, Dendrobium, Oncidium* (De, 2020). Epiphytic and most lithophytic orchids have clinging roots for anchorage, absorbing roots that penetrate the humus on bark and the aerial roots hang free in the air and help in the absorption of moisture (De, 2020). Orchids generally have simple leaves with parallel veins, although some Vanilloideae have a reticulate venation. They may be ovate, lanceolate, or orbiculate and very variable in size. Epiphytic orchids are characterized by thick and succulent leaves with thick cell walls, cuticles, and small substomatal chambers, whereas those of terrestrial species are thin (Sailo *et al*., 2014). Orchids are monocotyledonous plants bearing flowers with seven floral parts- three sepals, three petals and the column or gynostemium. The orchid flowers consist of three outermost floral parts- the sepals are similar in appearance. The inner whorl of three segments is called the petals. The two lateral petals are alike and the other one, called the lip or labellum, is highly modified and enlarged. The labellum is the most prominent and distinctive part of the orchid flower. The column or gynostemium is located at the center of the flower and is the unique structure distinguishing the orchids from all other kinds of plants. It is the reproductive part of the flower formed by the union of the male and female organs (De, 2020).

Materials and Methods

The experiment was conducted using all vegetatively propagated plants of 42 orchid species namely *Acampe rigida, Acampe papillosa*, *Aerides rosea, Arachnis labrosa, Arundina graminifolia*, *Ascocentrum ampullaceum, Calanthe masuca, Cattleya maxima, Coelogyne elata, Coelogyne flaccida, Coelogyne fuscescens, Coelogyne nitida, Coelogyne ovalis, Coelogyne suaveolens, Coelogyne orchracea, Coelogyne graminifolia, Coelogyne cristata, Coelogyne barbadense, Cleisocentron trichonum, Cottonia peduncularis, Cymbidium cyperifolium, Dendrobium nobile alba, Dendrobium farmerii, Diplomeris hirsuta, Epidendrum radicans, Epidendrum secundatum, Epidendrum xanthium, Eria bambusifolia, Eria coronaria, Eria flava, Eria suaveolens, Gastrochilus bellinis*, *Lycaste cruentus, Lycaste macrophylla Paphilionanthe vandarum, Paphiopedilum spicerianum, Phaius flavus, Phaius wallichii, Phalaenopsis mannii, Renanthera imschootiana, Thunia marshalliana* and *Vanda motesiana*.

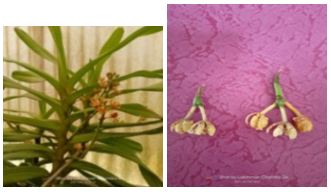
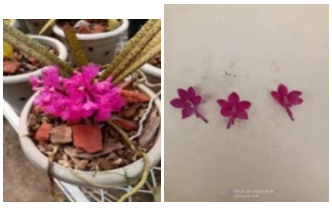
For all the species, full-grown 20 plants with at least two pseudobulbs/shoots were selected for study. Usually, healthy and insect pest and disease-free plants are required for testing, for taking morphological observations without any chemical and bio-physical treatment. The experiment was conducted for two similar flowering seasons at two different places under greenhouse conditions, ensuring satisfactory growth for the expression of the relevant characteristics of the variety and species. All observations were taken by measuring or counting on 10 plants or parts taken from each of the 10 plants. Normally, growth regulators are not applied. All observations were taken of the shoot on the flowering shoot, of the leaf on the longest leaf of a flowering shoot, of the inflorescence and the flower at the time when 50% of the flowers on the inflorescence have opened and on the most recently fully opened flower on the inflorescence before fading of colour, of the length and width of the flower and parts of the flower in the spread out position, of the colour of sepal, petal, lip and column on the inner side. For the assessment of colour characteristics, the Royal Horticultural Society (RHS) colour chart was used.

Results and Discussion

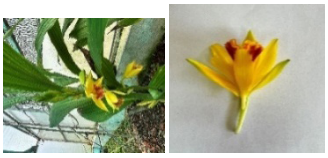
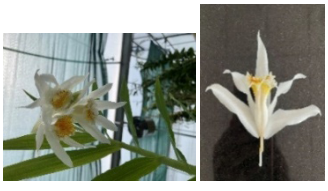
A unique landrace or farmer variety can be registered if it fulfils essentially the criteria of Distinctiveness, Uniformity, and Stability, which means the candidate variety or species must be distinguishable by at least one essential characteristic from a variety that is sufficiently uniform in its expression of its essential characteristics, which should remain fixed even after repeated propagation. The variety should also have a single and distinct denomination (Henke, 2008). In the present investigation, morphological descriptors of 42 orchid species namely *Acampe rigida, Acampe papillosa*, *Aerides rosea, Arachnis labrosa, Arundina graminifolia*, *Ascocentrum ampullaceum, Calanthe masuca, Cattleya maxima, Coelogyne elata, Coelogyne flaccida, Coelogyne fuscescens, Coelogyne nitida, Coelogyne ovalis, Coelogyne suaveolens, Coelogyne orchracea, Coelogyne graminifolia, Coelogyne cristata, Coelogyne barbadense, Cleisocentron trichonum, Cottonia peduncularis, Cymbidium cyperifolium, Dendrobium nobile alba, Dendrobium farmerii, Diplomeris hirsuta, Epidendrum radicans, Epidendrum secundatum, Epidendrum xanthium, Eria bambusifolia, Eria coronaria, Eria flava, Eria suaveolens, Gastrochilus bellinis*, *Lycaste cruentus, Lycaste macrophylla Paphilionanthe*

vandarum, Paphiopedilum spicerianum, Phaius flavus, Phaius wallichii, Phalaenopsis mannii, Renanthera imschootiana, Thunia marshalliana and *Vanda motesiana* were studied (Table 1).

Table 1: Morphological characteristics of some orchid species

Name of the species	*Characteristics*	*Illustrations*
Acampe rigida	Plants are robust with oblong, rigid horizontal clasping leaves measuring 22.5 to 32 cm length and arranged with 3 to 7 erect and dense inflorescences bearing 20-36 scented flowers. Flowers are yellow, 1-1.3 cm across and last for 15-30 days (Table 2).	
Arachnis labrosa	These are monopodial epiphyte, with leathery strap shaped leaves, 30-60 cm tall bearing branched or simple raceme inflorescence. Inflorescence is 110-115 cm long bearing 57-60 yellow scented flowers (Table 3).	
Ascocentrum ampullaceum	These are dwarf monopodial epiphytes and characterized by large spur hangs from the tip. The plants are small, compact with small strap shaped leaves and short stalked erect and cylindrical covered with many closely spaced flowers. Flowers are 1.3 to 2.2 cm diameter, rose-purple in colour (Table 4).	
Calanthe masuca	An evergreen species with elliptic –ovate to lanceolate leaves. Inflorescence is axillary or terminal, 55-64 cm tall crowded with 14 to 25 small blue violet flowers. Flowers are 3.0 to 3.5 cm long and 5.0 to 5.5 cm width (Table 5).	
Cattleya maxima	The plants possess elongated pseudobulbs and may be of unifoliate or bi-foliate. The leaves are thick and leathery. Plants are 30-35 cm tall arranged with 20cm long inflorescence bearing 2-3 flowers per inflorescence. Flowers are 11.0-11.5 cm long and 9.5-10 cm wide undulate crisped lips and are purple in colour	
Coelogyne barbadense	These are sympodial and ovoid pseudobulbous orchids. The pseudobulbs are topped by 2 to 4 lanceolate leaves, slender in size and arranged along with creeping rhizomes. The leaves are coriaceous, thick and leathery with pronounced stalks. Inflorescences are 70-75 cm long, arching or pendulous bearing white flowers with brown lips (Table 6).	
Cottonia peduncularis	A tropical epiphyte with strap shaped arching leaves and horizontal inflorescence bearing 4 to 6 bee shaped flowers. Flowers are yellow with purple-brown lips (Table 7).	

Cont...

Cymbidium cyperifolium	Plants are arranged with conical pseudobulbs and linear leaves, bearing greenish-yellow flowers with red spotted lips. Sepals and petals are lanceolate. Inflorescence 40-50 cm long bearing 6 to 9 flowers. Flowers are 3.6 to 4.0 cm long and 1.5 to 1.7 cm wide, scented.	
Dendrobium nobile alba	Plants are 35-45 cm tall with clavate fleshy pseudobulbs. The pseudobulbs are arching or erect with caduceus, leathery, glossy green leaves with 5-20 inflorescences. Inflorescence is 1 to 3 flowered, short and arises from the upper nodes of the old leafless pseudobulbs. The flowers are fragrant, long lasting, 6.5 to 7.5 cm in diameter, waxy, and lip with white margin.	
Dendrobium farmerii	Plants are 22-25 cm tall with 4 angled club shaped pseudobulbs and 2-3 lance or elliptic shaped leaves. Inflorescences borne on the cylindric pendulous racemes arise from the apices of the mature new growth. Flowers are 3.5 to 5.0 cm in diameter with white sepals and petals and orange lips.	
Eria cruenta	Plants are 25-35cm tall with conical peudobulb bearing 2 leaves. Leaves are lanceolate shaped, 20-25 cm long. The inflorescence is 25-30 cm tall arranged with 10-15 flowers. Flowers are white, 3-35cm across (Table 8).	
Paphiopedilum spicerianum	Plants are 15-20 cm tall bearing 4-5 leaves. The leaves are broad, linear oblong, dark green with wavy margins and purple on the underside. The inflorescence is 20-30 cm long, purple, slender and erect. The flowers are 7.5-8.0 cm across, glossy and long lasting. The dorsal sepal is broad, pure white and greenish at the base. The petals are deflexed, yellowish green with speckles and a red central line. The lip is bell shaped and crimson brown in colour.	
Phaius flavus	Plants are 45-60 cm tall and arranged with 8-10 lanceolate leaves. Flowers are borne terminally, yellow with a reddish-brown banded lip, scented. Pseudobulbs are conical, topped with 60-70 cm long plicate variegated leaves.	
Phalaenopsis mannii	Plants are small, arranged with 22-28 cm long narrow obovate leathery leaves bearing raceme inflorescence. Flowers are 2.8 3.0 cm in diameter, deep red and dark golden yellow, the white lip is highlighted by yellow throat markings.	
Thunia marshalliana	These are terrestrial sympodial orchids. Plants are 50-60 cm tall having 15-20 fleshy canes 20-25 cm long horizontal lanceolate leaves. Inflorescence is 15-20 cm long bearing 5-10 flowers in one direction. Flowers are borne terminally, pendulous, white, 7-9 cm in diameter.	

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Vanda motesiana	Leaves are strap shaped, 28-30 cm long inflorescence, 35-40 cm long develop from the leaf axils with 5 to 10 flowers. The flowers are 4-6 cm across, greenish yellow, with spotted sepals and petals, and striped or streaked lips.	

Table 2: Morphological descriptors of *Acampe rigida*

S. No.	*Characters*	*Acampe rigida*
1	Internode length	3.0–4.0 cm
2	Stem diameter	1.5–1.7 cm
3	Root location	All along the stem
4	Leaf type	Strap
5	Leaf length	22.5–32.0 cm
6	Leaf breadth	4.3–5.5 cm
7	Leaf shape	Oblong
8	Leaf apex	Acute
9	Leaf orientation	Horizontal
10	Leaf colour	Green
11	Leaf sheath pigmentation	Green
12	No. of inflorescences/year/ plant	03–07
13	Inflorescence length	22.5–25.5 cm
14	Peduncle length	3.0–6.5 cm
15	Inflorescence orientation	Erect
16	Inflorescence nature	Dense
17	No. of flowers/inflorescence	20–36
18	Orientation of flowers	Facing all directions
19	Flower width	1.0–1.3 cm
20	Flower fragrance	Present
21	Flower longevity on the plant	15–30 days
22	Flower predominant colour	Yellow (7-D)
23	Dorsal sepal size (l × b)	1.1–1.4 × 1.2–1.5 cm^2
24	Dorsal sepal shape	Elliptic
25	Dorsal sepal curvature	Incurved
26	Dorsal sepal apex	Obtuse
27	Lateral sepal length (l × b)	1.0–1.2 × 0.6–0.7 cm^2
28	Lateral sepal shape	Elliptic
29	Lateral sepal curvature	Incurved
30	Lateral sepal apex	Obtuse
31	Sepal colour (nos.)	Two
32	Sepal colour pattern	Brindled
33	Petal size (l × b)	1.0–1.1 × 0.4–0.5 cm^2
34	Petal shape	Obovate
35	Petal curvature	Incurved with a straight apex
36	Petal apex	Obtuse
37	Petal margin	Entire
38	Petal colour (nos.)	Two
39	Petal colour pattern	Brindled
40	Lip length	1.0 cm
41	Lip width	0.4–0.6 cm
42	Lip: apical lobe shape	Ovate
43	Lip: lateral lobe shape	Orbicular
44	Lip curvature	Deflexed
45	Lip apex	Obtuse
46	Keels no.	Two
47	Lip surface	Glabrous
48	Lip colour (nos.)	Two
49	Lip colour pattern	Blotched
50	Column length	0.3 cm
51	Column colour pattern	Uniform
52	Pedicel length	1.1–1.5 cm
53	Spur type	Conical
54	Spur length	0.3–0.5 cm

Cont...

Table 3: Morphological descriptors of *Arachnis labrosa*

S. No.	*Characters*	*Arachnis labrosa*
1	Internode length	3.0 cm
2	Stem diameter	1.0 cm
3	Root location	All along stem
4	Leaf type	Strap
5	Leaf length	28.5 cm
6	Leaf breadth	28 cm
7	Leaf apex	Retuse
8	Leaf orientation	Arching
9	Leaf colour	Green
10	Leaf sheath pigmentation	Absent
11	No. of inflorescences/year/plant	05
12	Inflorescence length	114 cm
13	Peduncle length	42 cm
14	Inflorescence orientation	Horizontal
15	Inflorescence nature	Lax

Cont...

S. No.	*Characters*	*Arachnis labrosa*
16	No. of flowers/inflorescence	57
17	Orientation of flowers	Facing all directions
18	Flower width	1.9 cm
19	Flower fragrance	Present
20	Flower longevity on the plant	15–30 days
21	Flower predominant colour	Yellow
22	Dorsal sepal size (l × b)	1.6 × 0.3 cm^2
23	Dorsal sepal shape	Linear
24	Dorsal sepal curvature	Incurved with a straight apex
25	Dorsal sepal apex	Obtuse
26	Lateral sepal length (l × b)	2.0 × 0.3 cm^2
27	Lateral sepal shape	Linear
28	Lateral sepal curvature	Deflexed
29	Lateral sepal apex	Acute
30	Sepal colour (nos.)	Double
31	Sepal colour pattern	Blotched
32	Petal size (l × b)	1.4 × 0.3 cm^2
33	Petal shape	Obovate
34	Petal curvature	Incurved with a straight apex
35	Petal apex	Acute
36	Petal margin	Entire
37	Petal colour (nos.)	Double
38	Petal colour pattern	Blotched
39	Lip length	1.1 cm
40	Lip width	0.3 cm
41	Lip: mid lobe shape	Lanceolate
42	Lip: lateral lobe shape	Orbicular
43	Lip curvature	Straight
44	Lip apex	Obtuse
45	Keels no.	Absent
46	Lip surface	Glabrous
47	Lip colour (nos.)	Two
48	Lip colour pattern	Streaked/ Shaded
49	Column length	0.5 cm
50	Column colour pattern	Uniform
51	Pedicel length	2.3 cm
52	Spur type	Conical
53	Spur length	0.4 cm
54	Flowering season	Rainy

Table 4: Morphological descriptors of *Ascocentrum ampullaceum*

S. No.	*Characters*	*Ascocentrum ampullaceum*
1	Internode length	0.6–0.8 cm
2	Stem diameter	1.5–1.8 cm
3	Root location	Only at stem base
4	Leaf type	Strap
5	Leaf length	12.0–14.0 cm
6	Leaf breadth	2.0–2.2 cm
7	Leaf apex	Praemorse
8	Leaf orientation	Straight
9	Leaf colour	Green
10	Leaf sheath pigmentation	Present
11	No. of inflorescences/year/ plant	03–06
12	Inflorescence length	10.0–11.5 cm
13	Peduncle length	1.5–2.0 cm
14	Inflorescence orientation	Erect
15	Inflorescence nature	Dense
16	No. of flowers/inflorescence	20–26
17	Orientation of flowers	All directions
18	Flower width	1.3–2.2 cm
19	Flower fragrance	Absent
20	Flower longevity on the plant	15–30 days
21	Flower predominant colour	Red purple (72-B)
22	Dorsal sepal size (l × b)	0.9–1.2 × 0.6–0.8 cm^2
23	Dorsal sepal shape	Elliptic
24	Dorsal sepal curvature	Straight
25	Dorsal sepal apex	Acute
26	Lateral sepal length (l × b)	1.0–1.1× 0.6–0.7 cm^2
27	Lateral sepal shape	Elliptic
28	Lateral sepal curvature	Incurved with a straight apex
29	Lateral sepal apex	Acute
30	Sepal colour (nos.)	Single
31	Sepal colour pattern	Uniform
32	Petal size (l × b)	1.1–1.2 × 0.6–0.7 cm^2
33	Petal shape	Elliptic
34	Petal curvature	Incurved with straight apex
35	Petal apex	Obtuse
36	Petal margin	Entire
37	Petal colour (nos.)	Single
38	Petal colour pattern	Uniform
39	Lip length	0.6–0.7 cm
40	Lip width	0.8–0.25 cm
41	Lip: mid lobe shape	Oblong
42	Lip: lateral lobe shape	Lanceolate

Cont...

S. No.	Characters	Ascocentrum ampullaceum
43	Lip curvature	Straight
44	Lip apex	Obtuse
45	Keels no.	Absent
46	Lip surface	Smooth
47	Lip colour (nos.)	Single
48	Lip colour pattern	Uniform
49	Column length	0.2 cm
50	Column colour pattern	Uniform
51	Pedicel length	1.5–1.8 cm
52	Spur type	Tubular
53	Spur length	0.9–1.0 cm
54	Flowering season	Spring

Table 5: Morphological descriptors in *Calanthe masuca*

S. No.	Characters	Calanthe masuca
1	Plant height	32.5–68.5 cm
2	Nature of bulb	Conical
3	Bulb size (l × b)	3.0–3.1 × 2.2–3.0 cm^2
4	No. of leaves	07–08
5	Leaf length	30–47 cm
6	Leaf width	13.5–14.0 cm
7	Leaf apex	Acuminate
8	Leaf shape	Elliptic
9	Inflorescence no./plant	01–02
10	Inflorescence orientation	Erect
11	Inflorescence length	55–64 cm
12	Peduncle length	35–48 cm
13	Peduncle bract	Present
14	Peduncle colour	Green
15	Peduncle diameter	0.5–0.9 cm
16	No. of flowers/inflorescence	14–26
17	Flower length	2.9–3.5 cm
18	Flower width	5.0–5.5 cm
19	Flower bract	Present
20	Flower fragrance	Absent
21	Dorsal sepal length	2.5–4.6 cm
22	Dorsal sepal width	1.0–1.1 cm
23	Dorsal sepal shape	Lanceolate
24	Dorsal sepal apex	Acute
25	Dorsal sepal curvature	Reflexed
26	Lateral sepal length	2.5–3.1 cm
27	Lateral sepal width	1.0–1.1 cm
28	Lateral sepal shape	Lanceolate
29	Lateral sepal apex	Acuminate
30	Lateral sepal curvature	Reflexed
31	Sepal main colour	Purple (84-A)
32	Sepal curvature	Deflexed
33	Petal length	2.0–2.4 cm
34	Petal width	0.8–0.9 cm
35	Petal shape	Elliptic
36	Petal apex	Acute
37	Petal main colour	Purple (84-A)
38	Petal colour no.	Single
39	Petal colour pattern	Shaded
40	Petal curvature	Straight
41	Apical lip length	2.0 cm
42	Apical lip width	1.5–1.7 cm
43	Apical lip shape	Orbicular
44	Apical lip apex	Lobed
45	Lip curvature	Straight
46	Lip lobation	Present
47	Lip main colour	Purple (N81-A)
48	Lip colour no.	Two
49	Lip colour pattern	Shaded
50	Lateral lip length	0.8–1.0 cm
51	Lateral lip width	0.3 cm
52	Lateral lip shape	Lanceolate
53	Lateral lip apex	Obtuse
54	Lip emargination	Shallow
55	Lip callus	Present
56	Lip surface texture	Glabrous
57	Column length	0.3–0.5 cm
58	Column width	0.4–0.5 cm
59	Column main colour	White (153-C)
60	Column colour pattern	Uniform
61	Anther cap colour	White
62	Pedicel ovary length	3.9–4.0 cm
63	Flowering season	Rainy
64	Flower longevity on the plant	15–30 days
65	Spur/mentum length	3.4–4.4 cm

Hundreds of natural inter-generic, inter-specific, or intra-specific natural hybrids of commercially important orchid species are found in nature. Most of the Indian orchid species studied have great potential in breeding programmes, especially in producing primary hybrids due to their inherent attractiveness coupled with their ability to transmit these characteristics to hybrids. In epiphytic orchids, offspring of reciprocal crosses show variations in characteristics like cane length and flower colour, flower size, flowering season, and flower yield (Mc Connel and Kamemoto 1983; De 2020). In some orchid species, fragrance is the most

Table 6: Morphological descriptors of some *Coelogyne* species

S. No.	*Characters*	*Coelogyne barbadense*	*Coelogyne cristata*	*Coelogyne flaccida*
1	Pseudobulb size (l × b)	7.5 × 3.5 cm^2	10.5–12.6 × 1.6–2.0 cm^2	6.8–8.9 × 1.3–2.7 cm^2
2	Pseudobulb shape	Ovoid	Clavate	Conical
3	No. of leaves/pseudobulb	02	02	02
4	Leaf length	47 cm	32.8–40.1 cm	12.7–22.6 cm
5	Leaf width	5.9 cm	4.0–4.7 cm	2.9–3.8 cm
6	Leaf shape	Lanceolate	Lanceolate	Lanceolate
7	Leaf apex	Acute	Acute	Acute
8	Inflorescence no./plant	10	08–09	01–06
9	Inflorescence orientation	Arching	Pendulous	Pendulous
10	Inflorescence length	72 cm	26.5–30.9 cm	21.7–26.8 cm
11	Peduncle length	50 cm	8.5–9.9 cm	4.0–6.5 cm
12	Peduncle bract	-	Present	Present
13	Peduncle colour	-	Greenish brown	Light brown
14	Peduncle diameter	-	0.6–0.7 cm	0.5–0.6 cm
15	No. of flower/ inflorescence	11	08–09	08–11
16	Flower length	6.0 cm	5.9–6.2 cm	3.5–3.8 cm
17	Flower width	8.5 cm	6.3–6.5 cm	3.9–4.0 cm
18	Flower fragrance	Present	Present (mild)	Present
19	Dorsal sepal length	5.0 cm	3.1–3.4 cm	2.0–2.3 cm
20	Dorsal sepal width	1.5 cm	1.0–1.1 cm	0.8–0.9 cm
21	Dorsal sepal shape	Lanceolate	Lanceolate	Lanceolate
22	Dorsal sepal apex	Acute	Acute	Acute
23	Lateral sepal length	4.7	3.3 cm	2.2–2.6 cm
24	Lateral sepal width	1.4	1.0 cm	0.7–0.8 cm
25	Lateral sepal shape	Lanceolate	Lanceolate	Lanceolate
26	Lateral sepal apex	Acute	Acute	Acute
27	Sepal main colour	White (155-C)	White (155-C)	Yellow (11-C)
28	Sepal colour pattern	Uniform	Uniform	Uniform
29	Sepal curvature	Deflexed with straight apex	Incurved with reflexed apex	Incurved with straight apex
30	Petal length	4.6	3.0 cm	2.1–2.3 cm
31	Petal width	0.5	0.7–0.8 cm	0.4 cm
32	Petal shape	Linear	Lanceolate	Narrow lanceolate
33	Petal apex	Acute	Acute	Acute
34	Petal main colour	White (155-C)	White (155-C)	Yellow (11-C)
35	Petal colour pattern	Uniform	Uniform	Uniform
36	Petal curvature	Reflexed	Reflexed	Reflexed
37	Lip length	4.0 cm	2.7–2.8 cm	2.1–2.2 cm
38	Lip width	3.0 cm	2.5–2.6 cm	1.9 cm
39	Lip shape	Orbicular	Ovate	Ovate
40	Lip apex	Obtuse	Acute	Acute
41	Lip curvature	Reflexed	Reflexed	Reflexed
42	Lip lobation	Present	Present	Present
43	Lip margin	Entire	Entire	Entire
44	Lip main colour	White (155-C)	White (155-C)	White (155-C)

Cont...

S. No.	Characters	Coelogyne barbadense	Coelogyne cristata	Coelogyne flaccida
45	Lip colour pattern	Striped/Shaded	Striped/streak	Striped/streak
46	Lip callus/keels	03	03	03
47	Lip no. of colour	Double	Double	Triple
48	Lip surface texture	Pubescent	Glabrous	Glabrous
49	Column length	2.5 cm	2.0–2.1 cm	1.4–1.6 cm
50	Column width	0.7	0.8 cm	0.4–0.6 cm
51	Column main colour	White (155-C)	White (155-C)	White (155-C)
52	Column colour pattern	Uniform	Striped	Striped/streak
53	Anther cap colour	-	White + Brown	Light yellow
54	Pedicel ovary length	1.7 cm	2.7–2.8 cm	1.5–1.9 cm
55	Spur/mentum	Absent	Absent	Absent
56	Flower longevity on plant	15–30 days	15–30 days	15–30 days
57	Flowering season	Autumn (Sept-Oct)	Spring (March)	Spring (March)

Table 7: Morphological descriptors of *Cottonia peduncularis*

S. No.	Characters	Cottonia peduncularis
1	Internode length	0.1–1.2 cm
2	Stem diameter	0.6–0.7 cm
3	Root location	All along stem
4	Leaf type	Strap
5	Leaf length	10.2–10.7 cm
6	Leaf breadth	1.3–1.5 cm
7	Leaf apex	Retuse
8	Leaf orientation	Arching
9	Leaf colour	Green
10	Leaf sheath pigmentation	Absent
11	No. of inflorescences/year/plant	01–02
12	Inflorescence length	16.7–17.5 cm
13	Peduncle length	13.9–14.2 cm
14	Inflorescence orientation	Horizontal
15	Inflorescence nature	Lax
16	No. of flowers/inflorescence	04–06
17	Orientation of flowers	All directions
18	Flower width	1.1–1.4 cm
19	Flower fragrance	Absent
20	Flower longevity on the plant	>30 days
21	Flower predominant colour	Yellow
22	Dorsal sepal size (l × b)	1.1–1.2 × 0.6–0.7 cm
23	Dorsal sepal shape	Obovate
24	Dorsal sepal curvature	Straight
25	Dorsal sepal apex	Obtuse
26	Lateral sepal length (l × b)	1.1 × 0.6–0.7 cm^2
27	Lateral sepal shape	Elliptic
28	Lateral sepal curvature	Incurved with straight apex
29	Lateral sepal apex	Obtuse
30	Sepal colour (nos.)	02
31	Sepal colour pattern	Streak/Striped
32	Petal size (l × b)	1 × 0.4 cm^2
33	Petal shape	Obovate
34	Petal curvature	Incurved with straight apex
35	Petal apex	Obtuse
36	Petal margin	Entire
37	Petal colour (nos.)	02
38	Petal colour pattern	Streak/Striped
39	Lip length	1.4–1.5 cm
40	Lip width	1.1 cm
41	Lip: mid lobe shape	Orbicular
42	Lip: lateral lobe shape	Orbicular
43	Lip curvature	Straight
44	Lip apex	Bilobed
45	Keels no.	01
46	Lip surface	Glabrous
47	Lip colour (nos.)	Two
48	Lip colour pattern	Uniform
49	Column length	0.6 cm
50	Column colour pattern	Blotched
51	Pedicel length	1.6–2.0 cm
52	Spur type	Absent
53	Spur length	-
54	Flowering season	Rainy

Cont...

Table 8: Morphological descriptors of *Eria cruenta*

S. No.	*Characters*	*Eria cruenta*
1	Plant height	31–35 cm
2	Nature of bulb	Conical
3	Number of leaves/shoot	02
4	Leaf length	20–25 cm
5	Leaf width	3.8–5.0 cm
6	Leaf shape	Lanceolate
7	Leaf apex	Acute
8	Inflorescence number/plant	01–02
9	Inflorescence orientation	Arching
10	Inflorescence length	30 cm
11	Peduncle length	5.5 cm
12	Peduncle bract	Present
13	Peduncle colour	Green
14	Peduncle diameter	0.3 cm
15	Flower no./ Inflorescence	10–15
16	Flower length	1.5–3.0 cm
17	Flower width	3.0–3.4 cm
18	Flower fragrance	Present
19	Dorsal sepal length	2.0–2.5 cm
20	Dorsal sepal width	0.4–0.5 cm
21	Dorsal sepal shape	Narrow lanceolate
22	Dorsal sepal apex	Acuminate
23	Lateral sepal length	2.0–2.3 cm
24	Lateral sepal width	0.4–0.5 cm
25	Lateral sepal shape	Narrow lanceolate
26	Lateral sepal apex	Acuminate
27	Sepal main colour	White (155-C)
28	Sepal colour pattern	Spotted
29	Sepal curvature	Straight
30	Petal length	1.7–2.0 cm
31	Petal width	0.4 cm
32	Petal shape	Narrow lanceolate
33	Petal apex	Acuminate
34	Petal main colour	White (155-C)
35	Petal colour pattern	Uniform
36	Petal curvature	Incurved
37	Lip length	1.2–1.3 cm
38	Lip width	0.6–0.7 cm
39	Lip shape	Oblong
40	Lip apex	Acute
41	Lip curvature	Deflexed
42	Lip lobation	Present
43	Lip main colour	White (155-C)
44	Lip colour (nos.)	02
45	Lip colour pattern	Striped/Shaded
46	Lip callus	Present
47	Lip surface texture	Glabrous
48	Column length	0.8 cm
49	Column width	0.3–0.4 cm
50	Column main colour	White (155-C)
51	Column colour pattern	Striped/Shaded
52	Anther cap colour	White
53	Pedicel ovary length	1.0–1.2 cm
54	Flowering season	Rainy
55	Flower longevity	15–30 days
56	Spur/Mentum length	0.5 cm

Cont...

important characteristic sought after by breeders (Singh, 1984). Thomas (2001) viewed the requirements for flower forms of commercial orchids as strong, self-supporting, erect inflorescences, long duration of blooms, compact plant size, wide temperature tolerance, disease resistance, firm substances and consistency of colours.

Conclusion

A good morphological study is required for the conservation and utilization of endangered orchids. Native species can be effectively utilized for the development of inter-generic, inter-specific, or intra-specific natural hybrids of commercial orchid genera like *Ascocentrum*, *Cattleya, Cymbidium, Dendrobium, Mokara, Oncidium, Paphiopedilum, Phaius, Phalaenopsis,* and *Vanda* and their compatible alliances, which would be market-driven, having export value as well as being tolerant to biotic and abiotic stresses. Investigations on the morphological diversity of indigenous species could open up avenues for the identification of new and elite germplasm for pot culture, cut flowers, dry flowers, herbal preparations, and exhibits for market displays.

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RESEARCH ARTICLE

Documentation of Exploration, Collection and Extraction of *Chironji* (*Buchanania lanzan* Spreng.): A Potential Nut of Bundelkhand Region, India

DP Semwal*, OP Dhariwal and PK Singh*

Abstract

Almondette (*Buchanania lanzan* Spreng.), locally known as "*Chironji*," is a dry fruit traditionally used for culinary and medicinal purposes by local communities and tribal groups in the Bundelkhand region of India. This species thrives in various habitats within Bundelkhand and several states in central India. Local communities and tribal groups in the area continue to maintain traditional knowledge regarding the extraction of kernels from *Chironji* nuts. During an exploration conducted in May-June 2024, we documented a traditional practice of kernel extraction with the assistance of local informants. Our visit to Bijawar village in Chhatarpur district, Madhya Pradesh, revealed that the locals have traditionally employed old decorticating tools called «Chhanna,» made of iron, to process *Chironji* nuts. After processing and grading, the *Chironji* kernels are sold in the local market at a high price, ranging from ₹1,800 to ₹2,200 per kilogram. *Chironji* is facing a severe threat to its existence due to extensive exploitation and needs to be protected in *in-situ* conservation areas for sustainable utilization.

Keywords: Bundelkhand region, *Chironji*, Economic Potential, Traditional seed processing.

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Introduction

The Buchanania lanzan Spreng., locally known as '*Chironji*', *Achar'*, "*Char', 'Charoli',* and *'Chawar',* is a potential minor fruit tree species belonging to the angiosperm family Anacardiaceae (Mabberley, 2017). Internationally, it is commonly known as the Chirauli nut, Chirauli nut tree, or almondette in French. In southern India, it is also known as Calumpang nut, Cuddapah almond, and is used as an alternative to almonds, especially in Shrikhand sweetmeats (Wiersema and Leon, 2013; Mabberley, 2017). It is a highly heterozygous and cross-pollinated species (Malik *et al.,* 2010). The Indian subcontinent is recognized as the primary centre of origin of *Chironji* (Zeven and de Wet, 1982; Pradheep *et al.,* 2014). In India, the species is distributed across tropical dry deciduous forests in Andhra Pradesh, Bihar, Chhattisgarh, Gujarat, Jharkhand, Madhya Pradesh, Maharashtra, Rajasthan, Uttar Pradesh and ascends up to 1300 m elevation in the sub-Himalayan tract with its sparse presence in Uttarakhand (Shetty and Singh, 1987; Chandra and Mukherjee, 2000).

Chironji has significant potential as a tree component in agro-forestry and social forestry systems in India. It plays an important role in the development of wastelands and dry-land horticulture due to its diverse uses and ability to withstand harsh climatic conditions. Currently, it grows wild in forested areas as a minor potential fruit species, providing economic benefits to tribal communities in specific regions. Therefore, it serves as a valuable

resource and a boon for these communities (Singh, 1982; Singh *et al.,* 2006).

Medicinal plants have been utilized since ancient times and are crucial in generating foreign exchange through international trade (Singh *et al.,* 2006). Non-timber forest products (NTFPs) account for 70% of India's forest product exports. The demand for phyto-chemicals is expected to increase, creating new trade opportunities (Kumar *et al.,* 2013). *Chironji*, an important source of raw materials for various traditional health practices, including Unani, Ayurveda, Homeopathy, Siddha, Sowa-Rigpa, and Naturopathy, is widely used in community-specific folk medicine and by local medical practitioners (Kumar *et al.,* 2013). In Andhra Pradesh and Telangana, its gum is dissolved in cow's milk and used to treat rheumatic pain (Wealth of India, 1988).

Although the distribution of this species occurs in ten states, detailed information on its area and production in India is not readily available. It is primarily cultivated in limited regions such as Jhansi, Lalitpur, Banda, and Chitrakoot in Uttar Pradesh, as well as Chhatarpur, Tikamgarh, and Panna districts in Madhya Pradesh. Production is mostly concentrated in drier areas, where villagers and tribal communities collect and sell the nuts and them in local markets. The kernels are highly nutritious, containing 25 to 30% protein, and are used as a dry fruit in various food products (Singh *et al.,* 2006). *Chironji* has significant potential for inclusion in dry-land horticulture and can be utilized to cover arid and semi-arid areas, resource-poor regions, and rangeland.

Traditionally, the methods for collecting and extracting seeds vary among different crops or fruit species. Kumar *et al.* (2012) mentioned the collection and extraction of *Chironji* seeds. Pamarthi *et al.* (2022) noted that the seeds of *Arivela viscosa* are traditionally extracted and cleaned using locally made winnowers. Dangi *et al.* (2021) have reported the morphological characterization of sweet cherry (*Prunus avium* L.), and Srivastava (2022) has provided a detailed discussion on the management of horticultural genetic resources in India. Scented Amber is collected from silver oak trees (*Grevillea robusta* A.Cunn. ex R.Br., Family Proteaceae) traditionally in Chhattisgarh and Odisha for making dhoop sticks and incense sticks.

Materials and Methods

Study sites

Following a comprehensive gap analysis of the collected germplasm, seven districts in central India were identified for the exploration and collection of minor fruit species, including *Buchanania lanzan* and several others (*Cordia myxa, Embelica officinalis, Feronia limonia, Syzygium cumini, Ziziphus mauritiana,* etc.). The selected districts included Jhansi, Lalitpur, Banda, and Chitrakoot in Uttar Pradesh, along with Chhatarpur, Tikamgarh, and Panna in Madhya Pradesh (Fig. 1). The exploration and germplasm collection activities were conducted between May and June 2024.

Survey and information gathering

The primary objective of this study was to document indigenous knowledge related to *Chironji* by gathering information from local communities through surveys and direct interactions. This included recording the local names of the plant and its various parts, along with their traditional uses. Data were collected using a standardized questionnaire developed by Bhatt and Shah (2016). To ensure comprehensive coverage, we engaged with elderly

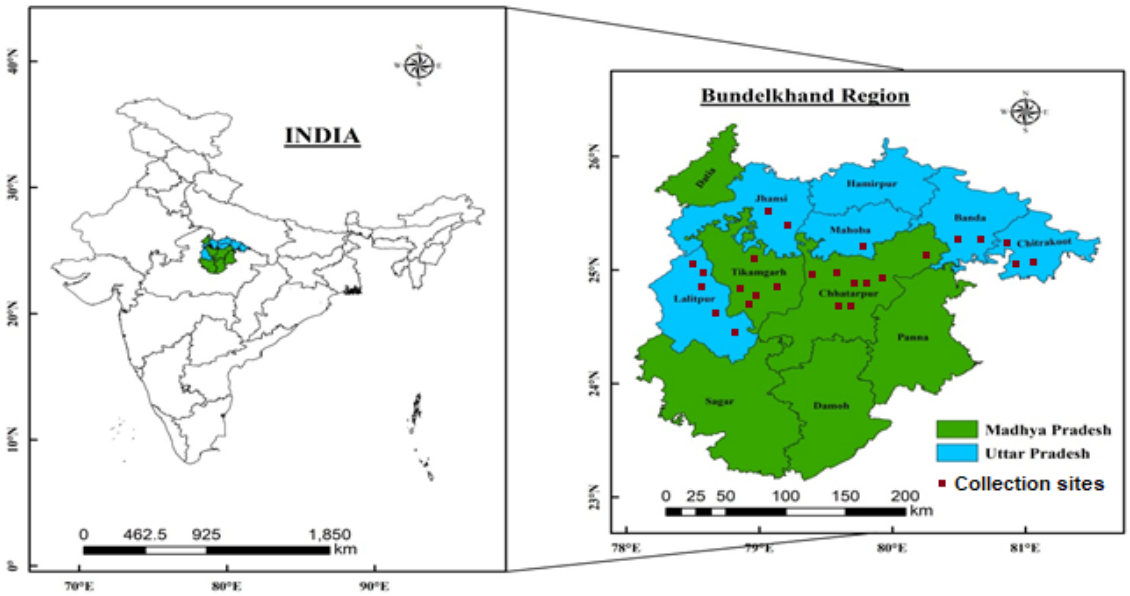

Fig. 1: Germplasm collection sites in the Bundelkhand region, covering districts in Madhya Pradesh and Uttar Pradesh, India

and knowledgeable individuals, including men and women, as well as traditional medicine practitioners. Additionally, relevant literature was reviewed to document the common and vernacular names of the plant and its ethnobotanical uses. A herbarium specimen (HS-26338) was prepared and deposited at the National Herbarium of Cultivated Plants (NHCP), ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR), New Delhi (NHCP Database, 2024).

Results and Discussion

Exploration and germplasm collection

A total of 289 accessions of *Chironji*, collected from various regions of India, are conserved in the National Genebank at ICAR-NBPGR, New Delhi (NGB Database, 2025). During an exploration mission conducted from May 30 to June 5, 2024, we successfully collected several minor and rare fruit species, including *B. lanzan* (documented accessions: IC653432, IC653434, IC653435, and IC653436). This exploration took place during an extreme summer, as temperatures reached the highest in the past fifty years. Natural populations of *Chironji* were mainly found in forests, marginal lands, and the field bunds of cultivated areas in Lalitpur, Tikamgarh, and Chhatarpur.

Harvesting, drying and processing of nuts

Fruit harvesting primarily occurs from April to June. The fruits are harvested manually using locally available cord and wood stairs, with small twigs being dragged from the branches (Fig. 2). Tribal inhabitants use baskets of various sizes to collect *Chironji* fruits, which are later stored in large earthen pots. However, it was observed that unorganized harvesting practices, coupled with the frequent cutting of *B. lanzan* tree branches to gather fruits from the natural habitats, contribute significantly to genetic erosion. After consuming the flesh of mature fruits, the nuts are sun-dried (Fig. 2). These dried nuts, locally known as '*Guthali*', are either broken manually or, when available in large quantities, taken to traditional Chhanna (decorticating tools), where the nuts are mechanically split to extract the kernels. Ripe fruits fetch a price of ₹200–300/kg in the local market, while the processed seed kernels were sold at the rate of ₹1,800 to 2,200/kg in the local mandi (unorganized village market). Approximately 70% of local producers sell their harvest at the farm level to local vendors, retailers, and pre-harvest contractors. Some people continue to use traditional methods for extracting the kernel from the nut (Fig. 3).

The *Chironji* nuts are notably hard to crack, and traditionally, dried nuts were broken by rubbing them between stones to separate the kernels from the hulls. During the exploration, an innovative method of kernel extraction was observed in the village of Bijawar, located in Bijawar Tehsil of Chhatarpur district, Madhya Pradesh. In discussions with residents, Mr. Kamlesh Sahoo shared

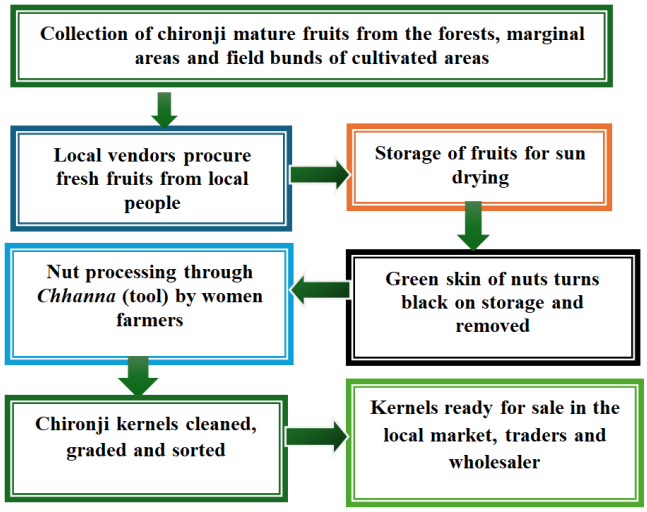

Fig. 2: Flow chart illustrating the processing steps of Chironji nuts

Fig. 3: (A-B) *Chironji* tree in natural habitat; (C) A woman selling *Chironji* fruits in local market place; (D) Close-up view of raw and mature fruits; (E) Display of washed seed; (F) Putting nuts on Chhanna; (G-H) Weighing and separation of kernels; (I) Grading of processed kernels; and (J) *Chironji* kernels ready for sale

insights into a traditional decorticating technique using a tool known as Chhanna. He explained that this method of processing *Chironji* nuts has been practiced in his family for nearly 60–70 years, originally started by his father to sustain their livelihood. In this method, *Chironji* seeds are filled into bags of varying sizes (20, 40, and 50 kg), and both male and female farmers participate in the extraction process using the Chhanna tool (Fig. 3). Each worker, particularly women, has a defined role in the process. The nuts are initially placed in a basket and then fed into a mechanical device that separates the kernels from the hulls, with the kernels collected in steel tubs (Fig. 3). The extracted kernels often contain a mixture of seed coats and shell fragments, which are then handed over to women farmers for final manual sorting. Once cleaned, the sorted kernels are packed and prepared for sale in the local market.

Traditional uses of Chironji in the Bundelkhand region

Chironji possesses significant potential due to its wide range of applications, with various parts of the plant, including roots, leaves, fruits, seeds, and gum, being used for medicinal and cosmetic purposes (Table 1). Among Gujarat's tribal communities, the kernels are traditionally consumed as a brain tonic. An ointment prepared from the kernels is applied to relieve skin itchiness and reduce facial blemishes. The plant's root extracts serve as an expectorant and are also used in the treatment of blood disorders and biliousness. Crushed leaves or leaf powder are commonly applied to wounds as a natural dressing. The seed kernel of *Buchanania lanzan* is an important food source for Bundelkhand region local people. The seed kernel of *Hodgsonia heteroclita* (locally known as Kathai) is an important food source for tribal populations in India's North-Eastern Hill (NEH) region (Semwal *et al.,* 2014). The oil of the kernel also serves as an effective substitute for almond and olive oils (Table 1).

Establishment of in-situ conservation areas

The native wild population of *Chironji* has been under constant pressure for decades due to the absence of standardized management practices and the high market demand for fresh fruits and seed kernels. This has led to extensive exploitation of the species in the wild. Consequently, the genetic resources of *B. lanzan*

Table 1: Documentation of major uses of *Chironji* in India

Major uses	*References*
Food	
The oily kernels are used in the preparation of puddings.	Singh, 1982
The mesocarp of the fruit is edible and particularly enjoyed by children.	Munde *et al.,* 2003
The oil is non-repellent, non-toxic, and suitable for human consumption.	Banerjee and Jain, 1988
Chironji kernels contain 34 to 47% fatty oil.	Rajput *et al.,* 2018
Seed kernel and extracted kernel oil are used for the preparation of several Indian dishes.	Malik *et al.,* 2012
The flesh of ripe *Chironji* fruit is highly palatable and is commonly eaten raw or roasted.	Present study
Local communities prepare refreshing juice from the pulp of *Chironji* fruits.	
Popular dishes *viz.,* halwa and kheer made with *Chironji* kernels.	
Local people use the seeds to prepare "*Chironji* ki burfi.»	
Medicinal	
Treating burns, blood disorders, asthma, diarrhoea, dysentery, fever, ulcers, bodily sensations, and snakebites.	Rajput *et al.,* 2018
In Andhra Pradesh, the gum dissolved in cow's milk is consumed to alleviate rheumatic pains.	Wealth of India, 1988
Extract of the root is also used as an expectorant and for curing biliousness and blood diseases.	Rajput *et al.,* 2018
The gum after mixing with goat milk is used as an analgesic.	Pandey, 1985
Bark or leaf paste of *B. lanzan* and *Diospyros melanoxylon* mixed with a glass of water is given twice daily to treat snakebite.	Shukla *et al.,* 2001
Gum from the cut-bark is soluble in water and used internally for the treatment of intercostals pain and diarrhoea.	Rajput *et al.,* 2018
Other uses	
Chironji provides quality timber for various purposes.	Singh *et al.,* 2010
Varnish is extracted from bark of *Chironji* and used in tanning in Kerala.	

face a significant threat in their natural habitat. These threats have caused a scattered species distribution, low natural regeneration rates, and unsustainable harvesting practices. Local and tribal communities often cut small and large branches of the trees to collect wild fruits for personal consumption or to sustain their livelihoods, further aggravating the decline in *Chironji* diversity.

The genotypic composition of germplasm conserved in the National Genebank (NGB) remains static, whereas global efforts are now shifting towards dynamic conservation methods, such as *in-situ conservation*. *These methods allow* for adaptation to changing climates and the continuation of natural evolutionary processes. Given the high market demand for *Chironji* kernels and the immediate threat to its natural populations, there is an urgent need to identify and establish *in-situ* conservation areas in the Bundelkhand region and other states. This approach will help safeguard this minor yet potentially fruit species before they are lost forever. Additionally, model nurseries should be developed to provide high-quality planting material and agrotechniques suited to diversified farming systems. Efforts should focus on standardizing high-density planting and canopy management under various tropical semi-arid ecosystems across the country.

ICAR-NBPGR has established the first *Citrus Gene Sanctuary* in 1981 in the Garo Hills of Meghalaya, encompassing a vast area of 10,266 hectares (Singh, 1981) and recently, the institute has identified a wild rice (*Oryza rufipogon*) habitat in Borjuli, Tezpur, Assam and a natural stand of guava (*Psidium guajava* L.) in the East Ramganga River Valley, Pithoragarh district, Uttarakhand, as *in-situ* conservation sites (Semwal *et al.,* 2024). These initiatives highlight the Institute's capability and know-how in establishing *in-situ* conservation areas in different parts of the country. Such a conservation site may also be located for *Chironji*.

Conclusion

The tremendous potential of *Chironji* has been documented, owing to its significant food, medicinal and industrial value. The indigenous communities have preserved a wealth of traditional knowledge about their collection, usage, and value addition since time immemorial. However, due to a narrow distribution range, over-exploitation, indiscriminate harvesting (including lopping and branch cutting), and low natural regeneration have seriously threatened its survival. There is an urgent need to implement protective measures, like the establishment of *in-situ* conservation areas, *on-farm* conservation sites and habitat management areas for *Chironji,* on priority. Such initiatives would ensure sustainable livelihood security for local communities while conserving this valuable species.

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RESEARCH ARTICLE

Physio-biochemical Evaluation of Mulberry (*Morus* Spp.) Genotypes under High Alkaline Soil for Identification of Stress-Tolerant Genotypes

Thulasy Gayathri*, Subrahmaniam Gandhi Doss, Tanmoy Sarkar, Melur Kodandaram Raghunath and Pankaj Tewary

Abstract

Mulberry (*Morus* spp.) leaf yield and quality are the major factors in sericulture since mulberry foliage is the only source of food for the silkworm (*Bombyx mori* L.). High soil alkalinity will adversely affect the plant's physio-biochemical processes such as nutrient uptake, chlorophyll biosynthesis and photosynthesis, cell membrane integrity, etc. About 21 mulberry genotypes were evaluated under high alkaline soil conditions and estimated chlorophyll, photosynthetic rate and measured activities of antioxidant enzymes (SOD and POX) as well as quantified accumulation of non-enzymatic antioxidants (ascorbic acid, reduced glutathione and phenols) and osmolyte (proline). Based on morphological and physio-biochemical responses of the plants during alkalinity stress for a period of 120 days, four genotypes *viz.,* Sahana (MI 0524), Bheria dangi-1 (MI 0822), T-36 (MI 0226), and Kanthaloor-2 (MI 0449) highly tolerant to soil alkalinity stress (pH> 9.0) were identified in the study along with adaptive responses /traits for future crop improvements in mulberry.

Keywords: Alkalinity, Antioxidants, Adaptive traits, Crop improvement, Mulberry, Osmolyte.

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Introduction

Growth and development of the silkworm, *Bombyx mori* (L.), solely depend on the mulberry leaf quality. Mulberry leaf yield and quality may largely depend on various environmental factors such as temperature, availability of nutrients, soil moisture content and rainfall and soil pH, relative humidity and other soil parameters. In India, the majority of cultivatable areas are under semi-irrigated or rain-fed conditions and soil alkalinity is a major problem in these areas due to poor rainfall and the presence of soluble salts in the soil. In India, around 7.421 million hectares of land are affected by salinity and alkalinity (Sharma *et al*., 1998). Large areas under semi-irrigated conditions exhibit alkalinity in India due to poor rainfall and poor quality of irrigation water. In India, 3.79 million hectares of area are affected by alkalinity, of which 1.48 lakh hectares are in Karnataka. Salt-affected soils have been categorized into three different types: saline, saline-alkali and alkaline soils, based on total concentration of soluble salts (electrical conductivity), soil pH and exchangeable sodium percentage (ESP). Sodic/alkaline soil is remarkable by high pH, i.e., more than 8.5 or 9.0, exchangeable sodium percentage is greater than 15 and electrical conductivity is less than 4 mmhos/cm. Accumulation of high concentrations of alkaline salts such as $NaHCO_3$ and Na_2CO_3 in the soil results in increased pH and the presence of these salts in excess amounts may cause damage to the plants by ionic toxicity, especially by sodium toxicity. The main problem in salt-affected soils is nutrient deficiency due to the changes in availability, absorption and transport of nutrients

within the plants as a result of salt stress (Munns and Tester, 2008). Availability of nutrients in soil is generally affected by physicochemical properties of the soil such as structure, texture, moisture content, temperature, pH and availability of nutrients (Meena *et al.,* 2017).

High alkaline conditions can adversely affect the plant's physio-biochemical processes such as nutrient uptake, chlorophyll biosynthesis and photosynthesis, cell membrane integrity etc. It will also lead to the production of reactive oxygen species (ROS) and cellular damage. In mulberry, maximum availability of major nutrients is in the range of soil pH 6.5-7.5 and the availability of micronutrients is more in the acidic range than in neutral or alkaline pH (Dandin and Giridhar, 2014). High pH also causes significant damage to the roots, which makes sprouting difficult and if sprouted, then the growth is very much retarded or stunted. Alkalinity causes decreased leaf area and, therefore, during stress, a considerable reduction in the area of energy captured and it decreases the fixing of CO_2/unit area also. Therefore, a low rate of carbon assimilation due to alkalinity and osmotic stress leads to partial stomatal closure, which in turn causes a decrease in stomatal conductance and transpiration. The rate of photosynthesis will also be reduced significantly under alkaline soil (Yang *et al*., 2007).

Plants generally adapt to high pH by accumulating micromolecule metabolites with buffering function, such as organic acid, proline, glycine betaine, etc., for adjusting the internal pH value. Adjusting pH value is a process of consuming energy, which may decrease intracellular pH and inhibit the growth of plants simultaneously. Plants adjust to intracellular pH by maintaining the micro-environment pH around the root to keep a relative balance of metabolism and ions. Plants can adjust pH in the micro-environment outside roots by excreting H^+, organic acid and discharging CO_2 through respiration (Wang *et al.,* 2009). Intracellular pH can be adjusted mainly by accumulating organic acid largely in vacuoles (Shi and Sheng, 2005). The large accumulation of organic acids in the cells of oat seedlings under alkaline stress can compensate for water and adjust intracellular pH. Therefore, it was stated that accumulating organic acids in large amounts was a physiological response mechanism of most plants during alkaline stress.

In the present study, 21 short-listed mulberry genotypes were evaluated under high alkaline soil (pH>9.0), and various physio-biochemical parameters were recorded during the stress period. Chlorophyll content, photosynthetic rate and accumulation of antioxidants were estimated in these genotypes and identified tolerant genotypes and physio-biochemical adaptive traits for alkalinity stress tolerance in mulberry. The information and data generated under this study could be utilized for crop improvement programmes in mulberry. The four alkaline-tolerant genotypes were identified and these genotypes can be utilized as resource materials (parents) for developing stress tolerant varieties through various plant breeding approaches.

Materials and Methods

Saplings of 21 short-listed mulberry genotypes (for low input and alkaline conditions) namely S34, AR12, Sahana, S 1635, RC1, RC2, S13, Mysore Local, K2, RFS 175, V1, Saranath-3 (MI 0764), Bheria dangi-1 (MI 0822), Kanthaloor-2 (MI 0449), T-36 (MI 0226), Pouri-2 (MI 0652), Hosur-C16 (MI 0836), Baragarh -2 (MI 0437), Chirayinkizh (MI 0762), MS-2 (MI 0027) and Madhopur-4 (MI 0670) were raised in nursery beds and after 4 months healthy saplings were transplanted to earthen pots filled with alkaline soil and soil with optimum pH (7.0-7.2, control) and maintained for conducting the experiment. Alkaline soil was collected from an alkaline hot spot area (Kinakanahalli, Karnataka) and filled in earthen pots and used for growing the experimental plants. All saplings (3 saplings per genotype) of short-listed genotypes were simultaneously evaluated under high alkaline soil with pH 9.44 and soil with optimum pH (7.0–7.2). Experimental plants, grown in alkaline soil, were irrigated with alkaline water (initially 15 days with pH 8.5 and from the 16th day onwards with pH 9.0–9.2 water) to maintain the alkaline soil pH. Alkaline water was prepared by adding $NaHCO_3$ and $NaCO_3$ to the irrigation water (10:1 for pH 8.5 and 3:1 for pH 9.2) and used for irrigating the experimental plants.

Control plants were irrigated with normal water (pH 7.5–8.0) and the irrigation frequency was once in three days. Experimental plants were evaluated under alkalinity stress continuously for a period of 120 days. Among the 21 genotypes, 4 susceptible genotypes (Baragarh -2 (MI 0437), Chirayinkizh (MI 0762), MS-2 (MI 0027) and Madhopur-4 (MI 0670) did not survive in high alkaline soil. Fresh leaves were collected from the experimental plants (control and alkaline) for biochemical analysis of antioxidants and osmolytes.

The following physio-biochemical parameters were recorded during the alkalinity stress experiments between the 60th to 75th day of the treatment.

Physiological Parameters

Chlorophyll content

Chlorophyll content was measured using a Soil Plant Analysis Development (SPAD) chlorophyll meter. Chlorophyll content was recorded in the leaves of experimental plants (3 plants per genotype and 3 readings per plant) grown in alkaline soil and the normal soil. Chlorophyll content in leaves was expressed as SPAD value.

Photosynthetic rate

Photosynthetic rate was recorded using a portable photosynthesis system LI-6400 XT (LI-COR Inc., Lincoln, NE, USA) in fully expanded leaves of all experimental plants in pots grown and maintained at alkaline soil and normal soil.

Leaf net photosynthetic rate (P_N) was expressed as µmol $m^{-2}s^{-1}$.

Biochemical Parameters

Antioxidant enzymes

- *Superoxide dismutase (SOD)*

SOD enzyme extraction and activity assay were carried out by the method described by Dhindsa *et al* (1981). SOD was extracted from the leaf tissue using 50mM potassium phosphate buffer (pH 7.8). Enzyme activity was measured by recording the decrease in absorbance of formazan produced by superoxide-nitro blue tetrazolium (NBT) complex. Absorbance was recorded at 560 nm and one unit of enzyme activity was taken as the quantity of enzyme that reduced the absorbance reading of samples by 50% in comparison with tubes lacking enzymes. SOD activity was expressed in Units g^{-1}FW.

Peroxidase (POX)

POX enzyme activity was estimated according to Putter (1974). POX enzyme extracted using 0.1M phosphate buffer (pH 7.0). Peroxidase activity was assayed using guaiacol solution as substrate. Assay mixture consisted of guaiacol solution, crude enzyme extract and H_2O_2 solution. The time required to increase the absorbance from 0.05 to 0.1 at 436 nm was noted and the enzyme activity was calculated using the extinction coefficient of guaiacol dehydrogenation product at 436 nm. Enzyme activity was expressed in Units g^{-1}FW.

Non-enzymatic antioxidants

Reduced glutathione (GSH)

The levels of reduced glutathione (GSH) were estimated by the method of Moron *et al.,* (1979). Plant tissue was homogenized in 5% TCA. The homogenate was immediately acidified by adding 25% TCA to prevent aerial oxidation of glutathione. Precipitate was centrifuged at 10,000 rpm for 10 minutes and the supernatant was used for estimation. Supernatant was diluted with 0.2 M sodium phosphate buffer (pH 8.0), followed by the addition of 2 mL of freshly prepared DTNB (5,5'-dithiobis- (2-nitrobenzoic acid) solution and the intensity of the yellow color was read at 412 nm. A standard curve was plotted using known concentrations of GSH. The quantity of reduced glutathione was expressed in mg g^{-1}FW.

Phenols

Total phenols were estimated according to Malick and Singh (1980). Phenols were extracted with 80% ethanol and quantified by the addition of Folin–Ciocalteu reagent. The absorbance was recorded at 650 nm. Total phenols were calculated using catechol as a standard and the quantity of phenolic compounds in leaves was expressed in mg g^{-1}FW.

Osmolytes

Proline

Proline was extracted with 3% sulfosalicylic acid estimated by the method of Bates *et al*. (1973). Absorbance was recorded at 520 nm using toluene as a blank. The proline concentration was determined from a standard curve and the concentration was expressed in µmol g^{-1}FW.

Statistical Analysis

One-way ANOVA (analysis of variance) and Duncan multiple range test were carried out at a significance level of $p < 0.05$ using SPSS (version 16.0; Chicago, IL, USA). Three independent experiments were done in each analysis, and the values were expressed as mean ± standard error.

Results and Discussion

Physiological Parameters

Chlorophyll (SPAD) and photosynthetic rate were recorded in experimental plants under alkalinity stress. All genotypes showed comparatively low chlorophyll (SPAD value) content in alkaline soil. In control (optimum pH), RC1 (40.90), Bheria dangi-1 (37.70), T-36 (36.43), S34 (35.93) and S1635 (34.16) showed high chlorophylls. V1, Saranath-3 and Kanthaloor-2 showed almost similar chlorophyll content. Low chlorophyll was recorded in Sahana (27.93), AR12 (30.86), S13 (31.20) and RC2 (32.16). In alkaline soil, T-36 (31.60) and AR12 (30.30) showed high chlorophyll followed by S 1635 (28.86), Bheria dangi-1 (28.30), Kanthaloor-2 (27.90, V1 (27.33), S34 (26.56), Sahana (25.96) and Saranath-3 (25.71). Low chlorophyll was observed in Pouri-2 (14.22), RC2 (15.23), S13 (17.50) and RC1 (18.16) (Table 1).

Data represented here are the mean value of replicates of three independent analyses and SE within a column, followed by the same letter, are not significantly different ($p < 0.05$) as determined by Duncan Multiple Range Test (DMRT)

The photosynthetic rate was recorded in experimental plants of both the control and the alkaline soil. In control, among the genotypes, maximum photosynthetic rate was recorded in Bheria dangi-1 and Kanthaloor-2 (16.84 µmol $m^{-2}s^{-1}$), Saranath-3 (16.23 µmol $m^{-2}s^{-1}$), Pouri-2 (15.71 µmol $m^{-2}s^{-1}$), T-36 (15.27 µmol $m^{-2}s^{-1}$), S 1635 (13.87 µmol $m^{-2}s^{-1}$) and V1 (13.78 µmol $m^{-2}s^{-1}$). Minimum photosynthetic rate was recorded in RC2, S13 and S34 (5.23-6.90 µmol $m^{-2}s^{-1}$). Under alkaline soil, all genotypes except K2 recorded a reduced photosynthetic rate. In K2, photosynthetic rate was almost same in control (10.57 µmol $m^{-2}s^{-1}$) and treatment (9.75 µmol $m^{-2}s^{-1}$). Photosynthetic rate was high in Kanthaloor-2 (12.14 µmol $m^{-2}s^{-1}$), K2 (9.75 µmol $m^{-2}s^{-1}$), S 1635 (7.74 µmol $m^{-2}s^{-1}$), T-36 (5.65 µmol $m^{-2}s^{-1}$), Bheria dangi-1 (4.35 µmol $m^{-2}s^{-1}$), Sahana (4.23 µmol $m^{-2}s^{-1}$) and AR12 (3.99 µmol $m^{-2}s^{-1}$). Low photosynthetic rate was recorded in Pouri-2 (MI 0652) (0.49 µmol $m^{-2}s^{-1}$),

Table 1: Variation in chlorophyll (SPAD value) content among different mulberry genotypes

Genotype	*SPAD value at Optimum pH*	*SPAD value at Alkaline pH*
S13	31.20 ± 1.30 ^{cd}	17.50 ± 2.72 ^{de}
Mysore Local	37.30 ± 0.40 ^{ab}	22.17 ± 2.79 ^{bcde}
S34	35.93 ± 4.78 ^{abc}	26.56 ± 2.93 ^{ab}
RC1	40.90 ± 1.72 ^{a}	18.16 ± 2.11 ^{cde}
RC2	32.16 ± 0.62 ^{bcd}	15.23 ± 1.50 ^{de}
AR12	30.86 ± 1.04 ^{cd}	30.30 ± 2.54 ^{ab}
V1	33.36 ± 0.75 ^{bcd}	27.33 ± 1.03 ^{ab}
K2	31.26 ± 0.77 ^{cd}	23.03 ± 3.58 ^{abcd}
S1635	34.16 ± 1.04 ^{bc}	28.86 ± 1.14 ^{ab}
Sahana	27.93 ± 0.82 ^{d}	25.96 ± 3.58 ^{abc}
Saranath-3	33.10 ± 0.75 ^{bcd}	25.71 ± 0.81 ^{abc}
Bheria dangi-1	37.70 ± 0.46 ^{ab}	28.30 ± 4.88 ^{ab}
Kanthaloor-2	33.66 ± 2.78 ^{bc}	27.90 ± 0.83 ^{ab}
T-36	36.43 ± 1.28 ^{abc}	31.60 ± 2.52^{a}
Pouri-2	34.10 ± 1.02 ^{bc}	14.22 ± 0.58 ^{e}

Mysore Local, RC2 and S13 (<2 µmol $m^{-2}s^{-1}$) (Table 2). Fully expanded healthy leaves were not present in RFS 175 and Hosur-C16 for recording of photosynthetic rate.

Data represented here are the mean value of replicates of three independent analyses and SE within a column, followed by the same letter, are not significantly different (p <0.05) as determined by Duncan Multiple Range Test (DMRT)

Biochemical Parameters

Antioxidant enzymes (superoxide dismutase and peroxidase) activities were estimated in leaves of experimental plants grown in alkaline (treatment) and normal soil (control). In control plants, maximum SOD was recorded in T-36 (45.79 Units/g), RC1 (32.46 Units/g), Kanthaloor-2 (32.16 Units/g), Hosur-C16 (25.67 Units/g), S13 (22.69 Units/g), Pouri-2 (21.45 Units/g), Bheria dangi-1 (19.88 Units/g), S1635 (19.61 Units/g), Sahana (18.99 Units/g), AR12 (18.55 Units/g) and S34 (18.35 Units/g). Minimum enzyme activity was observed in RC2 (5.73 Units/g) and Mysore Local (8.54 Units/g). SOD enzyme activity was significantly reduced in plants grown under alkaline soil. High activity was recorded in Sahana (28.36 Units/g), T-36 (24.32 Units/g), AR12 (17.71 Units/g), RFS 175 (17.49 Units/g), S 1635 (10.95 Units/g) and Bheria dangi-1 (10.14 Units/g). RC1 (4.31 Units/g), Kanthaloor-2 (5.07 Units/g) and Pouri-2 (5.51 Units/g) showed low SOD activity. In the other seven susceptible genotypes (Mysore Local, S13, S34, RC2, V1, K2 and Hosur-C16), SOD activity is not observed in leaves under alkaline soil conditions (Fig. 1).

Peroxidase enzyme activity was estimated in the leaves of control and alkaline soil-grown experimental plants.

Table 2: Variation in the photosynthetic rate among different mulberry genotypes (P_N-Photosynthetic rate)

Genotype	*P_N at Optimum pH*	*P_N at Alkaline pH*
S13	6.86 ± 1.20 ^{ef}	1.43 ± 0.16 ^{ghi}
Mysore Local	9.47 ± 0.14 ^{def}	0.85 ± 0.41 ^{lj}
S34	6.90 ± 1.82 ^{ef}	3.47 ± 1.45 ^{efgh}
RC1	10.29 ± 1.56 ^{cde}	2.99 ± 0.85 ^{efghi}
RC2	5.23 ± 0.64 ^{f}	1.19 ± 0.40 ^{lj}
AR12	6.63 ± 0.11 ^{ef}	3.99 ± 0.54 ^{defg}
V1	13.78 ± 2.33 ^{abc}	1.43 ± 0.38 ^{hij}
K2	10.57 ± 1.56 ^{cde}	9.75 ± 1.17 ^{b}
S1635	13.87 ± 1.14 ^{abc}	7.74 ± 0.54 ^{c}
Sahana	11.37 ± 0.58 ^{bcd}	4.23 ± 0.64 ^{def}
Saranath-3	16.23 ± 1.47 ^{a}	2.01 ± 0.31 ^{ghij}
Bheria dangi-1	16.84 ± 0.69^{a}	4.35 ± 0.51 ^{de}
Kanthaloor-2	16.84 ± 1.48^{a}	12.14 ± 0.33 ^{a}
T-36	15.27 ± 2.27 ^{ab}	5.65 ± 0.57 ^{d}
Pouri-2	15.71 ± 1.27 ^{ab}	0.49 ± 0.22 ^{j}

Results of the enzyme assay indicated that in control plants. In control plants, high peroxidase activity was recorded in RC1 (51.89 Units/g), followed by V1 (49.56 Units/g), S34 (48.48 Units/g), Sahana (40.59 Units/g), S13 (25.61 Units/g), T-36 (24.25 Units/g), Mysore Local (20.44 Units/g), AR12 (18.12 Units/g), and Bheria dangi-1 (16.38 Units/g). Low enzyme activity as observed in Pouri-2 (3.55 Unit/g), RC2 (5.06 Units/g), S 1635 (6.33 Units/g) and RFS 175 (7.19 Units/g). Under alkaline soil, all genotypes exhibited a low enzyme activity and among the genotypes, higher peroxidase activity was recorded in Bheria dangi-1 (6.65 Units/g), Sahana (5.84 Units/g), AR12 (4.5 Units/g) and K2 (2.3 Units/g). All other genotypes showed a least enzyme activity (<2 Units/g). In RC2 and S 1635, no enzyme activity was detected during the activity assay (Fig. 2).

Non-enzymatic antioxidants, phenols and reduced glutathione were estimated in all experimental plants and results indicated significant variation in the accumulation of these compounds under control and alkalinity stress conditions. In control plants, high phenol content was recorded in T-36 (36.36 mg/g), Sahana (29.76 mg/g), Pouri-2 and RFS 175 (25.50 mg/g), Bheria dangi-1 (24.66 mg/g), Kanthaloor-2 (20.6 mg/g), Hosur-C16 (19.83 mg/g) and AR12 (19.16 mg/g). Low phenols were observed in S13 (13.23 mg/g), RC2 (13.36 mg/g), S34 (14.23 mg/g) and Mysore Local (15.26 mg/g). Under alkaline soil, higher accumulation of phenol was recorded in T-36 (29.73 mg/g), Sahana (28.06 mg/g), Bheria dangi-1 (22.63 mg/g), Pouri-2 (21.16 mg/g), Hosur C16 (18.63 mg/g) and RFS 175 (18.36 mg/g). Mysore Local (11.26 mg/g), S13 (11.33 mg/g), S34 (12.06 mg/g) and RC1 (14.16 mg/g) showed the least phenol content (Fig. 3).

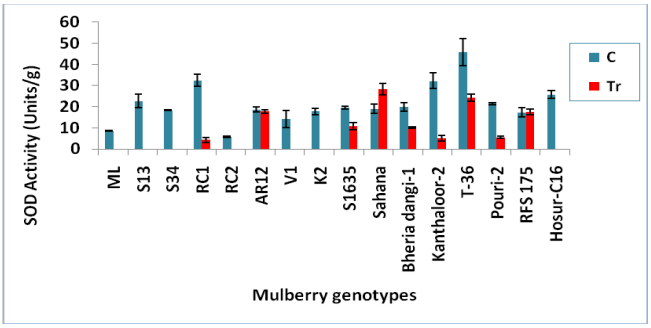

Fig. 1: SOD enzyme activity profile among different mulberry genotypes C- control, T-Treatment (high pH)

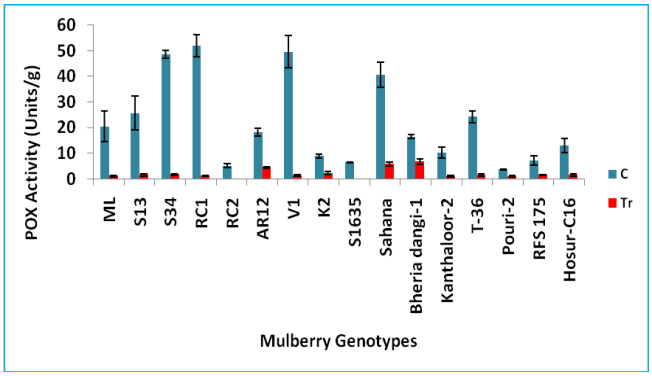

Fig. 2: POX enzyme activity profile among different mulberry genotypes. C- control, T-Treatment (high pH)

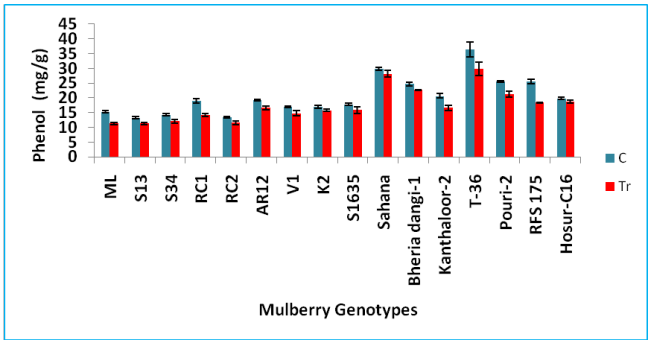

Fig. 3: Variation in quantity of phenols in different mulberry genotypes. C- control, T-Treatment (high pH)

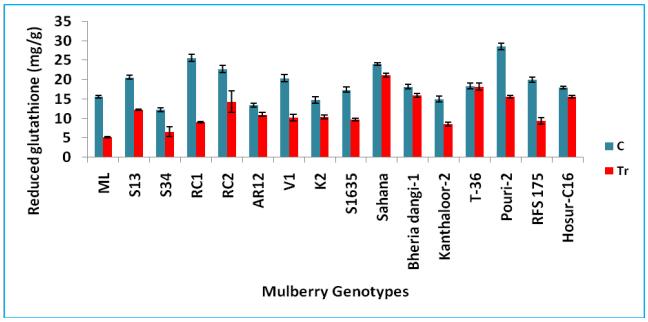

Fig. 4: Variation in quantity of reduced glutathione in different mulberry genotypes. C- control, T-Treatment (high pH).

Reduced glutathione (GSH) was estimated in all genotypes and the results indicated that in control plants, high reduced glutathione content was recorded in Pouri-2 (28.20 mg/g), RC1 (25.6 mg/g), Sahana (24.0 mg/g), RC2 (22.80 mg/g), S13 (20.60 mg/g), V1 (20.40 mg/g) and RFS 175 (20.0 mg/g). Low GSH was observed in S34 (12.20 mg/g), AR12 (13.40 mg/g), K2 (14.80 mg/g), Kanthaloor-2 (15.0 mg/g) and Mysore Local (15.60 mg/g). Under alkaline soil, maximum GSH content was observed in Sahana (21.20 mg/g), T-36 (18.20 mg/g), Pouri-2 and Hosur C16 (15.60 mg/g) and RC2 (14.33 mg/g). Minimum GSH was recorded in Mysore Local (5.14 mg/g), S34 (6.60 mg/g) and Kanthaloor-2 (8.60 mg/g). In other genotypes, it ranged from 9.05 to 12.20 mg/g. In genotypes T-36 (18.40 and 18.20 mg/g), Sahana (24.0 and 21.20 mg/g) and Bheria dangi-1 (18.20 and 16.0 mg/g), the GSH accumulation was almost the same in control and alkalinity stress conditions (Fig. 4).

Accumulation of osmolyte, proline, was estimated in experimental plants grown under optimum pH soil and alkaline soil during the experiment. Under optimal soil pH conditions, high proline accumulation was observed in Bheria dangi-1 (72.58 µmol/g), Sahana (40.45 µmol/g), K2 (39.28 µmol/g), T-36 (35.94 µmol/g), Kanthaloor-2 (34.61 µmol/g) and S1635 (33.97 µmol/g). Low proline was recorded in Mysore Local (9.44 µmol/g) and AR12 (10.82 µmol/g). Under alkaline soil, higher proline accumulation was recorded in Bheria dangi-1 (65.70 µmol/g), Sahana (39.60 µmol/g), Kanthaloor-2 (32.66 µmol/g), RFS 175 (26.48 µmol/g), T-36 (17.49 µmol/g) and V1 (17.06 µmol/g). Low accumulation of proline was observed in Mysore Local (4.88 µmol/g), Hosur C16 (10.82 µmol/g) and S13 (10.13 µmol/g) (Fig. 5).

Physiological parameters such as chlorophyll (SPAD) and photosynthetic rate were recorded in experimental plants grown under optimal soil pH and high alkaline soil. All genotypes showed a reduction in chlorophyll (SPAD value) in alkaline soil. The photosynthetic rate was significantly reduced in plants grown under alkaline soil. However, the tolerant genotypes, T-36 (31.60) and AR12 (30.30), S 1635 (28.86), Bheria dangi-1 (28.30), Kanthaloor-2 (27.90), V1 (27.33), S34 (26.56) and Sahana (25.96) showed high chlorophyll content. In susceptible genotypes such as Pouri-2, RC2, S13 and RC1, chlorophyll content was reduced under alkalinity soil.

Earlier reports confirmed that the photosynthetic rate of a plant decreases with increasing saline stress intensity (Yang *et al*., 2009a,b), and it has been reported that alkaline stress leads to limited photosynthesis in barley (Yang *et al*., 2009a). The present study also reported the low photosynthetic rate in susceptible genotypes and a comparatively higher photosynthetic rate in tolerant genotypes. High rate of photosynthesis was recorded in tolerant genotypes such as Kanthaloor-2, K2, S1635, T-36, AR12 and S34.

In high alkaline soil, deficiency of other nutrients will be observed in the soil due to the high concentration of

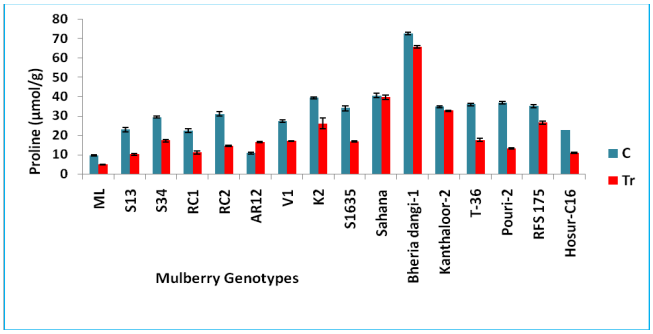

Fig. 5: Variation in accumulation of proline in different mulberry genotypes. C- control, T-Treatment (high pH)

Na^+ and other environmental factors, such as drought, which exacerbate this problem (Silberbush and Ben-Asher, 2001). Besides this, high Na^+ hampers the uptake of other nutrients by: (1) Na^+ interfering with transporters in the root plasma membrane, such as K^+-selective ion channels, and (2) reduction of root growth by high Na^+ concentration (Tester and Davenport, 2003). Thus, the uptake of water and growth-limiting nutrients (such as P, Fe, or Zn) and the growth of soil microorganisms, such as mycorrhizal fungi, can be inhibited and which leads to the stunted growth of the plant as observed in susceptible genotypes.

Normal growth, development, and physiological and biochemical metabolism of plants are severely disrupted during alkalinity stress conditions. The high concentration of sodium ions in the soil under saline-alkali stress can disrupt the dynamic balance of ions in cells, leading to a series of damaging effects on plants, such as destruction of the cell membrane structure, abnormal metabolons in cells and ionic toxicity (Hasegawa, 2013). Plants alleviate the toxicity of sodium ions mainly through excreting sodium ions from cells and sequestering them through ion antiporters such as NHX7 (also named SOS1) within the cell membrane and NHX1 within the vacuolar membrane, both of whose activity is regulated by calcium-dependent SOS2/SOS3 kinase complexes (Bahmani *et al.,* 2015).

Osmotic stress and ionic stress caused by saline-alkali stress lead to the generation of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH). These ROS accumulated in the cell during the stress will disrupt the normal physiological functions of cells and which may result in metabolic disorders. Generally, well-developed ROS scavenging systems were identified in tolerant plants, including antioxidant enzymes and antioxidants for reducing the oxidative damage of ROS in plants (Shumei Fang *et al*., 2021). To cope with these adverse stress conditions, plant cells synthesize and accumulate several small-molecule organic compounds, such as proline, soluble proteins, betaine, sugar, polyols and polyamines, to maintain intracellular water potential (Sun *et al*., 2019).

In tolerant plants, a well-organized antioxidant system was developed for mitigating the effects of reactive oxygen species (ROS) generated during the stress conditions. The present study also indicated that the antioxidant enzymes (SOD and POX) showed comparatively higher activities in tolerant genotypes. A recent study in mulberry under optimal growth conditions confirmed that antioxidant enzyme activities were varied among different genotypes and it was higher in stress-tolerant genotypes (Gayathri *et al*., 2022). SOD activity (17.49-28.36 Units/g) was high in genotypes like Sahana, T-36, AR12 and RFS 175. Higher POX activity (4.50- 6.65 Units/g) was recorded in Bheria dangi-1, Sahana and AR-12. SOD is the first line of defense of the antioxidant system in plants and can transform accumulated superoxide molecules into oxygen and H_2O_2, after which catalase, ascorbate peroxidase and peroxidase convert H_2O_2 into water and oxygen. In addition, these enzymes work together to scavenge MDA produced from lipid peroxidation to protect the membrane structure.

Non-enzymatic antioxidants also play an important role in stress tolerance response in higher plants. These antioxidants include mainly glutathione (GSH), ascorbic acid (ASA), mannitol, flavonoids, anthocyanins and vitamin E. These compounds are distributed in different parts of cells to regulate the balance of ROS in cells. Increased levels of these foliar antioxidants were reported during drought stress in mulberry (Guha *et al*., 2011). Antioxidant phenol was estimated in plants grown under alkalinity stress conditions and it was found that high quantity of phenol was recorded in T-36 (29.73 mg), Sahana (28.06 mg) and Bheria dangi-1 (22.63 mg). Low quantity was observed in susceptible genotypes (11.26-11.53 mg) such as Mysore Local, S13 and RC2. Reduced glutathione content was also high in these tolerant genotypes (18.20–21.20 mg) and least in the susceptible one (514–6.60 mg).

Alkalinity stress experiments indicated that in tolerant genotypes, there was a remarkable increase in the foliar antioxidants and compatible solutes (proline), which appear to play a major role in scavenging the reactive oxygen species generated during the stress. Changes in the accumulation of proline and glycine betaine, along with the activities of antioxidant enzymes such as peroxidase, catalase, glutathione reductase, were reported earlier (Ahmed *et al*., 2014) in two mulberry cultivars (S146 and Sujanpur) under sodium carbonate-induced alkalinity stress. This study reported the high activities of antioxidant enzymes in the tolerant cultivar S146. A comparative antioxidant enzyme activity and gene expression analysis were reported in rice (Khare *et al*., 2015) among salt-tolerant (Panvel-3) and salt-sensitive (Sahyadri-3) varieties and confirmed the high level of antioxidant enzymes in the tolerant variety. This report also indicated the additive effect of Na^+ and Cl^- ions under NaCl stress and the tolerant cultivar maintained lower Na^+/K^+ and ROS levels through an effective antioxidant mechanism. A recent study was reported in three halophytic grasses and comparative mineral nutrient analysis was carried out

under saline and alkaline stress conditions (Lata *et al*., 2022). The results of this study indicated that the Na+/K+ ratio is an important indicator of a plant's response to salt stress and low leaf Na+ concentration and low Na+/K+ can be considered as an indicator for stress tolerance.

Proline was an effective osmotic adjusting material of plants for responding to stress situations (Song *et al*., 2006; Yang *et al.,* 2008). A larger accumulation of proline represents a stronger osmotic adjusting ability and resistance to an adverse environment of plants (Yang *et al.,* 2008). Among all compatible solutes, proline and glycine betaine occur widely in higher plants and accumulate in considerable amounts in salt-stressed plants (Ahmad *et al.,* 2006; Koyro *et al.,* 2012; Ahmad *et al.,* 2012). In this study, proline and glycine betaine were increased markedly with an increase in external $NaHCO_3$ level and growth period. Relatively $NaHCO_3$-tolerant cv. S146 was superior to cv. Sujanpuri accumulates both osmoprotectants under alkaline regimes. This pattern of accumulation of the two osmoprotectants clearly shows that they could be used as potential indicators of alkalinity tolerance in mulberry, as was earlier reported in broad bean (Mohamed *et al.,* 2011), mustard (Ahmad *et al*., 2012), and chickpea (Saiema Rasool *et al*., 2012). Stress-tolerant responses in terms of grain yield and associated traits were evaluated in 102 Indian wheat cultivars under terminal heat stress conditions by Singh *et al*., (2020) and identified genotypes with stable yield performance for stress and non-stressed environments. Moisture stress tolerance studies were conducted in Desi, Kabuli and Wild chickpea accessions and confirmed the drought tolerance of the Desi variety due to its high root traits such as root area, root length and root weight. And these root traits were marked as selection indices for drought tolerance with high yield stability (Johal *et al.,* 2021).

The present study also reported the high activity of antioxidant enzymes (SOD and POX), accumulation of antioxidants, as well as proline in tolerant genotypes. Hence, the presence of these antioxidants (SOD, POX, reduced glutathione and phenols) and proline in higher quantities during the alkalinity stress could be utilized as an adaptive trait/response for alkalinity tolerance in mulberry and is useful for selecting tolerant genotypes. Alkalinity-induced symptoms such as necrosis, chlorosis, terminal bud burning, leaf senescence, stunted growth, etc. were observed regularly in the experimental plants grown under high alkaline soil. Based on physio-biochemical responses and appearance of alkalinity-induced symptoms in plant parts, four genotypes (Sahana, Bheria dangi-1, Kanthaloor-2 and T-36) were identified as tolerant to high alkalinity (pH >9).

21 short-listed mulberry genotypes were grown under high alkaline soil and recorded physio-biochemical parameters such as chlorophyll content, photosynthetic rate, activities of antioxidant enzymes and accumulation of antioxidants and osmolytes. Results of the study indicated that tolerant genotypes showed higher photosynthetic rate (>4 µmol $m^{-2}s^{-1}$) even under alkalinity stress. In susceptible genotypes, P_N was significantly reduced during the stress period (0.49 -3.47 µmol $m^{-2}s^{-1}$) and hence, photosynthetic rate can be considered as a physiological adaptive trait for identifying alkalinity stress-tolerant genotypes. Higher activities of superoxide dismutase and peroxidase enzymes were recorded in tolerant genotypes during stress conditions. Non-enzymatic antioxidants (reduced glutathione, ascorbic acids and phenols) and osmolytes (proline and glycine betaine) also exhibited an increased accumulation in tolerant genotypes (Sahana, Bheria dangi-1, Kanthaloor-2 and T-36) under alkalinity stress. Hence, these compounds can be used as biochemical indicators for identifying stress-tolerant genotypes in mulberry. Data on profiling of antioxidant enzymes and non-enzymatic antioxidants as well as the physiological parameters achieved in the study, provide useful insights into the physio-biochemical mechanism of alkalinity stress tolerance in mulberry. The present study also identified four genotypes tolerant to high alkaline soil (pH>9.0) based on physio-biochemical responses and alkalinity-induced symptoms in the leaves and plant parts under alkaline soil conditions.

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RESEARCH ARTICLE

Phenological Insights and Seed Trait Diversity in Fourteen Native Plant Species of Central India

Manish Kumar Vijay*, Shweta Tiwari, Lata Kahar, Deepandra Malviya and Neelu Singh

Abstract

Phenological events such as flowering, fruiting, and seed maturity are critical stages in the lifecycle of plants, playing a pivotal role in ecological processes and plantation programs. This study examines the phenological patterns and seed trait diversity of 14 native tree species from central India: *Buchanania cochinchinensis, Butea monosperma, Cochlospermum gossypium, Commiphora wightii, Feronia limonia, Hymenodictyon excelsum, Kydia calycina, Mallotus philippensis, Nyctanthes arbortristis, Putranjiva roxburghii, Pterospermum acerifolium, Semecarpus anacardium, Sterculia villosa,* and *Stereospermum chelonoides*. Conducted over three years (2021–2024) across diverse forested landscapes, the study identifies interspecific variation in flowering, fruiting, and seed maturation timelines. Using advanced image analysis, we quantified key morphometric parameters such as seed area, length, width, aspect ratio, average diameter, perimeter, convex perimeter, and roundness, alongside colorimetric attributes determined with the RHS color chart. The findings reveal a remarkable diversity in phenological and seed traits, offering valuable insights for ecological research, biodiversity conservation, and sustainable forest management. The outcomes of this study hold practical relevance for nursery managers, small-scale planters, and forest managers by providing critical information on fruiting periods and optimal seed collection timings for the propagation of desired tree species.

Keywords: Colorimetric analysis, Conservation biology, Ecological significance, Morphometric analysis, Phenology, Seed image analysis, Species diversity.

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Introduction

Phenological events such as flowering, fruiting, and seed maturation are critical determinants of reproductive success and long-term persistence in plant species. These events are closely regulated by environmental cues, including temperature, precipitation patterns, photoperiod, and biotic interactions (Heithaus, 1974; Frankie *et al*., 1975; Pulliam and Brand, 1975; Opler *et al*., 1976; Thompson and Willson, 1979; Stiles, 1980). In tropical and subtropical ecosystems, shifts in phenology due to climate variability can significantly alter reproductive timing, seed production, and subsequent seedling recruitment (Suresh and Sukumar, 2011).

Across Indian forest ecosystems, phenological studies have provided valuable insights into species-specific life history strategies. Research conducted in subtropical humid forests (Shukla and Ramakrishnan, 1982, 1984), Kumaun Himalayan forests (Ralhan *et al*., 1985; Pangtey *et al*., 1990), deciduous forests of Bandipur (Prasad and Hegde, 1986), and tropical moist forests of Karnataka (Bhat and Murali, 2001) has demonstrated that phenophases timing is not only taxon-specific but also responsive to microclimatic gradients and edaphic conditions.

Seed maturation represents a crucial terminal phenological stage, during which physiological and structural changes culminate in the attainment of maximum seed quality. Seeds harvested

at physiological maturity generally exhibit superior viability and vigor, which are essential for successful germination and seedling establishment (Edwards, 1980; Singh and Kachari, 2006). Morphological markers, such as fruit dehiscence, color changes, and firmness, serve as practical indicators for determining the optimal harvest window (Piña-Rodrigues and Aguiar, 1993). Tools such as the Royal Horticultural Society (RHS) Color Chart enhance the accuracy of maturity assessment and have proven particularly useful in seed quality assurance protocols (Tucker *et al*., 1991; RHS, 2015).

Seed morphometric traits, including size, shape, and surface characteristics, are important predictors of germination performance and ecological adaptability. These traits often reflect the plant's reproductive strategy and its ability to respond to local environmental pressures (Dell'Aquila, 2006; Oliveira *et al*., 2012; Amulya *et al*., 2022; Panda *et al.,* 2023). In rare, endangered, and threatened (RET) species, analysing seed variability offers insights into population-level genetic diversity and potential resilience to habitat degradation (Brus *et al*., 2011; Santana *et al*., 2013; Costa *et al*., 2016; Khumaeva *et al*., 2016). Advanced image-based phenotyping technologies now allow for rapid, non-destructive, and reproducible assessment of seed traits, enabling large-scale evaluation and supporting conservation-oriented research (Varma *et al*., 2013; Kapadia *et al*., 2017).

Central India is a biodiversity hotspot, home to a diverse assemblage of native tree species with ecological, medicinal, and socio-economic value. However, the lack of comprehensive data on the phenological behaviour and seed characteristics of these species, particularly RET taxa, hampers the development of targeted conservation and propagation strategies. Addressing this gap, the present study examines the phenological patterns and seed trait diversity of 14 native forest tree species of Central India-*Buchanania cochinchinensis*, *Butea monosperma*, *Cochlospermum gossypium*, *Commiphora wightii*, *Feronia limonia*, *Hymenodictyon excelsum*, *Kydia calycina*, *Mallotus philippensis*, *Nyctanthes arbor-tristis*, *Putranjiva roxburghii*, *Pterospermum acerifolium*, *Semecarpus anacardium*, *Sterculia villosa*, and *Stereospermum chelonoides*.

The study was conducted over four years (2020–2024) across representative forest landscapes. By integrating phenological observations with morphometric seed trait analysis, this work aims to contribute actionable knowledge for conservation planning, species restoration, and nursery-based propagation. The outcomes are expected to support forest managers, nursery practitioners, and researchers in optimizing seed collection timings, enhancing seed handling protocols, and improving the quality of planting stock for afforestation and ecological restoration initiatives.

Materials and Methods

Study area

The study was carried out in the forested regions of Central India, encompassing diverse ecological zones characterized by varying climatic conditions, soil types, and altitudinal gradients. Central India experiences a tropical climate with distinct wet and dry seasons. Summers are hot, with temperatures ranging from 30 to 45°C, while winters are mild, with temperatures between 10 and 25°C. Annual rainfall ranges from 75–150 cm, predominantly during the monsoon season (June-September), followed by a prolonged dry period. The region is predominantly covered by tropical dry deciduous forests growing on red, black, and alluvial soils of moderate fertility. Its topography includes plateaus and ranges such as the Vindhyas and Satpuras, which create localized climatic variations. Field surveys were conducted across multiple districts to document the phenological and seed trait diversity of the following 14 native tree species: *B. cochinchinensis, B. monosperma, C. gossypium, C. wightii, F. limonia, H. excelsum, K. calycina, M. philippensis, Nyctanthes arbor-tristis, P. roxburghii, P. acerifolium, S. anacardium, S. villosa,* and *S. chelonoides*. Field observations were carried out over four years (2020–2024) to record phenological events, including flowering, fruiting, and seed maturation. Data were collected monthly to capture intra- and inter-annual variations. Phenophases were identified using morphological indicators such as flower bud development, fruit color changes, and seed dehiscence.

Seed collection and morphometric analysis

Seeds were collected during their maturation phase from multiple trees across different populations to ensure genetic diversity. Maturity was assessed using morphological indicators such as fruit size and color. To ensure precision in documenting fruit and seed colors, the RHS color chart was utilized. A minimum of 50 seeds per species per population were collected for morphometric analysis. Phenological observations revealed the following patterns:

Flowering period

The flowering period varied among species.

Fruiting period

Fruiting began soon after flowering, depending on the species.

Time of mature fruit/seed collection

Mature fruit and seed collection were recorded, varying by species.

Fruit color

Mature fruit colors documented using the RHS color chart ranged

Seed color

Seed collars, also recorded with the RHS color chart

Fruit maturity period (days)

The time from fruit initiation to physiological maturity, depending on the species.

Seed image analysis

An automated image analysis system (Biovis Seed Image Analyzer PSM 3000) was used to characterize the morphometric properties of Flacourtia indica seeds. This system employs a digital camera or flat-bed scanner to capture high-resolution images. During the analysis, seed samples were evenly distributed on a specialized measuring plane, ensuring consistent image capture under optimal lighting conditions to avoid shadows and reflections. The images were processed using dedicated software, which automatically measured various morphometric parameters such as seed area, length, width, aspect ratio, average diameter, perimeter, convex perimeter, and roundness. These parameters were then statistically analysed to identify significant differences between different seed size classes. Descriptive statistics (mean and standard deviation) were calculated for all measured parameters.

Results and Discussion

Fruit and seed maturity markers

The phenological patterns of 14 tree species show diverse flowering, fruiting, and seed maturation periods, along with variations in fruit and seed color (Table 1).

Flowering and fruiting

Flowering and fruiting periods varied widely across species. Some species, such as *C. wightii* and *F. limonia*, exhibited extended flowering periods, while others, including *B. monosperma* and *C. gossypium*, had more defined, shorter periods of flowering and fruiting.

Fruit and seed colors

The fruit colors ranged from shades of violet, orange, and red to brown and yellow, with notable differences in the RHS color groups used for observation. Seed colors ranged from white and yellow to black and dark brown, varying according to the species.

Fruit maturity period

The time to reach fruit maturity ranged from 60 days (e.g., *B. monosperma*, *C. gossypium*) to 340 days (e.g., *P. acerifolium*). Species like *F. limonia* had longer maturity periods (240–260 days), while others, such as *S. villosa* had quicker maturation (70–90 days).

These observations underscore the importance of understanding species-specific reproductive cycles for effective seed collection and management in conservation programs.

Image analysis

The seed morphometric observations for the fourteen study species, using the seed image analyzer, reveal notable variations in seed size and shape across the species. The following summary outlines key seed attributes (Fig. 1; Table 2).

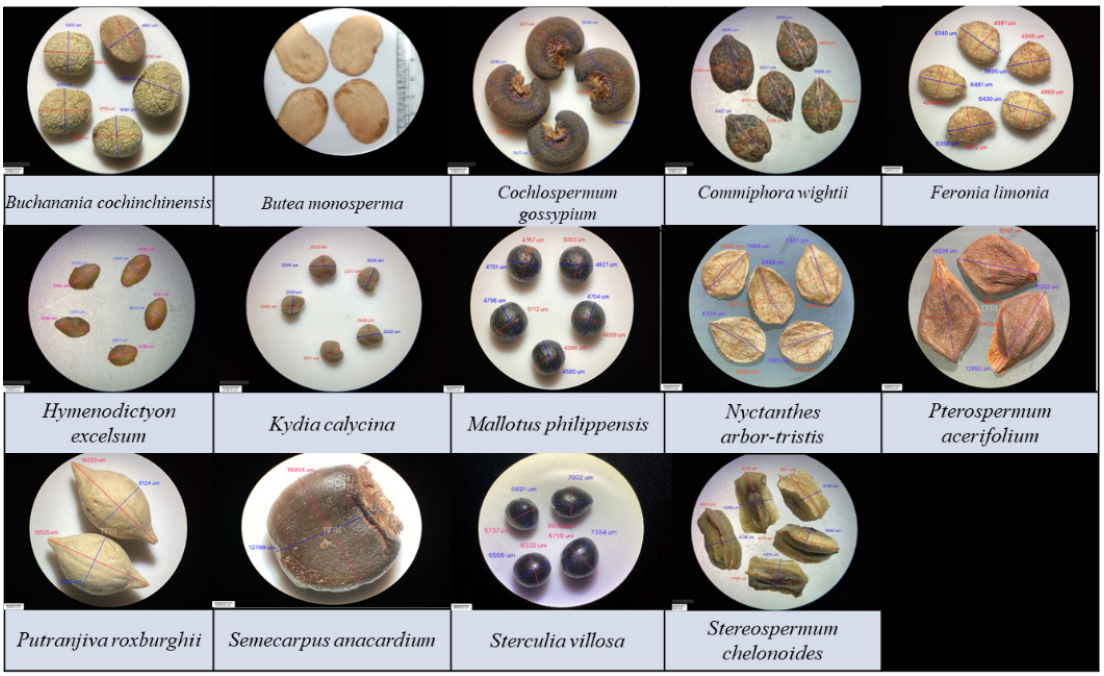

Fig. 1: Morphometric variability in seeds of the targeted plant species

Table 1: Phenological markers of fruit and seed maturity across native tree species

Species	*Flowering period*	*Fruiting period*	*Time of mature fruit/ Seed collection*	*Fruit color*	*Seed color*	*Fruit maturity period (Days)*
B. cochinchinensis (Lour.) Almei	Jan-March	April-May	May-June	Violet Blue Group N92 (Dark Greyish Purple A)	White Group155 (yellowish White A)	80–90
B. monosperma (Lamk.) Taub.	Feb-March	March-April	May-June	Greyed Orange Group 165 (Pale yellow D)	Greyed Orange Group 174 (Greyish Reddish orange B)	60–80
C. gossypium	Feb-March	March-April	April-May	Brown group (greyish reddish brown 200 B)	Brown Group 200 (Dark Greyish Reddish-Brown A)	60–80
C. wightii	Round the year	Oct-Nov	Nov-Dec, April-May	Red Purple Group 58 (Moderate purplish Red A)	Black group 203 (Black A)	90–120
F. limonia	Mar-April	Oct-Feb	Feb-March	Green white Group 157 (Pale Yellow Green B)	Greyed Orange Group 164 (Brownish Orange A)	240–260
H. excelsum	April-May	Jan-Feb	March-April	Greyed orange Group 177(Moderate Reddish Brown A)	Grayed orange Group N170 (Brownish Orange A)	140–160
K. calycina Roxb.	Aug-Oct	Sep-Dec	Jan-Feb	Greyed orange Moderate brown (165A)	Dark Greyed yellow N200A	90–110
M. philippensis (Lamk.) Mull-Arg.	Oct-Dec	Jan-Feb	Feb-March	Orange Red Group (Moderate Red N34 A)	Black group (Dark Greyish purple 202A)	140–170
Nyctanthes arbor-tristis (L.)	June-Sep	Sep-Oct	March-April	Greyed Orange Group 165 (Moderate Brown A)	Greyed brown group dark greyish yellowish (brown N199)	130–150
P. acerifolium (L.) Willd.	Mar-June	Apr-Sep	Dec-Feb	Grey brown Group 199 (Moderate Yellowish-Brown C)	Greyed Orange Group 177 (Moderate Reddish Brown A)	300–340
P. roxburghii Wall.	March-May	Feb-March	March- April	yellow group 8 Light greenish yellow C	Orange White Group159 (Pale Orange yellow C)	240–270
S. anacardium (L.) f.	April-Oct	Dec-March	April-May	Greyed Orange group N163 dark orange A	Black group 203 (Black D)	170–190
S. villosa	Aug-Sep	Oct-Nov	March-April	Red group strong reddish orange (42A)	Brown group dark greyish reddish brown (200A)	70–90
S. chelonoides	May-July	Oct-Dec	March-April	Grey brown Group 199(Light Olive Brown B)	Greyed Orange Group 164 (pale yellow D)	260–300

Area

The species with the largest seed area was *B. monosperma* (7.7255 ± 0.2913 cm^2), followed by *S. anacardium* (2.7311 ± 0.0846 cm^2), indicating that these species produce larger seeds compared to others, such as *H. orixense* (0.0080 ± 0.0253 cm^2), which has a notably small seed area.

Length and width

The longest seeds were observed in *B. monosperma* (3.8566 ± 0.0717 cm), with the widest being in *S. anacardium* (1.7920 ± 0.0264 cm). On the other hand, *H. orixense* had the smallest dimensions, both in length (0.3758 ± 0.0080 cm) and width (0.2405 ± 0.0072 cm).

Aspect ratio

The aspect ratio, indicating seed shape, was largest in *B. monosperma* (1.5097 ± 0.0338), suggesting a relatively elongated shape. In contrast, seeds of *H. orixense* and *K. calycina* had aspect ratios closer to 1, indicating more circular shapes.

Table 2: Seed morphometric observation in the targeted plant species using the seed image analyzer

Species	*Area (sq. cm)*	*Length (cm)*	*Width (cm)*	*Aspect (cm)*	*Diameter (Av cm)*	*Perimeter (cm)*	*Perimeter (Convex) (cm)*	*Roundness*
Buchanania cochinchinensis (Lour.) Almei	0.6886 ± 0.0245	1.0105 ± 0.0189	0.8954 ± 0.0187	1.1297 ± 0.0159	0.9084 ± 0.0172	3.1947 ± 0.0823	2.9693 ± 0.0515	0.8536 ± 0.0315
B. monosperma (Lamk.) Taub.	7.7255 ± 0.2913	3.8566 ± 0.0717	2.5579 ± 0.0551	1.5097 ± 0.0338	3.0974 ± 0.0591	10.8904 ± 0.2156	10.2987 ± 0.2096	0.8175 ± 0.0061
C. gossypium	0.3631 ± 0.0150	0.7337 ± 0.0192	0.6167 ± 0.0098	1.1884 ± 0.0170	0.6567 ± 0.0156	2.2492 ± 0.0574	2.1456 ± 0.0507	0.9005 ± 0.0113
C. wightii	0.2823 ± 0.0094	0.7035 ± 0.0186	0.5238 ± 0.0091	1.3467 ± 0.0424	0.5726 ± 0.0112	1.9700 ± 0.0378	1.8988 ± 0.0343	0.9123 ± 0.0084
F. limonia	0.3206 ± 0.0070	0.7864 ± 0.0117	0.5936 ± 0.0175	0.6337 ± 0.0069	0.5299 ± 0.0115	0.7738 ± 0.0122	2.3880 ± 0.0411	0.7119 ± 0.0196
H. excelsum	0.0080 ± 0.0253	0.3758 ± 0.0080	0.2405 ± 0.0072	1.5680 ± 0.0290	0.2950 ± 0.0074	1.0233 ± 0.0234	0.9763 ± 0.0228	0.8501 ± 0.0075
K. calycina Roxb.	0.0624 ± 0.0073	0.2753 ± 0.0068	0.2314 ± 0.0072	1.1953 ± 0.0185	0.2339 ± 0.0063	0.8071 ± 0.0205	1.0350 ± 0.0114	0.8648 ± 0.0191
M. philippensis (Lamk.) Mull-Arg.	0.1766 ± 0.0035	0.4838 ± 0.0063	0.4489 ± 0.0065	1.0790 ± 0.0065	0.4486 ± 0.0223	1.4988 ± 0.0048	1.4532 ± 0.0154	0.9888 ± 0.0054
Nyctanthes arbor-tristis L.	0.6067 ± 0.0205	1.0926 ± 0.0231	0.7886 ± 0.0144	1.3872 ± 0.0278	0.8713 ± 0.0138	3.2522 ± 0.1764	2.8798 ± 0.0512	0.7484 ± 0.0430
P. acerifolium (L.) Willd.	0.9766 ± 0.0287	1.5121 ± 0.0199	0.9417 ± 0.0153	1.6082 ± 0.0273	1.1098 ± 0.0165	3.9855 ± 0.0443	3.7468 ± 0.0433	0.7714 ± 0.0115
P. roxburghii Wall.	0.9713 ± 0.0454	1.4964 ± 0.0341	0.9861 ± 0.0263	1.5238 ± 0.0415	1.0951 ± 0.0247	4.5604 ± 0.1612	3.7840 ± 0.0766	0.5946 ± 0.0284
S. anacardium L. f.	2.7311 ± 0.0846	2.0854 ± 0.0414	1.7920 ± 0.0264	1.1634 ± 0.0161	1.8512 ± 0.0292	6.8140 ± 0.1660	6.0102 ± 0.0969	0.7438 ± 0.0253
S. villosa	0.3942 ± 0.0741	0.7447 ± 0.0797	0.6625 ± 0.0637	1.1240 ± 0.0516	0.6802 ± 0.0637	2.2680 ± 0.2127	-	0.9560 ± 0.2127
S. chelonoides	0.4351 ± 0.0253	0.9083 ± 0.0278	0.8202 ± 0.0266	1.1101 ± 0.0237	0.7374 ± 0.0210	2.9496 ± 0.0808	2.5441 ± 0.0729	0.6236 ± 0.0074

Diameter (average)

The species with the largest average seed diameter was *S. anacardium* (1.8512 ± 0.0292 cm), while *H. orixense* again had the smallest average diameter (0.2950 ± 0.0074 cm).

Perimeter

The species with the largest seed perimeter was *P. acerifolium* (4.5604 ± 0.1612 cm), while *H. orixense* had the smallest perimeter (1.0233 ± 0.0234 cm), correlating with its small seed size.

Convex perimeter

P. acerifolium also showed the largest convex perimeter (3.7840 ± 0.0766 cm), indicating a more irregular seed shape, while *H. orixense* again had the smallest value (0.9763 ± 0.0228 cm).

Roundness

M. philippensis had the highest roundness (0.9888 ± 0.0054), indicating nearly spherical seeds, while *P. acerifolium* exhibited the lowest roundness (0.5946 ± 0.0284), suggesting a less spherical, more elongated seed shape.

Overall, the data highlights the considerable diversity in seed size, shape, and other morphometric parameters across the fourteen species, with significant variability in seed dimensions, roundness, and perimeters. These variations may reflect different ecological adaptations or reproductive strategies within these species.

The phenological and seed morphometric data collected from the fourteen native tree species provide essential insights into their reproductive strategies and ecological adaptations, with potential implications for seed collection and conservation planning. The flowering and fruiting periods varied considerably among the species studied. For example, *C. wightii* and *F. limonia* exhibited extended flowering periods, potentially facilitating increased pollinator activity or optimized allocation of resources over time. In contrast, species like *B. monosperma* and *C. gossypium* displayed more synchronous and short flowering–fruiting cycles, which may be adaptive in seasonal environments to ensure reproductive success during limited windows of favourable conditions (Fenner, 1998).

Seed maturation periods also revealed significant interspecific variation. For instance, *F. limonia* demonstrated a longer seed maturation duration (240–260 days), whereas *S. villosa* matured seeds in 70–90 days. Such variation likely reflects species-specific ecological strategies and developmental physiology. Seed maturity indices, as emphasized by Edwards (1980) and Singh and Kachari (2006), are critical determinants of seed viability and germination potential. Our findings support this, as species with well-defined maturation periods tended to have higher germination rates and seedling vigor, emphasizing the importance of identifying species-specific collection windows to optimize propagation success.

Although environmental factors influencing seed maturation were not experimentally tested in this study, observational data and secondary literature indicate that temperature and soil moisture availability are key drivers (Piña-Rodrigues & Aguiar, 1993). This suggests the need for localized seed collection calendars that align with microclimatic conditions to enhance germplasm quality, especially under climate variability scenarios.

Fruit and seed color diversity observed among species may serve important ecological functions. Brightly colored fruits such as red, violet, and orange are generally associated with endozoochorous (animal-mediated) seed dispersal, particularly by birds and mammals (Howe & Smallwood, 1982). Seed morphometric traits-including size, shape, and roundness-also varied significantly among species. Larger-seeded taxa like *B. monosperma* and *S. anacardium* are presumed to allocate greater energy reserves to support initial seedling growth, a trait often associated with enhanced early survival (Westoby *et al*., 1996). Conversely, smaller-seeded species such as *H. orixense* appear to adopt a reproductive strategy favouring quantity over individual seed investment. Aspect ratio and roundness are associated with dispersal and germination behavior (Coomes & Grubb, 2003), and the variability noted among species supports the idea that seed morphology is tightly linked to adaptive reproductive ecology.

Seed image analysis was employed in this study as a modern tool to assess seed morphometry with greater accuracy and objectivity. Studies by Kapadia *et al*., (2017) and Dell'Aquila (2006) have demonstrated that image-based analysis can significantly improve precision in seed characterization. Our results corroborate these advantages, revealing clear associations between specific seed traits and predicted seed viability, germination success, and vigor. This suggests that imaging techniques can supplement traditional germination testing for quality assurance, particularly in large-scale seed programs.

Geographic and population-level differences in seed morphology were also evident, pointing to the influence of genotypic and environmental factors. Previous work by Brus *et al*., (2011) and Costa *et al*., (2016) has confirmed that local adaptation and population divergence contribute to variability in seed traits. While genetic analyses were beyond the scope of this study, the observed differences reinforce the importance of region-specific seed sourcing to maintain ecological suitability and adaptability in restoration efforts.

Conclusion

In conclusion, the study documents considerable variation in phenological and seed morphometric traits across fourteen native species of Central India. These differences reflect a spectrum of ecological adaptations, reproductive strategies, and dispersal mechanisms. By integrating maturity indices,

environmental cues, and advanced tools such as seed image analysis, this research contributes to a deeper understanding of seed biology. Such insights are critical for developing informed strategies for conservation, restoration, and sustainable use of native flora, particularly under changing climate regimes and anthropogenic pressures.

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RESEARCH ARTICLE

Genetic Variability, Character Association and Heterosis Studies in Intraspecific F_1 Hybrids and Open-Pollinated Progenies of *Moringa oleifera* for Seed Germination, Seedling Growth and Nutritional Characteristics

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Abstract

The present study aimed to assess intraspecific F_1 hybrids along with the open-pollinated progenies of *Moringa oleifera* for seed germination (%), seedling growth and nutritional characteristics at Punjab Agricultural University, Ludhiana, during 2021-22. Significant variations were noted among hybrids and open-pollinated progenies for the studied parameters. First and last germination traits were found to be highly influenced by the environment and others are highly heritable traits for which we can select the hybrids for further evaluation. The majority of nursery and seedling traits had a positive significant correlation, and nutritional and physiological traits had a negative significant correlation. However, some have a significant negative correlation with total carotenoids. Two out of 22 components showed an Eigenvalue greater than unity and elucidated a significant amount of variation, i.e., approximately. 100% of the total variation existed among the hybrids and progenies. Only two hybrid progenies, i.e., *PKM-1* × *Bhagya* (H_1) and *PKM-1*× *PKM-2* (H_2), had the maximum heterosis for the majority of traits. Hybrid H_1 exhibited maximum magnitude of heterosis for seedling height (cm), leaf width (cm), and chlorophyll content (mg/g FW), carbohydrates (g/100 g DM), crude protein (g/100 g DM) and crude fibre (g/100 g DM). Hybrid H_2 exhibited maximum magnitude of heterosis for seedling height, collar diameter, chlorophyll content, crude protein, crude fibre and crude fat. Thus, only two hybrid progenies of crosses H_1 and H_2 showed higher economic heterosis for a majority of traits.

Keywords: *Moringa oleifera*, Open-pollinated progenies, F_1 hybrids, Genetic variations, heterosis.

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Introduction

Moringa oleifera Lam. (syn. *M. ptreygosperma* Gaertn.; n=14) is one of the best-known and most commonly utilized and naturalized tree species that has a place in the monogeneric family Moringaceae (Nadkarni, 1976; Ramachandran *et al.,* 1980). It is native to the western and sub-Himalayan tracts, including India and Pakistan; Asia Minor, Africa and Arabia (Somali *et al.,* 1984), but now is distributed in several countries of Asia and Africa (Morton, 1991; Pradheep *et al.,* 2011). Among 13 species in the genus moringa, *M. oleifera* is well known and widely adapted to the tropics and subtropics owing to its fast growth, capabilities to survive in humid, dry hot climates with poor soils (Arora and Onsare, 2014). Next in importance are the *M. concanensis, M. sternoptala, M. peregrina, M. optera, M. arabica* and *M. zyleynica*. *M. oleifera* is grown on approximately. 43,600 hectares of land in India, yielding 12 lakh tonnes of pods annually and India is the world's largest producer of moringa (Sandeep *et al.,* 2019). Andhra Pradesh is the leading Indian state with the maximum area under moringa cultivation (15,665 ha), followed by Tamil Nadu (13,042 ha), Karnataka (10,280 ha) and the remaining 4,613 ha area occupied by other states (Sannagoudar *et*

al., 2022). Tamil Nadu is the pioneering state as it initiated its genetic improvement works with local selections and introductions from Sri Lanka.

The moringa is referred to as a "wonder-tree" due to its multiple uses, especially for nutritional, mineral and important phytochemical ingredients (Verma and Nigam, 2014). Besides its wider cultivation as quality fodder to small size livestock in south and central Indian states, *M. oleifera* is utilized for edible pods, therapeutic significance and seed oil (Fugli, 2005). *M. oleifera* is an important nutrient-rich plant and is normally considered versatile for food that can be eaten in different recipes (Kumssa *et al.,* 2017). Moringa leaves has adequate amount of nutrients (g/100g DM) like crude protein (14.80 ± 0.18; 24.50 ± 0.35), crude fibre (17.81 ± 0.36; 24.72 ± 0.52), total carbohydrates (71.46 ± 0.20; 59.19 ± 0.39), crude fat (3.52 ± 0.01; 5.01 ± 0.01) and total soluble sugars (9.69 ± 0.02; 6.43 ± 0.03) in tender and mature leaves, respectively (Dhakad *et al.,* 2024). Moringa is one of the significant perennial vegetables growing in India. Because it contains vital nutrients including vitamins, minerals, phytochemicals, and other substances, all of its parts, including its leaves, roots, flowers, pods, and seeds, exhibit a surprising range of characteristics (Dhakad *et al.,* 2019).

Genetic diversity is a crucial need for choosing parents for hybridization and developing high yielding genotypes in any crop breeding programme. Transgressive segregants are more likely to be produced when there is a higher genetic variety between the parents. The drumstick plant's genetic diversity may be caused by a variety of agro-ecological conditions, genetic drift, gene flow, introduction and interchange of genetic stocks at the national and international research institutes, as well as natural and artificial selection among the wild and cultivated cultivars. During the late 90s, three varieties (*PKM-1; PKM-2* and *ODC-3*) have been developed in Tamil Nadu, India, while, Bhagya was developed from UHS Bagalkot, Karnataka in 2011 to improve leaf biomass and pod production (Sannagoudar *et al.,* 2022). Morphological and nutritional characterization is a fundamental tool for the selection, conservation, breeding and creation of new varieties (Popoola *et al.,* 2016). The study of accessions under homogeneous environmental conditions allows detecting the variability in the growth, flowering, number and size of leaves and fruits and allows identifying the resistance to various types of biotic and abiotic stress (Resmi *et al.,* 2005). Despite the great adaptability of the moringa plant, deciduous populations have been found in subtropical climates (Folkard *et al.,* 1999).

M. oleifera is cross-pollinated. Therefore, high heterogeneity in morphological, physiological and quantitative traits is anticipated. This tree has a variety of forms, from semi-spreading to upright, annual to perennial. Additionally, some trees flower twice a year, while others flower throughout the year (Raja *et al.,* 2013). Depending on the variety and location, flowers take a wide range of times to mature. In regions with little seasonal fluctuation in temperature or precipitation, flowering may be more or less constant. Insects pollinate flowers, some birds, and bees (Jyothi *et al.,* 1990; Morton, 1991) leads to the chances of incorporating the characters of widely planted trees in an ecosystem. *M. oleifera* has extremely low fruit set rates, 0.31% in the wet season and 1.5% in the dry (Krieg *et al.,* 2017) and the cause of this is still unknown. Usually, the fruit set by open pollination is 11 to 15%, while artificial/controlled pollination yields 62 to 100% (Kanthaswamy, 2005).

Knowledge of morphological diversity in moringa can become a resource for its breeding through the selection of elite varieties adapted to local conditions and having high nutritional value which is directly correlated to the leaf biomass production (Leone *et al.,* 2015). It is a fact that the south Indian varieties produced more leaf biomass with only high cultivation practices and good management, while north Indian cultivars especially from the Punjab region produced limited leaf biomass with a high amount of essential minerals, medicinally important secondary metabolites, dry matter digestibility (Singh, 2021). Punjab local cultivars are well adapted under extreme summers and winters, and can withstand poor management and cultivation practices. Even though *M. oleifera* is widely cultivated under various agro-ecological systems, large-scale commercial plantations are still not reported in the northern Indian states including Punjab possibly due to less biomass potential of locally adapted sources. But these salient findings, i.e. biomass and nutritional contents, observed in different cultivars should bring together in one cultivar before recommending it at a commercial scale in sub-tropical climatic conditions of Punjab. Keeping in view the above facts and discrepancies, the present study aimed to evaluate intraspecific F_1 of *M. oleifera* with their open pollinated progenies for seed germination, growth and nutritional characteristics.

Materials and Methods

The experiment was conducted at the Department of Forestry and Natural Resources, Punjab Agricultural University, Ludhiana, Punjab, since 2020-21. The study area falls in the central zone of Punjab (30°54′ N latitude and 75°48′ E longitude, with 247 m above mean sea level) and is characterized by sub-tropical to tropical, semi-arid type of climate. The minimum temperature may go down to 4°C or even less, while the maximum temperature may be as high as more than 45°C during the summer season. The occurrence of frost is not common. The soils are deep, well-drained, sandy loam in texture with low humus content. The soil pH is neutral. The average annual rainfall in Ludhiana is 760 mm, about three-fourths of which is contributed by the south-west monsoon during July to September.

The experimental was carried out in nursery evaluation of 10 open pollinated progenies of *M. oleifera* and its 4 F_1 hybrids of *PKM-1* × *Bhagya* (H_1), *PKM-1*× *PKM-2* (H_2), *PKM-2* × *ODC-3* (H_3) and *PKM-2* × Mandya local, Karnataka (H_4) established since July 2022 in polythene bags in three replications with plot size of 5 seeds following complete randomized design (CRD). The data on germination parameters, seedling growth and nutritional characteristics of seedling leaves were recorded. The data for leaf morphometric traits were recorded as per the procedure laid down for AVRDC-GRSU characterization record sheet developed by the World Vegetable Centre, Taiwan. The observations for seedlings growth traits and leaf characteristics were recorded on plants having 3-months of growth. Only healthy, mature and complete leaves were subjected to data collection and further nutritional and physiological evaluation.

The data collected from the seed sources of *M. oleifera* on different parameters were subjected to statistical analysis. Coefficient of variability at phenotypic and genotypic level (%), heritability (%) in broad sense, expected genetic advance (%) and genetic gain (%) expressed as genetic advance per cent of mean were calculated as per the formula suggested by Burton and De Vane (1953). Genotypic and phenotypic correlation coefficients were calculated according to the methods suggested by Goulden (1952). Genotypic and phenotypic parameters were statistically analyzed using OPSTAT software. SPSS software, version 16.0, was used to perform principal component analysis on data recorded for germination parameters, seedling growth and nutritional characteristics. The latent root criterion (eigenvalues greater than one) was applied for estimating the number of principal components. A bi-plot display of the first three principal components was used for grouping genotypes, illustrating the relationship between genotypes and indices (Yang and Kang, 2003) using JMP Pro 10.0 software. The superiority of F_1 hybrids over the standard commercial variety/cultivar, i.e. *PKM-1, PKM-2, ODC-3*, PAU local and Mandya local, known as standard heterosis or economic heterosis, were estimated by using the heterosis for replicated data with check(s) function of TNAUSTAT software (Nadarajan *et al.,* 2016).

Results and Discussion

Estimates of genetic variability

A key to progress in tree improvement and breeding programmes is the degree and extent of variation present in a species and germplasm. For planning of strategies for improvement and breeding of any species, the coefficient of variation (%) is most importance. Heritability (%) and genetic advance give information of the variations present in planting material as well as environmental influences on those traits. The components of variance with high genetic gain and high heritability can be used to generate an idea and scope for response of selection to various characters (Chauhan *et al.,* 2004).

In general, the phenotypic coefficient of variation was higher than the corresponding genotypic coefficient of variation for all characters, reflecting the sufficient genetic variations for the characters studied among hybrid and open-pollinated progenies (Table 1). High genotypic variance was observed in seed weight (29.85%), followed by crude fat (21.88%), survival percent (21.84%), and leaf length (20.22%). Total energy value (1.337%), followed by total carbohydrates (4.91%), ash content (5.318%) and crude fiber (4.82%) had the lowest amount of genotypic variance across progenies. A high heritability indicates that much of the variation for a given characteristic observed in the population is genetic in origin. The majority of the traits had very high heritability (>60%) except first and last germination (<30%) traits, which were highly influenced by environmental conditions. It means that the traits were under the strong influence of additive gene action, and selection would be quite effective.

In the current study, the trend of genetic advance as per cent of the mean shows a huge scope for genetic improvement in *M. oleifera*. In fact, moderate genetic gain was observed in survival percent (19.60%), followed by germination percent (18.89%), while the minimum was observed in total soluble energy (0.015%), first germination (0.468%) and last germination (0.722%). Similar results were reported by Verma *et al.* (2019), when compared to other characteristics, pod weight (g) had moderate heritability (51.32%). And genetic advance as a percentage of mean was observed lowest for number of seeds per pod (4.49) and pod girth 6.21, and a similar result was found by Balaguru *et al.* (2020) genetic advance as percentage of mean was observed lowest for number of seeds per pod and pod girth in *M. oleifera*.

Correlation studies

Correlation provides an insight to the deep complexity and the amount of inter-relationship existing between different traits. Because some traits are inter-linked related to one another, knowledge of associations is important when choosing one or more traits. It provides an excellent base for indirect selection, particularly for below-ground or biomass traits which require destructive sampling. The amount of genetic gain obtained from indirect selection, *i.e.* correlated response gives a better understanding of degree and magnitude of genetic association and must be given the due weightage while formulating the selection criteria in any tree improvement programme.

Significant correlations were recorded for the traits studies and presented in Tables 2 and 3. The majority of nursery and seedling traits had a positive significant, and nutritional and physiological traits had a negative significant correlation. However, some have a significant negative correlation with total carotenoids. Carotenoids had positive significant correlation with seedling weight (0.658**), survival percent (0.468**), plant percent (0.342**), leaf length

Table 1: Genetic variability estimates for seed germination, seedling growth and nutritional characteristics of open-pollinated progenies and F_1 hybrids of *Moringa oleifera* Lam.

Parameter	*Mean*	*Range*	*Coefficient of variation*		*Heritability (%)*	*Genetic advance (%)*	*Genetic gain (% of mean)*
			Phenotypic (%)	*Genotypic (%)*			
SW	16.01 ± 0.67	03.64-31.91	31.77	29.84	88.22	11.32	57.75
FG	06.82 ± 0.91	06.00-8.00	28.96	10.39	12.86	0.46	07.67
LG	06.82 ± 0.91	10.00-15.00	26.21	09.29	12.56	0.72	06.78
GP	54.50 ± 3.06	28.57-66.66	14.96	14.52	94.24	18.89	29.05
SP	34.72 ± 1.72	14.28-42.85	22.71	21.84	92.44	19.60	43.26
PP	23.96 ± 2.29	00.00-36.66	20.30	18.70	84.82	10.06	35.48
SH	60.09 ± 1.49	47.00-82.00	16.71	15.85	89.91	17.19	30.96
CD	07.22 ± 0.59	04.88-09.24	19.52	14.85	57.90	01.46	23.29
LL	20.18 ± 2.28	15.00-30.80	23.81	20.21	72.09	06.84	35.36
LW	12.31 ± 0.90	09.00-21.80	25.22	19.78	61.52	03.68	31.97
PL	06.93 ± 0.46	04.75-09.50	20.59	17.31	70.72	02.02	30.00
NoP	06.66 ± 0.55	06.00-08.20	17.20	13.09	57.89	01.55	20.51
DM	24.64 ± 0.29	22.98-25.74	09.19	08.98	95.39	04.43	18.07
CP	37.39 ± 0.27	32.17-40.63	10.54	10.47	98.63	07.43	21.42
CFt	04.60 ± 0.19	03.33-05.43	22.38	21.87	95.52	02.05	44.04
Ash	10.24 ± 0.10	09.47-10.93	05.86	05.31	82.15	01.02	09.93
CFb	22.24 ± 0.14	20.48-23.37	06.50	05.85	81.23	02.48	10.87
TCb	12.55 ± 0.19	12.07-13.06	08.04	07.99	98.84	08.24	16.38
TEV	382.03 ± 0.84	370.8-388.4	01.38	01.33	93.38	10.16	02.66
TSS	00.04 ± 0.00	0.03-0.04	18.04	17.50	94.12	00.01	34.99
TCH	47.77 ± 0.19	12.07-13.06	05.21	04.91	88.65	01.19	09.52
CART	38.59 ± 0.27	33.39-42.50	08.21	08.09	96.99	06.46	16.42

Note 1: SW- Seed Weight (g/100seeds); FG- First Germination (%); LG- Last Germination (%); GP- Germination Percentage (%); SP- Survival Percentage (%); PP- Plant Percentage (%); SH- Seedling Height (cm); CD- Collar Diameter (mm); LL- Leaf Length (cm); LW- Leaf Width (cm); PL- Petiole Length (cm); NOP- Number of Pinnae; DM- Dry Matter (g/100g); CP- Crude Protein (g/100g DM); CFt-Crude Fat (g/100g DM); Ash- Ash Content (g/100g DM); CFb- Crude Fiber (g/100g DM); TCb- Total Chlorophyll (mg/g FW); TEV- Total Energy Value (Mcal/kg); TSS- Total Soluble Sugars (g/100g DM); TCH- Total Carbohydrates (g/100g DM): CART- Total Carotenoids (g/100g DM).

(0.305*), petiole length (0.345*), total carbohydrates (0.517**), while significant negative correlation with all nutritional traits and all physiological traits except total carbohydrates at phenotype level. However, carotenoids showed only a positive significant correlation with seedling weight (0.711**), survival percent (0.494**), and total carbohydrates (0.542*) at the genotype level. Seedling weight showed positive correlations with germination percentage (0.471**), seedling height (0.479**), leaf width (0.484**), petiole length (0.434**), number of pinnae (0.500**) and total carotenoids (0.658**) at the phenotype level. Seedling weight showed positive correlations with germination percent (0.519**), survival percent (0.620**), seedling height (0.531**), leaf length (0.601**), leaf width (0.625**), petiole length (0.505), number of pinnae (0.786**), total crude fibre (0.356**) and total carotenoids (0.711**) at genotype level. Germination percent had a high correlation with survival percent, plant percentage and a low correlation with petiole length at the phenotype level. Germination percentage had a high correlation with survival percent, number of pinnae and a low correlation with petiole length at the genotype level. Maximum correlations show crude fat with total energy value at the phenotype level. At the genotype level, maximum correlations show first germination and last germination.

It was clearly evident from the Table 2 and 3 that the magnitude of genotypic correlation was higher than the corresponding phenotypic correlation which might be due to strong inherent linkage of traits at gene level or pleiotropic effect of a gene, suggested that any change in the gene locus of one trait may alter the genetic expression of the associated traits. The results are in agreement with the findings of Lastin *et al.* (2019), who observed that the genotypic correlation coefficient was higher than the

Table 2: Genotypic Correlations among seed germination, seedling growth and nutritional characteristics of open-pollinated progenies and F_1 hybrids of *M. oleifera* Lam.

	SW	*FG*	*LG*	*GP*	*SP*	*PP*	*SH*	*CD*	*LL*	*LW*	*PL*	*NoP*	*DM*	*CP*	*CFt*	*Ash*	*CFb*	*TCb*	*TEV*	*TSS*	*TCH*
FG	-0.052^{NS}																				
LG	-0.402**	2.800**																			
GP	0.519**	-0.343*	-0.613**																		
SP	0.620**	-0.431**	-0.733**	0.824**																	
PP	0.377*	-0.204^{NS}	-0.341*	0.616**	0.666**																
SH	0.531**	0.364*	0.009^{NS}	0.193^{NS}	0.011^{NS}	-0.188^{NS}															
CD	-0.166^{NS}	1.063**	0.908**	-0.097^{NS}	-0.510**	-0.414**	0.737**														
LL	0.601**	0.376*	0.037^{NS}	0.069^{NS}	0.131^{NS}	-0.374*	0.844**	0.503**													
LW	0.625**	0.050^{NS}	0.130^{NS}	0.071^{NS}	0.272^{NS}	-0.379*	0.660**	0.293^{NS}	1.065**												
PL	0.505**	-0.029^{NS}	-0.167^{NS}	0.017^{NS}	0.212^{NS}	-0.303^{NS}	0.805**	0.299^{NS}	0.904**	0.877**											
NoP	0.786**	0.105^{NS}	-1.058**	0.648**	0.591**	-0.004^{NS}	0.712**	0.047^{NS}	0.749**	0.792**	0.704**										
DM	-0.490**	-0.119^{NS}	-0.036^{NS}	0.371*	0.017^{NS}	-0.020^{NS}	-0.312*	0.098^{NS}	-0.483**	-0.434**	-0.643**	-0.166^{NS}									
CP	-0.054^{NS}	0.317*	0.578**	0.004^{NS}	-0.230^{NS}	0.230^{NS}	0.509**	0.696**	0.130^{NS}	-0.110^{NS}	0.016^{NS}	-0.165^{NS}	0.035^{NS}								
CFt	0.207^{NS}	0.566**	-0.378*	0.383*	0.315*	0.250^{NS}	0.130^{NS}	-0.070^{NS}	0.138^{NS}	0.289^{NS}	0.019^{NS}	0.312*	0.130^{NS}	-0.022^{NS}							
Ash	0.312*	-0.304^{NS}	-0.577**	0.455**	0.138^{NS}	0.333*	0.198^{NS}	0.000^{NS}	-0.148^{NS}	-0.133^{NS}	-0.348*	0.136^{NS}	0.328*	0.399**	0.209^{NS}						
CFb	0.356*	-0.058^{NS}	-0.145^{NS}	0.172^{NS}	0.254^{NS}	0.255^{NS}	-0.038^{NS}	-0.308*	0.125^{NS}	0.137^{NS}	0.247^{NS}	0.055^{NS}	-0.503**	-0.249^{NS}	-0.173^{NS}	-0.299^{NS}					
TCb	-0.046^{NS}	-0.388*	-0.348*	-0.162^{NS}	0.109^{NS}	-0.317*	-0.519**	-0.610**	-0.132^{NS}	0.045^{NS}	0.028^{NS}	0.052^{NS}	-0.109^{NS}	-0.951**	-0.261^{NS}	-0.550**	0.309*				
TEV	0.072^{NS}	0.695**	-0.128^{NS}	0.186^{NS}	0.255^{NS}	0.106^{NS}	0.044^{NS}	-0.070^{NS}	0.202^{NS}	0.345*	0.169^{NS}	0.253^{NS}	-0.011^{NS}	-0.194^{NS}	0.907**	-0.222^{NS}	-0.044^{NS}	-0.024^{NS}			
TSS	0.098^{NS}	0.193^{NS}	-0.037^{NS}	-0.390*	-0.408**	-0.158^{NS}	-0.030^{NS}	-0.119^{NS}	-0.050^{NS}	-0.155^{NS}	-0.104^{NS}	-0.034^{NS}	-0.386*	-0.196^{NS}	-0.190^{NS}	-0.198^{NS}	0.111^{NS}	0.252^{NS}	-0.104^{NS}		
TCH	0.213^{NS}	-0.302^{NS}	0.237^{NS}	0.284^{NS}	0.554**	0.534**	-0.050^{NS}	-0.066^{NS}	0.124^{NS}	0.088^{NS}	0.124^{NS}	-0.030^{NS}	-0.144^{NS}	0.155^{NS}	-0.021^{NS}	-0.083^{NS}	-0.030^{NS}	-0.123^{NS}	0.015^{NS}	-0.373*	
CART	0.711**	-0.071^{NS}	0.317*	0.307*	0.494**	0.355*	0.298^{NS}	-0.047^{NS}	0.339*	0.356*	0.360*	0.234^{NS}	-0.450**	0.092^{NS}	-0.146^{NS}	0.300^{NS}	0.342*	-0.087^{NS}	-0.275^{NS}	-0.324*	0.542**

Note 1: Genotypic correction coefficient is at left lower side and phenotypic correction coefficient is at right upper side of table (bold red digits)
Note 2: *. Correlation is significant at the 0.05 level (2-tailed); **. Correlation is significant at the 0.01 level (2-tailed).
Note 3: SW- Seed Weight (g/100seeds); FG- First Germination (%); LG- Last Germination (%); GP- Germination Percentage (%); SP- Survival Percentage (%); PP- Plant Percentage (%); SH- Seedling Height (cm); CD- Collar Diameter (mm); LL- Leaf Length (cm); LW- Leaf Width (cm); PL- Petiole Length (cm); NOP- Number of Pinnae; DM- Dry Matter (g/100g); CP- Crude Protein (g/100g DM); CFt-Crude Fat (g/100g DM); Ash- Ash Content (g/100g DM); CFb- Crude Fiber (g/100g DM); TCb- Total Chlorophyll (mg/g FW); TEV- Total Energy Value (Mcal/kg); TSS- Total Soluble Sugars (g/100g DM); TCH- Total Carbohydrates (g/100g DM): CART- Total Carotenoids (g/100g DM).

Table 3: Phenotypic Correlations among seed germination, seedling growth and nutritional characteristics of open-pollinated progenies and F_1 hybrids of *M. oleifera* Lam.

	SW	*FG*	*LG*	*GP*	*SP*	*PP*	*SH*	*CD*	*LL*	*LW*	*PL*	*NoP*	*DM*	*CP*	*CFt*	*Ash*	*CFb*	*TCb*	*TEV*	*TSS*	*TCH*
FG	0.055NS																				
LG	-0.105NS	0.035NS																			
GP	0.471**	-0.050NS	-0.260NS																		
SP	0.579**	-0.244NS	-0.186NS	0.755**																	
PP	0.321*	-0.148NS	-0.113NS	0.534**	0.600**																
SH	0.479**	0.158NS	0.037NS	0.181NS	0.014NS	-0.155NS															
CD	-0.150NS	0.281NS	0.350*	-0.079NS	-0.356*	-0.285NS	0.518**														
LL	0.478**	0.130NS	0.037NS	0.066NS	0.116NS	-0.221NS	0.648**	0.250NS													
LW	0.484**	0.292NS	-0.171NS	0.075NS	0.181NS	-0.273NS	0.541**	0.003NS	0.769**												
PL	0.434**	-0.070NS	-0.153NS	0.002NS	0.178NS	-0.201NS	0.614**	0.169NS	0.664**	0.605**											
NoP	0.500**	-0.120NS	-0.263NS	0.481**	0.473**	0.009NS	0.492**	0.048NS	0.458**	0.465**	0.495**										
DM	-0.442**	-0.072NS	0.028NS	0.341*	0.027NS	-0.016NS	-0.298NS	0.096NS	-0.411**	-0.386*	-0.536**	-0.072NS									
CP	-0.054NS	0.087NS	0.183NS	-0.001NS	-0.212NS	0.218NS	0.482**	0.544**	0.096NS	-0.083NS	0.049NS	-0.106NS	0.030NS								
CFt	0.198NS	0.256NS	-0.133NS	0.358*	0.297NS	0.222NS	0.136NS	-0.067NS	0.127NS	0.235NS	0.020NS	0.194NS	0.120NS	-0.029NS							
Ash	0.300NS	-0.009NS	-0.019NS	0.387*	0.091NS	0.299NS	0.155NS	0.022NS	-0.080NS	-0.084NS	-0.291NS	0.042NS	0.307*	0.343*	0.201NS						
CFb	0.257NS	-0.137NS	0.033NS	0.126NS	0.193NS	0.248NS	-0.045NS	-0.170NS	0.106NS	-0.042NS	0.140NS	0.041NS	-0.396**	-0.233NS	-0.173NS	-0.220NS					
TCb	-0.047NS	-0.143NS	-0.128NS	-0.149NS	0.102NS	-0.298NS	-0.494**	-0.478**	-0.107NS	0.027NS	-0.005NS	0.040NS	-0.104NS	-0.947**	-0.261NS	-0.512**	0.288NS				
TEV	0.057NS	0.256NS	-0.123NS	0.175NS	0.251NS	0.082NS	0.063NS	-0.076NS	0.162NS	0.270NS	0.153NS	0.172NS	-0.022NS	-0.186NS	0.893**	-0.260NS	-0.070NS	-0.023NS			
TSS	0.058NS	0.112NS	-0.029NS	-0.373*	-0.400**	-0.131NS	-0.023NS	-0.032NS	-0.021NS	-0.122NS	-0.121NS	-0.021NS	-0.353*	-0.193NS	-0.175NS	-0.149NS	0.132NS	0.242NS	-0.104NS		
TCH	0.195NS	-0.092NS	0.095NS	0.264NS	0.517**	0.443**	-0.041NS	0.011NS	0.097NS	0.045NS	0.133NS	-0.016NS	-0.136NS	0.150NS	0.006NS	-0.048NS	-0.023NS	-0.130NS	0.028NS	-0.328*	
CART	0.658**	-0.016NS	0.031NS	0.303NS	0.468**	0.342*	0.269NS	-0.038NS	0.305*	0.296NS	0.345*	0.165NS	-0.439**	0.096NS	-0.131NS	0.263NS	0.284NS	-0.092NS	-0.250NS	-0.305*	0.517**

Note 1: *. Correlation is significant at the 0.05 level (2-tailed); **. Correlation is significant at the 0.01 level (2-tailed).

Note 2: SW- Seed Weight (g/100seeds); FG- First Germination (%); LG- Last Germination (%); GP- Germination Percentage (%); SP- Survival Percentage (%); PP- Plant Percentage (%); SH- Seedling Height (cm); CD- Collar Diameter (mm); LL- Leaf Length (cm); LW- Leaf Width (cm); PL- Petiole Length (cm); NOP- Number of Pinnae; DM- Dry Matter (g/100g); CP- Crude Protein (g/100g DM); CFt-Crude Fat (g/100g DM); Ash- Ash Content (g/100g DM); CFb- Crude Fiber (g/100g DM); TCb- Total Chlorophyll (mg/g FW); TEV- Total Energy Value (Mcal/kg); TSS- Total Soluble Sugars (g/100g DM); TCH- Total Carbohydrates (g/100g DM): CART- Total Carotenoids (g/100g DM).

Table 4: Percentage of variance explained by different principal components among open-pollinated progenies and F_1 hybrids of *M. oleifera* Lam.

Characteristics	*Principal Components*	
	1	*2*
SW	0.000	-1.000
FG	0.915	0.402
LG	-0.915	-0.402
GP	-0.693	0.721
SP	0.994	0.000
PP	0.780	-0.626
SH	0.787	0.617
CD	0.594	0.804
LL	0.000	-0.995
LW	0.857	-0.516
PL	-0.971	0.000
NoP	0.696	0.718
DM	0.911	-0.412
CP	0.959	0.000
CFt	-0.568	0.823
Ash	-0.985	0.000
CFb	0.420	-0.907
TCb	-0.915	-0.402
TEV	0.448	0.894
TSS	0.888	-0.459
TCH	0.000	0.959
CART	0.900	0.436
Eigen value	12.834	9.166
Percent of variability	58.330	41.664
Cumulative percent of variability	58.330	~100

Note 2: SW- Seed Weight (g/100seeds); FG- First Germination (%); LG- Last Germination (%); GP- Germination Percentage (%); SP- Survival Percentage (%); PP- Plant Percentage (%); SH- Seedling Height (cm); CD- Collar Diameter (mm); LL- Leaf Length (cm); LW- Leaf Width (cm); PL- Petiole Length (cm); NOP- Number of Pinnae; DM- Dry Matter (g/100g); CP- Crude Protein (g/100g DM); CFt-Crude Fat (g/100g DM); Ash- Ash Content (g/100g DM); CFb- Crude Fiber (g/100g DM); TCb- Total Chlorophyll (mg/g FW); TEV- Total Energy Value (Mcal/kg); TSS- Total Soluble Sugars (g/100g DM); TCH- Total Carbohydrates (g/100g DM): CART- Total Carotenoids (g/100g DM).

phenotypic correlation coefficient. Similarly, number of seeds per fruit pod was significantly correlated with fruit/pod length, while 100 seed weight was significantly correlated with fruit/pod length, pod weight, number of seed per locule and number of seed per pod and significant relationship between days to 50% flowering and days to 50% maturity is indicative that there is a strong relationship between the stage of plant growth at which flowering is initiated and the time taken to complete the crop's life cycle (Akinyele and Osekita, 2006; Aremu, 2011).

Principal component analysis

Principal component analysis is a statistical data reduction technique to reduces a large data set with a number of correlated variables into a significantly lower set of new variables that comprises most of the variation present in the traits, so that the interpretation of results becomes feasible. The principle components of the correlation matrix having less than one Eigen value should be dropped as these components are less informative (Kaiser, 1958). It provides an insight into complex relationship existing between different parameters and reflects their contribution to the every character towards total variation (Kumari *et al.,* 2025).

Since, it is difficult to conceive 22 different characters, principle component analysis was performed and the summarized in Table 4. It was observed that two out of 22 components showed eigenvalues greater than unity and elucidated a significant amount of variation, *i.e.,* ~100 % of the total variation. The scree plot of eigenvalues is furnished in Figure 4.1. The first principal component (λ1= 12.834) explained 58.336 % variation of the total variation, with a maximum loading recorded for survival percent (0.994), crude protein (0.959), first germination (0.915) and dry matter (0.911). The second principal component (λ2= 9.166) explains 41.664 % of the total variation, with the highest loading for total carbohydrates (0.959) followed by total energy value (0.894), crude fat (0.823), and collar diameter (0.804). The principal component analysis had indicated some of the important components associated with reproductive and crossability traits in *M. oleifera,* which could be utilized for optimizing the productivity of elite *M. oleifera* progenies. Thus, maximum weightage should be given to survival percentage with maximum variable loading (0.994) followed by crude protein and total carbohydrates (0.959), first germination (0.861) and length (0.853), number of leaflets (0.832), leaf width (0.915) and dry matter (0.911), since they showed the highest contribution towards total genetic variability present in the hybrid and open pollinated progenies of *M. oleifera*. Similarly, Zeru and Hassen (2022), while evaluating provenance variation in Moringa, reported that principal component analysis revealed distinct population differentiation associated with variability for plant height and seedling survival rate.

Heterosis studies

In the present study, the results obtained with respect to the magnitude of heterosis have been presented in Tables 5 to 8. Hybrid H_1 exhibited maximum magnitude of heterosis for seedling height, leaf width, and chlorophyll content, carbohydrates, crude protein and crude fibre. Hybrid H_2 exhibited maximum magnitude of heterosis for seedling

Table 5: Standard heterosis (%) of progeny of *PKM-1* × *Bhagya* (H_1) of *M. oleifera* Lam. over their parents and check varieties for growth and nutritional characteristics

Characters	*Mean*	*% over Bhagya*	*% over PKM-1*	*% over PAU Local*
Growth characteristics				
Seedling height (cm)	62.44	16.92**	51.55**	-11.57**
Collar diameter (mm)	7.15	19.96ns	50.74**	-8.80ns
Leaf length (cm)	22.05	16.55ns	55.26**	8.60ns
Leaf width (cm)	13.60	31.78*	44.63**	16.24ns
Petiole length (cm)	7.38	10.15ns	38.81**	-4.98ns
Number of pinnae	6.76	-6.45ns	-6.45ns	-29.20**
Nutritional characteristics				
Dry matter (g/100g)	24.38	-0.73 ns	1.99 ns	13.02**
Ash content (g/100g DM)	10.93	5.13*	7.89**	1.86 ns
Crude protein (g/100g DM)	37.29	-4.02**	12.04**	-3.19**
Crude fibre (g/100g DM)	20.48	-4.07**	-16.29**	-4.12**
Crude fat (g/100g DM)	5.43	120.27**	7.24ns	3.82 ns
Carbohydrates (g/100g DM)	13.06	-5.79**	3.68*	7.96**
Total soluble sugars (g/100g DM)	0.04	-7.76 ns	-19.55**	7.00 ns
Total energy value (Mcal/kg)	383.43	3.43**	-0.36 ns	0.05 ns
Chlorophyll content (mg/g FW)	46.35	-4.01**	-16.39**	1.82*
Total carotenoids (g/100g DM)	42.50	-0.65 ns	19.96**	13.59**

Table 6: Standard heterosis (%) of progeny of *PKM-1*× *PKM-2* (H_2) of *M. oleifera* Lam. over their parents and check varieties for growth and nutritional characteristics

Characters	*Mean*	*% over PKM-1*	*% over PKM-2*	*% over PAU Local*
Growth characteristics				
Seedling height (cm)	63.92	55.14**	28.60**	-9.47**
Collar diameter (mm)	7.32	54.25**	34.42**	-6.68 ns
Leaf length (cm)	20.67	45.54**	11.53 ns	1.81 ns
Leaf width (cm)	9.00	-4.25 ns	-23.70 ns	-23.05 ns
Petiole length (cm)	6.80	27.90**	9.68 ns	-12.45 ns
Number of pinnae	6.85	-5.17 ns	-5.21 ns	-28.26**
Nutritional characteristics				
Dry matter (g/100g)	22.98	-3.88**	-18.75**	-18.03**
Ash content (g/100g DM)	10.23	0.99 ns	-3.46 ns	-4.66*
Crude protein (g/100g DM)	40.63	22.10**	38.87**	5.50**
Crude fibre (g/100g DM)	23.37	9.43**	-4.51**	9.38**
Crude fat (g/100g DM)	4.60	-9.21*	32.69**	-12.10**
Carbohydrates (g/100g DM)	12.07	-4.21*	7.19**	-0.25 ns
Total soluble sugars (g/100g DM)	0.05	9.77**	12.31**	46.00**
Total energy value (Mcal/kg)	382.07	-0.71**	1.90**	-0.30 ns
Chlorophyll content (mg/g FW)	44.53	-19.66**	-19.69**	-2.17*
Total carotenoids (g/100g DM)	39.65	11.90**	2.35 ns	5.96**

Table 7: Standard heterosis (%) of progeny of *PKM-2* × *ODC-3* (H_3) of *M. oleifera* Lam. over their parents and check varieties for growth and nutritional characteristics

Characters	*Mean*	*% over PKM-1*	*% over PKM-2*	*% over ODC-3*	*% over PAU Local*
Growth characteristics					
Seedling height (cm)	51.50	25.00**	3.62 ns	-14.02**	-27.06**
Collar diameter (mm)	7.09	49.54**	30.31**	20.23 ns	-9.52 ns
Leaf length (cm)	17.17	20.92 ns	-7.34 ns	-10.79 ns	-15.42 ns
Leaf width (cm)	9.00	-4.25 ns	-23.70 ns	-19.23 ns	-23.05 ns
Petiole length (cm)	5.57	4.76 ns	-10.16 ns	-17.07 ns	-28.28
Number of pinnae	6.00	-16.97 ns	-17.01ns	-28.03**	-37.19**
Nutritional characteristics					
Dry matter (g/100g)	25.74	7.65**	-9.00**	0.55 ns	-8.19**
Ash content (g/100g DM)	10.33	1.97 ns	-2.52 ns	-8.82**	-3.73 ns
Crude protein (g/100g DM)	39.45	34.32**	29.40**	11.81**	2.42*
Crude fibre (g/100g DM)	22.47	5.21**	-8.19**	-5.03**	5.16**
Crude fat (g/100g DM)	5.03	-0.66 ns	45.19**	13.53**	-3.82 ns
Carbohydrates (g/100g DM)	12.32	-2.20 ns	9.44**	-3.47*	1.85 ns
Total soluble sugars (g/100g DM)	0.05	3.01 ns	5.38 ns	0.74 ns	37.00**
Total energy value (Mcal/kg)	383.83	-0.25 ns	2.37**	1.86**	0.16 ns
Chlorophyll content (mg/g FW)	45.19	-18.48**	18.51**	-7.69**	-0.73 ns
Total carotenoids (g/100g DM)	33.39	-5.77**	-13.81**	-26.63**	-10.77**

Table 8: Standard heterosis (%) of progeny of *PKM-2* × Mandya, Karnataka (H_4) of *M. oleifera* Lam. over their parents and check varieties for growth and nutritional characteristics

Characters	*Mean*	*% over PKM-1*	*% over PKM-2*	*% over Mandya Local*	*% over PAU Local*
Growth characteristics					
Seedling height (cm)	47.00	14.08**	-5.43ns	-21.54**	-33.43**
Collar diameter (mm)	7.61	60.44**	39.80**	28.98*	-2.93ns
Leaf length (cm)	16.33	15.02ns	-11.85ns	-15.14ns	-19.54ns
Leaf width (cm)	9.33	-0.74ns	-20.90ns	-16.27ns	-20.23ns
Petiole length (cm)	5.83	9.72ns	-5.91ns	-13.15ns	-24.89**
Number of pinnae	6.33	-12.36ns	-12.40ns	-24.03**	-33.71**
Nutritional characteristics					
Dry matter (g/100g)	25.47	6.53**	-9.96**	11.50**	-9.16**
Ash content (g/100g DM)	9.47	-6.58**	-10.69**	-16.47**	-11.80**
Crude protein (g/100g DM)	32.17	-3.33**	9.56**	-8.81**	-16.46**
Crude fibre (g/100g DM)	22.6	5.99**	-7.51**	-4.33**	5.94**
Crude fat (g/100g DM)	3.33	-34.21**	-3.85 ns	-24.81**	-36.31**
Carbohydrates (g/100g DM)	12.73	1.06 ns	13.08**	-0.26 ns	5.23 **
Total soluble sugars (g/100g DM)	0.05	1.50 ns	3.85 ns	-0.74 ns	35.00**
Total energy value (Mcal/kg)	378.80	-1.56**	1.03**	0.52 ns	-1.16**
Chlorophyll content (mg/g FW)	55.03	-0.73 ns	-0.76 ns	12.41**	20.88**
Total carotenoids (g/100g DM)	38.81	9.53**	0.18 ns	-14.72**	3.71*

height, collar diameter, chlorophyll content, crude protein, crude fibre and crude fat. Hybrid H_3 exhibited maximum magnitude of heterosis for seedling height, chlorophyll content, crude protein and crude fibre, and total carotenoids. Hybrid H_4 exhibited maximum magnitude of heterosis for seedling height, collar diameter, chlorophyll content, dry matter, ash content, crude protein and crude fibre, total energy value and total carotenoids.

The importance of intra- and inter-specific hybridization in the genetic improvement of Willows has been demonstrated by significant and stable heterosis/hybrid vigour expressed in F1 hybrids. The vigour in terms of growth of hybrids depends on the selection of parental species/genotype and filial generation of hybrids (Zsuffa, 1984). There were indications of heterosis in hybrids already produced between *S. matsudana* and *S. alba* which had showed 100 per cent superiority in diameter growth and 30 per cent in height growth over the growth of either parents (Hathaway, 1979). Superiority of intra-specific hybrids of has been already demonstrated by earlier workers (Hathaway and Kraayenoord, 1979; Dongsen *et al.,* 1992; Li and Wu, 1997; Ronnberg and Gulberg, 1999; Ozel *et al.,* 2010 in Poplars. Earlier Stott (1984) reported better productivity and higher adaptability of *Salix alba* × *Salix alba* hybrids as compared to hybrids between species (*S. alba* × *S. fragilis*)

In conclusion, all the studied parameters of reproductive, crossability, pod, germination, seedling growth, nutritional, and physiological showed a significant amount of variation present in the seed sources. First and last germination traits were found to be highly influenced with the environment and others are highly heritable traits for which we can select the hybrids for further evaluation. Significant correlations were recorded for the traits studies. The majority of nursery and seedling traits had a positive significant correlation, and nutritional and physiological traits had a negative significant correlation. However, some have a significant negative correlation with total carotenoids. Two out of 22 components showed eigenvalues greater than unity and elucidated a significant amount of variation, i.e., ~100 % of the total variation existed among the progenies. Only two hybrid progenies, i.e., H_1 and H_2, had the maximum heterosis for the majority of traits. Hybrid H_1 exhibited maximum magnitude of heterosis for seedling height, leaf width, and chlorophyll content, carbohydrates, crude protein and crude fibre. Hybrid H_2 exhibited maximum magnitude of heterosis for seedling height, collar diameter, chlorophyll content, crude protein, crude fibre and crude fat.

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RESEARCH ARTICLE

Breaking Barriers in Maize Seed Production: The Success Story of DMRH 1308, DMRH 1301 and LQMH 1 Hybrids

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Abstract

Hybrid technology has significantly contributed to increasing maize production and productivity in the country. Seed is the basic and key input that determines the farm productivity of any cultivar. However, the private sector contributes significantly to the maize seed supply, but the public sector develops hybrids that contribute to regulating seed prices and quality. For *Kharif* 2025, the DAC Govt. of India, demand for public-bred maize hybrid breeder seeds reached its highest level ever (126 q). The DMRH 1301 (medium duration) and DMRH 1308 (medium duration), released in 2017, and LQMH 1 (short duration) in 2020 through the central varietal release committee from ICAR-IIMR, created a significant impact in terms of acreage. So far a total of 24 different private seed companies have taken up these hybrids by signing 36 MoUs with the institute. In the last four years, DMRH 1308 has consistently topped DAC maize breeder seed demand, with its share ranging from 20.1 to 34.9%. Based solely on DAC breeder seeds supplied till 2024, DMRH 1308 is estimated to cover around 11.57 lakh hectares, DMRH 1301, 4.10 lakh and LQMH 1, 0.70 lakh hectares of land since their release. In farmers' fields, these hybrids yield 6.0 to 10.5 t/ha of grain. Higher female productivity (2.0–3.5 t/ha), longer pollen availability (7–9 days), broader adaptability, tolerance to major diseases and insect pests, and input responsiveness are the main characteristics of these hybrid parental lines, which contribute towards the successful commercial seed production. The majority of hybrid maize seeds are produced in peninsular India, resulting in higher seed costs. It is therefore necessary to establish alternative farmer-led seed production hubs. Our study documented the success story of farmer-led seed production in three different states, especially in new sites in West Bengal. This model can be replicated to popularize other public-bred hybrids.

Keywords: Maize hybrids impact, Flowering synchrony, Hybrid seed production, Alternate seed hubs.

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Introduction

Maize is an important, versatile crop also known as the 'Queen of cereals' due to its highest yield potential among the cereals. It is a relatively climate-resilient crop and can be grown in tropical, subtropical, and temperate climates from sea level to 3000 metres above sea level. In India, maize cultivation is critical for supporting the nation's burgeoning population and diverse agro-industrial sectors, acting as a crucial element in achieving food, nutritional and industrial security (Kumar *et al.,* 2013). Since the beginning, abiotic and biotic stresses, upscaling of improved PoP at farmers' fields, availability of quality seeds and inputs at affordable prices, coverage under single cross hybrids, controlling post-harvest losses, and market volatility have remained major challenges in maize. During 1950-51, India used to produce 1.73 million tonnes of maize from a 3.16-million-hectare area with a productivity of around 0.55 t/ha. At present (2023–24), nearly 37.67 million tonnes of maize are being produced from a sizable area of 11.24 million hectares of land with a productivity of 3.35 t/ha (Figure 1; MoA & FW, 2024). In India, maize production has increased 22 times, productivity 6.5 times, and area 3.4 times since 1950-51, all

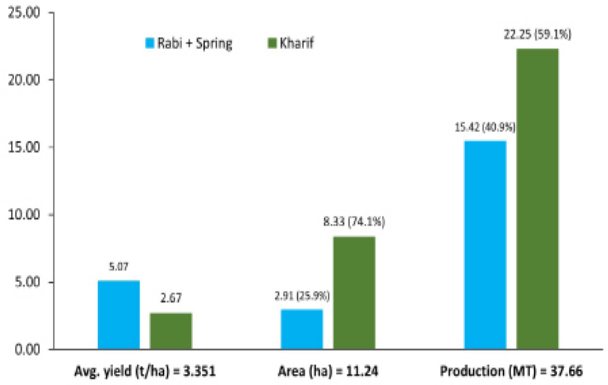

Figure 1: Seasonal-wise area, production, and productivity of maize in India during 2023-24

attributed to hybrid technology. Nearly 2.25 lakh tonnes of hybrid seed will be required to cover 11.24 million hectares (mha) of maize under hybrid/improved cultivars. Notably, *Rabi* and spring cultivation, despite occupying only 25.9% of the total 11.24 million hectares, achieve a higher yield of 5.07 t/ha, which contributes 40.9% (15.42 million MT) to the total 37.66 million MT production. This is possible because almost 100% of the maize acreage in these seasons is under hybrids. Conversely, *Kharif* season cultivation covers 74.1% of the area and accounts for 59.1% (22.25 million MT) of the total production (MoA & FW, 2024).

The significant increase in maize production and productivity has been possible due to policy interventions, cultivation of improved maize hybrids, adoption of best management practices and effective control of diseases and insect pests. In the country, single-cross hybrids have contributed significantly to the increase in maize production and productivity (DMR Vision 2030). The current per day productivity of maize in selected parts of the country is close to 65 kg/day, that of countries like the USA. During *kharif*, Madhya Pradesh has the highest area (2.02 mha) under maize, but Tamil Nadu has the highest *kharif* maize productivity (7.5 t/ha). West Bengal has the highest maize productivity in Rabi (8.0 t/ha) and spring (7.3 t/ha) (2022-23). With high energy value and less anti-nutritional properties like low fibre content, maize is used as food, feed, and fodder as well as a source of thousands of industrial/ processed products (Kumar *et al.,* 2013). About 60% of India's maize consumption goes to feed. A 20% share of maize is consumed as food, with 13% of it consumed directly and 7% in processed form (FICCI Maize Vision 2022; FAO 2023).

High-yielding, stress-tolerant hybrids are needed to meet the escalating demand for maize, driven by population growth and industrial expansion (Duvick, 2005). Additionally, seed production of newly developed maize hybrids plays an important role in improving production and productivity. To cover 11.24 mha of maize under hybrid/improved cultivars in India, nearly 2.25 lakh tonnes of hybrid seed will be needed. Private companies play a significant role in seed supply. The public sector also produces seeds and develops hybrids to control maize seed prices. For *Kharif* 2025, the DAC Govt. of India, demand for public-bred maize hybrid breeder seeds reached its highest level ever (126 q) (https://seednet.gov.in/ SATHI Portal). The DMRH 1301 (for the central western and north east plain zones) and 1308 (for Bihar and the central western zones) released from the ICAR-IIMR Ludhiana for *Rabi* season cultivation in 2017 have had a significant impact on farmers' fields. There should be high genetic purity within the line, a wide range of genetic diversity between the lines, high female productivity (> 3.0 t/ha), pollen availability for a longer period of time, flowering synchrony, broad adaptability, tolerance to major diseases and insect pests, and responsiveness to input. Seed growers and farmers consider all of these factors when producing and adapting hybrids.

Since most hybrid maize seeds are produced in peninsular India, transportation expenses contribute to higher seed costs. To minimize seed costs, alternative farmer-led seed production sites should be established in West Bengal, Bihar, Rajasthan, Madhya Pradesh, and Jharkhand. Important requirements for seed production at a specific location include availability of isolations, farmers' willingness, trained manpower, fertile soil, well-developed irrigation systems, mechanized cultivation, transportation, processing, and storage facilities (DSR, 2016). A lack of genetic purity and environmental factors hamper the widespread adoption of superior hybrids (Singh *et al.,* 2023). Nevertheless, estimating hybrids' impact and documenting their successful seed production can help other breeders design strategies for successful seed production and popularize their hybrids. The purpose of this study was to estimate the impact of selected maize hybrids developed and released from the ICAR-IIMR Ludhiana between 2017-2020, explore their seed production methodologies, and examine their adaptation based on the breeder seeds supplied into the seed chain. We also discussed the challenges and future prospects of strengthening maize seed production in the country through alternative seed hubs.

Materials and Methods

Materials

The selection of parental inbred lines is a foundational step in hybrid maize breeding. The two medium duration field corn hybrids, viz., DMRH 1301 and DMRH 1308, released in Aug. 2017 and notified on 24th January, 2018 [S.O.399(E) S.N.17] (Figure 2) (Kumar *et al.,* 2019) for *rabi* season cultivation, have the BML 6 as a common female and IML 418-1 and HKI 163, respectively, as male parents. The DMRH 1301 was recommended for North Eastern Plain Zone (NEPZ) comprising the states of Eastern UP, Bihar, Jharkhand, West Bengal and Odisha and Central and Western Zone (CWZ)

Figure 2: Photos of Hybrid DMRH 1308 (A), DMRH 1301 (B), LQMH 1 (C) and their ongoing seed production (D-F) from West Bengal, Andhra Pradesh and Telangana

comprising the states of Rajasthan, Gujarat, Chhattisgarh and Madhya Pradesh. Similarly, DMRH 1308 recommended for Bihar [S.O.3482 (E) S.N.50, 7th October, 2020], and Central Western Zone (CWZ) [S.O.399(E) S.N.19, Jan., 24, 2018] comprising the states of Rajasthan, Gujarat, Chhattisgarh and Madhya Pradesh. DMRH 1308 has also been included in the packages and practice of West Bengal for commercial cultivation. Besides, LQMH 1 (S.O. 3482 (E) S.N.38, 07.10.2020) (Figure 2), is the short duration biofortified hybrid released in 2020 (Kumar *et al.,* 2021) for *Kharif* season cultivation in Northern Hill Zone (NHZ) comprising the states of J&K, Himachal Pradesh, Uttarakhand (Hill region), and Northern Eastern Hill region (Meghalaya, Sikkim, Assam, Tripura, Nagaland, Manipur, Arunachal Pradesh). IML 343-1 is the female line and HKI 163 is the male line of this hybrid. The above-given parental lines and hybrids were the basic materials for large-scale seed production and commercial cultivation. Over the last 7 to 8 years, large-scale seed production has been undertaken for them in three states such as West Bengal, Andhra Pradesh, and Telangana.

Standardization of Seed Production

ICAR-IIMR, in collaboration with the state department of agriculture, West Bengal state seed corporation, and farmers groups in Nadia District, West Bengal, sowed the first batch of seed for DMRH 1301 from 3 to 5th November 2018. The female and male were planted in the ratio of 3:1 and 4:1 at four sites separated by >1000 m from each other. Three and four represent female lines, while one represents male lines. In total, 10 hectares of land were considered for sowing, with 2.5 hectares at each site. For both ratios, females and males were sown on the same date, while in another set, males, e.g., IML 418-1, were sown 5 days after females (BML 6) sowing. Since 2021-22, DMRH 1308 has been included in the package and practices of West Bengal state, and has been cultivated there during the rabi season. Seed production for this hybrid was taken up on 10 hectares of land directly in a 4:1 ratio of female to male sown at a 5-day interval,

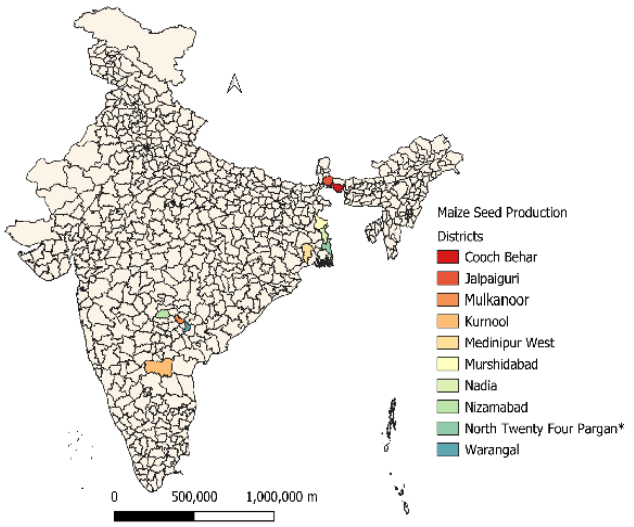

Figure 3: Representation of production sites on map where commercial seed production is going on for DMRH 1308, DMRH 1301 and LQMH1 in Andhra Pradesh, Telangana and West Bengal

e.g., male (HKI 163) was planted after 5 days of female (BML 6) sowing. Additionally, for both hybrids, seed production was started in the Kurnool district of Andhra Pradesh in Rabi 2020-21 and in the Warangal district of Telangana in 2021-22. State farmers were involved in the production through a cooperative located at Mulkanoor in Telangana, and two MSMEs in Andhra Pradesh. In both states, a 4:1 ratio was used for female and male sowing on the same day from 5 to 15th November. This was done with certain agronomical interventions, such as a spray of 2% urea only to females at the knee-high stage. Similarly, LQMH 1 seed production was started in *Rabi* 2021-22 in West Bengal as well as in Andhra Pradesh. The sowing of female and male was taken up on the same day in the ratio of 4:1 in Andhra Pradesh and with a one-week gap between male (sown before) and female in West Bengal. In Andhra Pradesh, 2% urea was sprayed on males at the knee-high stage, repeated the same at 10-day intervals. Now the seed production for all said hybrids have extended to various districts/locations in the state such as Jalpaiguri, Cooch Behar, Murshidabad, Medinipur West, and North 24 pargan in West Bengal and Warangal, Mulkanoor, and Nizamabad in Telangana (Figure 3). The details of the weather conditions, soil types, and geographical locations of all these sites have been provided in Table 1. All recommended packages of practices were followed in each state for growing a healthy crop.

Supporting hybrid seed production

The ICAR-Indian Institute of Maize Research, Ludhiana, also supports the production of hybrid seeds developed by their public partners. As part of their efforts to promote their products, the institute also displays public-bred hybrids *vis-à-vis* private in farmers' fields and at the institute's

Table 1: Meteorological data averaged over the past five years (2018-2022) for seed production sites during the crop period (November to April), along with topographical conditions

District	*State*	*Latitude*	*Longitude*	*Soil Type*	*Temp. (°C) (Max.)*	*Temp. (°C) (Min.)*	*Rainfall (mm/ day)*	*RH (%)*
Nadia	West Bengal	23.471°N	88.5565°E	Alluvial Soil	33.43	12.07	1.16	63.02
Cooch Behar	West Bengal	26.3234°N	89.3227°E	Alluvial Soil	30.21	12.16	1.62	63.02
Jalpaiguri	West Bengal	26.6967°N	88.7109°E	Alluvial Soil	30.87	12.74	1.45	58.97
Murshidabad	West Bengal	24.229°N	88.2461°E	Alluvial Soil	33.36	11.69	0.73	59.34
Medinipur West	West Bengal	22.424°N	87.319°E	Alluvial Soil	32.31	11.31	1.01	69.91
North Twenty Four Pargan	West Bengal	22.72°N	88.7°E	Alluvial Soil	33.57	12.81	1.31	65.83
Kurnool	Andhra Pradesh	15.6443°N	78.1108°E	Red Soil	37.1	16.52	0.39	55.32
Warangal	Telangana	17.9693°N	79.5926°E	Red Soil	35.78	14.62	0.32	54.66
Nizamabad	Telangana	18.6725°N	78.0941°E	Red Soil	36.20	14.50	0.15	45.97
Mulkanoor	Telangana	17.4879°N	80.14281°E	Red Soil	36.20	15.63	0.42	59.33

farm. To accelerate commercialization and large-scale seed production, we also planned visits by state and central government officials, representatives of private organizations, and farmers. The lead breeder of the hybrids and various experts from the institute, as well as state seed corporations, cooperatives, and boards, visit seed production sites in farmers'/government fields every year. They give training to seed growers, officers, and farmers on seed production and commercial maize cultivation technologies. ICAR-IIMR, Ludhiana, plays a leading role in providing training and providing breeders' seeds for further taking up seed production. Seed growers plan their seed production based on advance demands received from state, central, and other agencies for specific hybrids.

Estimating Acreage

For calculating acreage, only the breeder seed supplied through DAC, the Government of India demand, was considered every year. Even for the DAC breeder seed indent, we only considered the female seed quantity since hybrid seeds are purely harvested from female lines. To estimate the lowest acreage possible, each parameter of seed production was taken into account to the minimum extent possible. As an example, if we take 2.0 t/ha female productivity in each category of seed production, such as breeder to foundation and foundation to certified in a 3:1 ratio, then from 1-kg of breeder seeds we get 13333 kg of hybrid seeds. Among the criteria used to identify maize hybrids for release, the female productivity should be at least 2.5 t/ha for medium and late duration hybrids, and around 2.0 t/ha for short duration hybrids. In the rabi season, female line yields range from 2.0 to 3.5 t/ha for both medium duration maize hybrids, such as DMRH 1301 and DMRH 1308. In estimating the acreage, we took the lowest possible female productivity under any circumstances, e.g., 1.5 t/ha for these hybrids. For LQMH 1 hybrid, which is a short-duration biofortified hybrid, we considered 1.2 t/ha female productivity when estimating the acreage, but it produces 2.0 to 3.0 t/ha. Most of the seed production takes place in fertile land during productive seasons with good management practices, so yields will always be higher than what is considered here. Most of the commercial hybrid seeds are produced in a 4:1 ratio. Moreover, sufficient quantities of parental lines seed enter the seed chain every year as per non-DAC demand, e.g., directly received at the institute from seed-producing agencies; however, it was not included in acreage estimations. Therefore, we estimated the possible acreage of hybrids by using all parameters as minimally as possible.

Results and Discussion

The majority of maize hybrid seeds are produced in peninsular regions of the country, particularly in Andhra Pradesh and Telangana. It is a neutral kind of ecology free from the adverse effects of climate (Table 1). In this ecology, maize can be grown all year round. Further, in the regions, well-developed seed processing plants have been established and farmers are well trained. The soil is fertile and suited for maize cultivation (Table 1). Commercial seed production requires good transportation and irrigation facilities, both of which are well developed in these states. Now, West Bengal (WB) is stepping up in a big way, where new sites have been identified for maize seed production. Specifically for public sector hybrids, maize seed is being produced on a large scale. In the last five years, nearly 32465 q (including estimated production for *Rabi* 2024-25) of hybrid seed have been produced for DMRH 1308, DMRH 1301, and LQMH 1. Nadia, Jalpaiguri, Cooch Behar, Murshidabad, and Medinipur West, and North 24 Parganas are the districts of WB where seed production of DMRH 1301, DMRH 1308 and LQMH 1 has been well standardized and taken up on a large scale (Figure 3). November to April (*Rabi* season) is the favourable season for seed production

in the state. Due to excessive rainfall, it is not feasible to take production during the *kharif* season. For sowing, the 4:1 ratio for female to male during the first fortnight of November has been identified as ideal for commercial seed production. Pollen from the male parents (IML 418-1, HKI 163) of all three hybrids is available for 7 to 8 days continuously. As a result, the 4:1 ratio of females and males is highly successful and produces good hybrid seeds. There are two staggered sowings of males in cases of DMRH 1301 and 1308, e.g., one at the time of main sowing (with female), and another after 5 days. At some places, they only take one sowing of the male parent, which is 5 to 6 days after sowing the female parent. In staggered sowing, the 1st male sowing is done with a space of about 35 to 40 cm between plants so that the 2nd sowing can maintain around a 20 cm spacing between plants. They maintained a distance of approximately 60 to 65 cm between rows. The male is sown one week earlier than the female in LQMH 1. The seed production of these three hybrids, such as DMRH 1301, DMRH 1308 and LQMH 1 in Andhra Pradesh and Telangana starts on 20th October and continues till November. The commercial seed production of all three hybrids is carried out in the Kurnool district of Andhra Pradesh, and the Warangal and Nizamabad districts of Telangana. Female and male seeds are sown in ratios of 4:1 and 5:1. A foliar spray of 2% urea is applied at the knee-high stage to the female line in DMRH 1301 and DMRH 1308, i.e., without staggered sowing. For LQMH 1, some sow the male and female on the same day and spray 2% urea on the male at the knee-high stage, or they sow the female six days after the male.

Maize flowering is affected significantly by environmental factors, including temperature, water availability, humidity and photoperiod (day length), which can delay or accelerate flowering and affect yield (Khan *et al.,* 2022; Choquette *et al.,* 2023). Before starting large-scale seed production, it is advisable to check for the flowering synchrony of males and females at the new site. The farmers of these three states are perfectly trained for detasseling of female and other packages of practices for raising a healthy crop. The detasseling of female lines is an essential step in producing hybrid maize seeds (Singh *et al.,* 2021). After tassels emerged from the boot leaf, it continued for about 10 to 12 days. Ideally, this should be done before the anthers begin to burst or shed pollen. This is a must for avoiding selfing in females and hence to get pure hybrid seed. It ensures cross-pollination with the desired male line in hybrid seed production. Hybrid seed production in maize necessitates meticulous management to ensure genetic purity and high seed quality (Macrobert *et al.,* 2014). Controlled pollination techniques such as detasseling and isolation are fundamental practices. Isolation requirements, both spatial and temporal, minimize the risk of outcrossing and maintain the genetic purity of hybrid seeds.

In maize seed production, harvesting is an important activity that impacts seed quality. Physiological immaturity and high moisture content will strongly affect seed germination (Singh *et al.,* 2021). Thus, it should be done when the kernel attains complete physiological maturity, e.g., when a black layer gets fully developed at the kernel base and when the moisture content is < 25%. Harvesting begins in Andhra Pradesh and Telangana in mid-March and in West Bengal in mid-April. They first harvest males and remove them from the field for use as commercial grain, then they harvest females for use as hybrid seed in bulk. In general, farmers are harvesting 2.5 to 3.5 t/ha hybrid seed yields for DMRH 1308 and DMRH 1301. However, in the case of LQMH, which is a short-duration hybrid, they are able to harvest 2.0 to 2.5 t/ha of hybrid seed yield.

Every year, ICAR-IIMR Ludhiana is demonstrating public-bred hybrids *vis-à-vis* private at its experimental farms situated at Ludhiana, Delhi, Begusarai and Hyderabad. Stakeholders from all backgrounds are invited to showcase the strength of public-bred hybrids. By doing so, the institute has been able to popularize the technologies among various stakeholders. Through the DAC, the highest ever demand has been received for public sector maize bred hybrids in *Kharif* 2025 (126 q). The share of public sector-bred cultivars of maize has increased to nearly 25%. In recent years, public-bred hybrids have been commercialized in a significant way. 24 different private seed companies have adopted DMRH 1301, DMRH 1308 and LQMH 1 [DMRH 1301(12), DMRH 1308 (17) and LQMH 1(7) with the institute (Table 2). In the past four years, IIMR-bred hybrids topped the DAC maize breeder seed demand with shares ranging from 26.4 to 62.4% [Anonymous 2023 & 2024 (in press)]. In this, DMRH 1308 has consistently topped the country for the last four years with its breeder seed share ranging from 20.1 to 34.9% [Anonymous 2023 & 2024 (in press)]. The ICAR-IIMR acknowledged the active participation of West Bengal State Seed Corporation, Kolkata, the state department of Agriculture, WB, National Seeds Corporation, New Delhi, and Rajasthan State Seed Corporation, Jaipur. During the seed production of said hybrids, the lead breeder, Director IIMR, and various state and central officials visited seed production sites in the states and conducted trainings, held on farm discussions, delivered lectures on seed production, commercial maize cultivation, and interacted with a variety of stakeholders including farmers, cooperatives, MSMEs, and state officials. As of today, >10 readings have been delivered, and 7 training sessions have been conducted for the stakeholders involved. Each year, IIMR conducts training and pays visits to the various stakeholders who produce hybrid seeds directly in contact with the institute and collect the figures on hybrid seeds produced. As result, so far around 50,625 q (including the estimated figure for Rabi 2024-25 production) hybrids seeds are produced for these hybrids

Table 2: Details of breeder seed supplied as per DAC Govt of India demand as well as through non-DAC channels, hybrid acreage and MoUs signed by private seed companies

Year	*Total breeder seed demand through DAC (kg)*	*Female breeder seed demand through DAC (kg)*	*Parental seed* demand other than DAC (kg)*	*Estimated Area Coverage (000' Hectares) (based on female DAC breeder seed supplied)*	*MOU signed (No.)*
DMRH 1301					
2018-19	0.0	0.0	1405.0	0.0	0.0
2019-20	8.0	5.0	240.0	1.9	3.0
2020-21	220.0	140.0	1800.0	52.5	4.0
2021-22	255.0	150.0	345.0	56.3	3.0
2022-23	600.0	380.0	555.0	142.5	1.0
2023-24	555.0	350.0	0.0	131.3	0.0
2024-25	199.0	150.0	0.0	56.3	1.0
2025-26*	135.0	90.0	0.0	33.8	0.0
Total	1972.0	1265.0	4345.0	474.6	12.0
DMRH 1308					
2019-20	0.0	0.0	0.0	0.0	1.0
2020-21	30.0	20.0	1316.0	7.50	1.0
2021-22	55.0	35.0	6982.0	13.13	0.0
2022-23	963.0	642.0	4421.0	240.75	2.0
2023-24	1290.0	860.0	6910.0	322.50	3.0
2024-25	2290.0	1527.0	8936.0	572.63	4.0
2025-26*	2745.0	1830.0	0.0	686.25	0.0
Total	7373.0	4914.0	28565.0	1842.76	11.0
LQMH 1					
2021-22	15.0	10.0	30	2.4	2.0
2022-23	42.0	28.0	18	6.7	3.0
2023-24	0.0	0.0	0	0.0	0.0
2024-25	275.0	255.0	410	61.2	1.0
2025-26*	393.0	263.0	0	63.1	0.0
Total	725.0	556.0	458.0	133.4	6.0

Note*: 1 During 2021-22, 2794 Kg, during 2022-23, 160 Kg, in 2023-24, 510 kg, during 2024-25, 1400 Kg female line seed of DMRH 1308 was produced and sold @ breeder seed price through AICRP on Maize Hyderabad. 2. During 2021-22, 783 Kg, in 2022-23, 3174 Kg, in 2023-24, 6600 Kg and in 2024-25,6200 Kg parental lines seed of DMRH 1308 and 300 Kg of LQMH 1 in 2024-25 and 150 Kg of DMRH 1301 in 2023-24 was produced and sold @ foundation seed price through Zonal Adaptive Research Station Krishnagar Farm, Nadia WB. Rest of the quantity was produced at IIMR Farm Begusarai Bihar. For 2025-26, the advance DAC demand received from DAC Govt. of India for DMRH 1308, DMRH 1301 and LQMH 1 has been mentioned. It will be ready by May 2025 as it is being produced in Rabi 2024-25.

by selected agencies which collaborate and are in contact with ICAR-IIMR. In this total, nearly 75% of production is for DMRH 1308 and the remaining 24 and 1% are produced for DMRH 1301 and LQMH 1, respectively.

Using the DAC Govt of India female seed demand for each hybrid from release to 2024, the total estimated acreage for DMRH 1308, DMRH 1301 and LQMH 1 was calculated as 11.57, 4.41 and 0.70 lakh hectares, respectively (Figure 4a-c). In addition, the highest ever DAC breeder seed demand of DMRH 1308 (2445 Kg) has been received for *Kharif* 2025 as well. Based on female demand for it, the additional 6.86 lakh hectare acreage can be estimated over the given figure (Table 2, Figure 4a-c). Besides the DAC, sufficient quantities of parental seed were also supplied to various seed-producing agencies according to their demands (Figure 4a-c). To meet this demand, along with IIMR farms, production was also taken up in the AICRP's partners and state department farms (Table 2). A substantial contribution has been made to improve maize productivity and farmer income through the release and cultivation of DMRH 1308, DMRH 1301, and LQMH 1. Since release, there has been a good quantity of hybrid seed production and commercial cultivation for DMRH 1301 and DMRH 1308 in the recommended states. Further, LQMH 1 is also gaining popularity and is expected

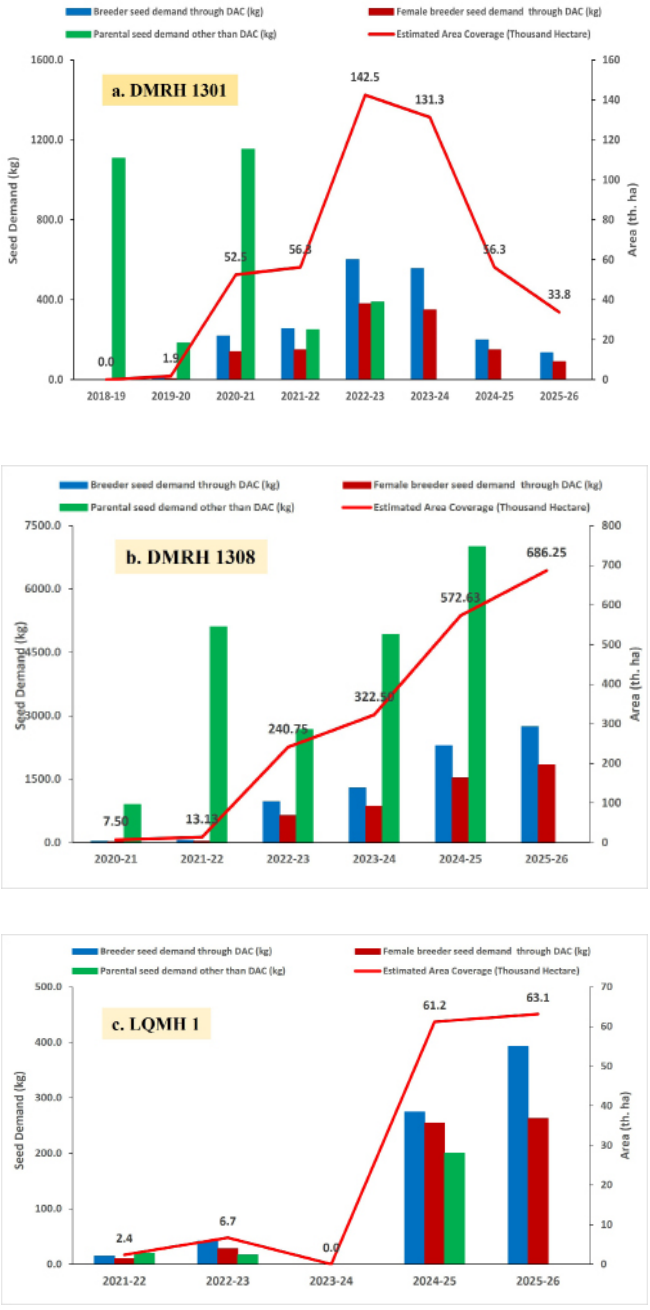

Figure 4: Details of year-wise acreage calculated based on female line breeder seed supplied as per the DAC, Govt. of India demand. The detailed procedure of same has been explained in materials and methods

to have more impact in the times to come. A significant impact is also seen in the fact that WB has the highest Rabi maize productivity (7.65 t/ha) in the country (DAC, 2021-22) where a significant quantity (around 65% of the total hybrid seed produced) of DRMH 1301 & 1308 hybrid seed is produced and supplied into the seed chain. We are working on identifying alternate seed hubs/new sites in states such as Uttar Pradesh, West Bengal, Punjab, and Rajasthan to produce maize seeds. It will help in controlling seed price and the timely supply of good-quality seed for farmers. The seed is the basic input in any crop and plays an important role in expressing its commercial potential. In order to avoid detasselling in hybrid maize seed production, the cytoplasmic male sterility system (CGMS) should be explored to cope with labour shortages. Yet challenges related to seed access, affordability, and equitable benefits remain critical considerations, particularly for smallholder farmers (Almekinders *et al.,* 2019). Integrating advanced breeding technologies, promoting sustainable agricultural practices and implementing supportive policy frameworks are crucial for ensuring better-quality seed in maize.

Conclusion

The private sector plays a major role in maize seed supply, while public institutions also develop hybrids to control prices and face climate change. The two hybrids, such as DMRH 1301 and DMRH 1308, developed and released from ICAR-IIMR Ludhiana in the year 2017, have created a significant impact. Since their release, these two hybrids are in the seed chain and have been taken up by 24 different private seed companies by signing a total of 29 MoUs with the institute. Based on the DAC breeder seed demand for females, the total estimated acreage was calculated as 11.57 and 4.41 lakh hectares for DMRH 1308 and DMRH 1301, respectively. Similarly, LQMH 1 is also gaining popular after its release (2020) and has an estimated coverage pf 0.70 lakhs hectares. This hybrid has been taken up by six different private seed companies. Still, there are challenges in seed access, affordability, and equitable benefits, especially for smallholders. Integrating advanced breeding, sustainable practices, and supportive policies is crucial for ensuring better-quality maize seed.

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REVIEW ARTICLE

The Potential Uses of *Indigofera* L. in India

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Abstract

Indigofera is an important genus of arid, semi-arid and temperate regions of India. Its species are significant as forage, particularly in hot arid regions and temperate zones, as they provide good grazing alongside native grasses. *I. oblongifolia* Forssk. and *I. cordifolia* B. Heyne ex Roth are important fodder species for sheep, goats, camels and cattle in hot arid regions. The seed of *I. cordifolia* is the source of food during times of scarcity and famine. The species *I. atropurpurea* Buch.-Ham. ex Hornem., *I. gerrardiana* Harv., *I. heterantha* Wall. ex Brandis and *I. pulchella* Roxb. are sources of good quality fodder and rich in protein in the Himalayan region. The research on the exploitation of *Indigofera* has been considerably less compared to other pasture legumes. Therefore, the present paper highlights the habits and potentials of *Indigofera* species for its forage, food, medicinal and other ethnobotanical information, and emphasizes its sustainable utilization and future prospects.

Keywords: Desert, Forage, *Indigofera*, Legume, Medicinal

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Introduction

The genus *Indigofera* Linn. belongs to the Leguminosae family, which is the third-largest genus encompassing approximately 700 - 750 species of annual and perennial herbs and shrubs found in warm regions (Schrire, 2005; Schrire *et al*., 2009). It is distributed in tropical and subtropical regions of the world with the major centers of diversity in Africa, Madagascar, Australia, the Sino-Himalayan region and the New World (Clark *et al*., 2015; Wilson and Rowe, 2015). In India, *Indigofera* is one of the five dominant genera, holding the fourth position in the tribe Indigofereae. It is also the second most prevalent endemic legume genera in India, with 21 endemic species (Rao and Chaudhary, 2002). This genus thrives in diverse forms across various niches in both tropical and temperate regions of India and represents 64 species (e-checklist, BSI). After that, two new species of *Indigofera* i.e. *Indigofera jaisalmerica* C.S. Purohit & Kulloli (Purohit and Kulloli, 2021; POWO, 2024) and *Indigofera jodhpurensis* Bhellum, Dhar & Magotra (Bhellum *et al*., 2023) reported from India. Despite their importance, the species of *Indigofera* have received limited attention in hot arid areas (Singh and Beniwal, 2005). Rajasthan state is home to a total of 29 reported species of *Indigofera* (Shetty and Singh, 1987). However, in western Rajasthan, the genus is well-represented, with 12 species occurring in different habitats (Bhandari, 1990; Purohit and Kulloli, 2021; Bhellum *et al*., 2023). These species exhibit remarkable resilience to harsh environmental conditions, such as extreme temperatures and drought in the Thar desert, and can withstand overgrazing

(Singh *et al*., 2023). These species thrive on a variety of soils and rainfall levels, adapting to a broad temperature range. *I. argentea* Burm.f. a typical dune pioneer legume, is primarily found on sand dunes in western Rajasthan. It possesses strong, long woody tap roots that descend deeply in search of water. *I. linifolia* (L.f.) Retz., grows prostrate on sand but becomes erect when grazed (Bhandari, 1993). *I. cordifolia* B.Heyne ex Roth, the most common annual species, emerges after the first rains on both open rocky slopes and sandy to gravelly ground. It is known to be an effective soil binder. *I. hochstetteri* Baker and *I. linnaei* Ali thrive on stable sandy soils. *I. oblongifolia* Forssk, an erect arid shrub, occupies open dry areas with stable sandy soils in the arid region.

The genus *Indigofera* thrives across a wide range of habitats, even in areas with low rainfall and it is also known for its rich nutrient content (Singh *et al*., 2006b). *Indigofera* species serve as crucial fodder sources in arid, temperate, and subtropical Himalayan regions. Most *Indigofera* species exist in the wild and are grazed by animals, particularly sheep and goats. They are also harvested as cut-and-carry fodder. Due to heavy grazing pressure, many species and their ecotypes are on the verge of extinction, especially in the Thar desert of western Rajasthan. During times of scarcity and famine, the seeds of some species serve as food sources. Chatterji and Baxi (1964) studied the germination behavior of seeds from five *Indigofera* species. The genus *Indigofera* has garnered attention previously in the realm of chemotaxonomy (Bhalla and Dakwale, 1978; Mishra *et al*., 1981) as well as for anatomical studies (Kumar, 1983). Subudhi and Mitra (1999) explored *Indigofera* as an underexploited forage legume. In arid regions, some species exhibit high seed yields even in poor soil conditions. Nataraja *et al*. (2002) observed abundant seed production in *I. cordifolia* with a significant portion of the seeds returning to the soil, contributing to its dominance in seed composition. Many of these seeds persist in the soil seed bank, demonstrating dormancy as a natural mechanism for ecological maintenance and long-term persistence. However, little research has been conducted on its potential as a fodder, medicinal and other ethnobotanical utilization. This paper presents the current state of knowledge on the economic importance of *Indigofera*, highlights key species with potential, and identifies future research needs, particularly for hot arid and temperate regions.

Table 1: Traditional food usage of *Indigofera* species in India

Species	*Habit*	*Food*
I. atropurpurea Buch.-Ham. ex Hornem.	Erect shrub	Gujjars in Uttarakhand cook the flowers as a vegetable (Prakash and Singh, 2002).
I. cassioides Rottler ex DC.	Under shrub	Flowers and flower buds are used as vegetables by tribal people in Maharashtra, Madhya Pradesh and Odisha (Datt, 1996; Snehalatha *et al*., 2022). Pods are also utilized as vegetables in Haryana and sold in local markets (Jain, 1984). Snehalatha *et al*. (2022) reported this plant as wild medico food plant. Its roots are used as a tonic by the tribal women after delivery. Tender leaves are used to cure cough by the Bhuian tribe. The decoction of flowers and stems is used as a tonic by tribal communities of Sundargarh and Mayurbhanj areas of Odisha.
I. cordifolia B. Heyne ex Roth	Annual herb	Seeds are mixed with sorghum (jowar) and pearl millet (bajra) to make bread in arid regions. This bread is palatable and poses no harmful effects on health (Gupta and Kanodia, 1968).
I. dosua Buch.-Ham. ex D.Don	Erect shrub	Utilized as a food source in the Himalayas.
I. glandulosa J.C.Wendl.	Annual herb	Tribals incorporate the seeds with other grains during periods of scarcity. The plant is harvested from the wild for local uses as a nutritive tonic. In times of need the plant is harvested from the Wild for its seeds which are an emergency source of food. The seed flour was used to prepare roti during extreme. (Mhaske and Kulkarni, 2022).
I. hebepetala Benth. ex Baker	Erect shrub	Sour flowers are used with vegetables in Bihar (Sen *et al*., 1986) and immature seed pods are cooked as a vegetable or are pickled (Tyagi *et al*., 2019).
I. heterantha Wall ex Brand. var. *gerardiana*	Erect shrub	Flowers are boiled and pickled and consumed in the Himalayas (Tyagi *et al*., 2019).
I. hochstetteri Baker	Annual herb	During times of scarcity, seeds are combined with other grains. They are ground and mixed with flour from other grains for consumption (Bhandari, 1990).
I. linifolia (L.f.) Retz.	Annual herb	The seeds, such as Italian millet, are threshed and ground into flour to make bread during periods of scarcity in arid regions (Gupta and Kanodia, 1968).
I. linnaei Ali	Annual herb	Tribal in Rajasthan consume the fruits raw (Katewa, 2003).
I. suffruticosa Mill.	Shrub	Utilized as a vegetable (Sen *et al*., 1986).
I. arrecta Hochst. ex A.Rich.	perennial herb	Young leaves are cooked and eaten as a vegetable (Tyagi *et al*., 2019).

Utilization Potential of Indigofera

The species of *Indigofera* genus has much potential as food, fodder, medicine, dye, green manure, fuel wood and others. The detailed species-wise utilization potential and traditional uses are described as under.

Food

Indigofera species have traditionally been used as food in India, especially during periods of scarcity (Table 1). The seeds of some *Indigofera* species are mixed with other food grains to make bread during times of famine. The seeds of *I. glandulosa* J.C.Wendl. are rich in valuable food ingredients like proteins, carbohydrates, essential amino acids and vitamins (Ghane *et al*., 2010). According to Mankad (2002), its nutritive seeds contain approximately three times more protein than wheat. Seeds of *I. cordifolia* are also used in arid regions. Additionally, the flowers and flower buds of *I. cassioides* Rottler ex DC. are consumed as vegetables and its pods are sold in local markets in Haryana (Jain, 1984).

Fodder

Several species of *Indigofera* are utilized as fodder in various regions of India, particularly in temperate and arid areas (Table 2). In western Rajasthan, *Indigofera* species hold significant importance in grazing lands, especially for sheep and goats. Farmers have been collecting and storing them as hay for an extended period. In this region, most of the herbaceous species of *Indigofera* are referred to generically as Bekaria, such as Bekaria (*I. cordifolia*), Sidio bakerio (*I. linnaei*), Aadio bekario (*I. hochstetteri*), Lambio bekario (*I. linifolia*) among others. *I. cordifolia,* locally known as Bekaria, plays a crucial role in the natural grasslands and pastures of western Rajasthan, often forming dominant colonies after the onset of monsoon rains. Sheep, goats and cattle in this region prefer it as forage. It is harvested, dried and stacked for stall-feeding during lean periods in Rajasthan, sometimes mixed with other feeds (Mertia, 1992; Kulhari and Joshi, 1992). Pigeons and ants also show a special preference for the pods of *I. cordifolia* (Chatterji and Baxi, 1964). In Rajasthan, *I. linnaei*

Table 2: Fodder usage of *Indigofera* species in India

Species	*Habit*	*Animal preference in descending order*
I. argentea Burm.f.	Perennial herb	Grazed by various animals and believed to enhance milk production (Singh *et al*., 2006b).
I. astragalina DC.	Annual herb	Grazed by goats and sheep.
I. atropurpurea Buch.-Ham. ex Hornem.	Erect shrub	Given to milking cattle in Garhwal (Rana *et al*., 2002).
I. caerulea Roxb. var. *caerulea*	Perennial herb/ under shrub	Used as fodder in western India and Bihar
I. cassioides Rottler ex DC.	Under shrub	Given to milking cattle and goats in Garhwal (Rana *et al*., 2002).
I. cordifolia B.Heyne ex Roth.	Annual herb	Considered good fodder for sheep, goats and cattle. Stored for stall feeding (Singh *et al*., 2006b).
I. cylindracea Graham ex Baker	Erect shrub	Browsed by animals in the Himalayas.
I. dosua Buch.-Ham. ex D. Don	Erect shrub	Eaten by buffaloes, sheep, and goats in Himalayas.
I. glandulosa J.C.Wendl.	Annual herb	Preferred by cattle in the green stage.
I. hebepetala Benth. ex Baker	Erect Shrub	Given to milking cattle in Garhwal (Rana *et al.,* 2002).
I. heterantha Wall. ex Brandis var. *heterantha*	Erect shrub	Eaten by sheep, goats, and horses in the temperate and subtropical Himalayas
I. heterantha Wall. ex Brandis	Erect shrub	Believed to be good fodder for sheep and goats in Garhwal (Negi *et al.,* 1993).
I. hirsuta L.	Annual herb	Grazed by all animals and also used as hay.
I. linifolia (L.f.) Retz.	Annual herb	Grazed by all animals.
I. linnaei Ali	Annual herb	Eaten by sheep, goat and cattle across India
I. oblongofolia Forssk.	Erect shrub	Browsed by sheep, goats and camels in Rajasthan (Singh *et al*., 2023).
I. sessiliflora DC.	Annual herb	Grazed by animals in western Rajasthan (Singh and Beniwal, 2005), believed to enhance milk yield and fat content in goats, sheep and, camels (Singh *et al*., 2006b).
I. spicata Forssk.	Perennial herb	Grazed by cattle
I. suffruticosa Mill.	Erect shrub	Utilized as fodder
I. uniflora Buch.-Ham. ex Roxb.	Perennial herb	Preferred by cattle
I. jaisalmerica C.S. Purohit & Kulloli	Prostrate herb	Local people used as a fodder for cattle in Jaisalmer area of Rajasthan (Purohit and Kulloli, 2021).

is highly favored by sheep, leading shepherds to refer to it as *Bhed-ka-chara*; infect, sheep often graze on it instead of many other good grasses (Paul, 1966). The substantial presence of phytochemicals such as total phenolic, total flavonoids, and total antioxidant capacity in the leaves of *I. oblongifolia* promotes it as a valuable browse species in the rangelands of the Indian hot arid region, particularly in western Rajasthan (Singh *et al*., 2023).

Equally significant are the temperate and subtropical species of *Indigofera*, such as *I. atropurpurea* Buch.-Ham. ex Hornem., *I. gerrardiana* Harv.*, I. heterantha* Wall. ex Brandis*, I. pulchella* Roxb., collectively referred to as *Sakina* in Uttrakhand. These species serve as good quality fodder, rich in protein. Notably, *I. hirsuta* L. is a self-seeding erect annual species widely distributed across India. It is grazed by various animals and is particularly valued for semi-arid

Table 3: Medicinal value of *Indigofera* species

Species	*Habit*	*Ethno-medicine*
I. argentea L.	Herb	The roots and leaves are bitter and tonic. The seeds are considered anthelmintic and are used to expel the taenia (Caius, 1989).
I. aspalathoides Vahl ex DC.	Erect undershrub	A decoction made from tender shoots, leaves and flowers is used for treating leprosy and cancerous conditions. The root is chewed to alleviate toothache, while the leaves are applied to abscesses (Caius, 1989).
I. cordifolia Heyne ex Roth.	Annual herb	It is reported to be useful for lung and kidney diseases in Pakistan (Rahim, 1988).
I. glabra L.	Annual Herb	The leaves are bitter, tonic, and febrifuge, and they are also applied externally as an emollient (Caius, 1989).
I. glandulosa J.C. Wendl.	Annual herb	The seeds are used as a nutritive tonic (Caius, 1989; Ghane *et al*., 2010).
I. heterantha Wall. ex Brandis var. *gerardiana* Ali	Erect shrub	Caius (1989) mentioned that the roots are anthelmintic. Leaves possess irritant and purging properties and are administered for inflammation of the liver.
I. hirsuta L.	Annual herb	The leaf decoction is employed in Gold Coast as a lotion for yaws, while the fruit is utilized in treating eye disease in Senegal (Caius, 1989).
I. linifolia (L.f.) Retz.	Annual herb	Used as a remedy for the eyes in combination with other drugs in Madhya Pradesh (Sen *et al.,* 1986). Root given for bowel complaints in West Bengal (Sen *et al.,* 1986). The whole plant is used in post-delivery fever and spermatorrhoea by the Kols of Uttar Pradesh. Plant juice is employed as an alternative and diuretic, and is considered an alternative in old venereal affections. Santhals use the plant in amenorrhoea along with *Euphorbia thymifolia* (Caius, 1989).
I. linnaei Ali	Annual herb	Root decoction is given for antifertility; plant decoction is used for diarrhea, dysentery, and stomachache in Andhra Pradesh (Venkata Raju, 2002). Leaf juice is used for the reddening and inflammation of eyes by the Kols of Uttar Pradesh. The decoction of the whole plant is used in epilepsy. The plant juice is a diuretic, alterative and it is also used to treat chronic venereal diseases. Leaf juice is used as eye drops to alleviate migraines. Seed powder with milk is taken as a tonic for general health (Kumar *et al*., 2005).
I. oblongofolia Forssk.	Erect shrub	It is considered an antidote to all kinds of poison. The root is used as a purgative, and the stem decoction is used as a gargle in mercurial salivation (Caius, 1989). It is also used for toothbrushes and to alleviate stomachache in Rajasthan (Bhandari, 1990).
I. pulchella Roxb.	Shrub	The Santhals used root decoction to cure cough, and root powder used to cure chest pains (Caius, 1989). The root is also used to cure cough in Manipur (Sinha, 1987).
I. tinctoria L.	Erect shrub	The root is used for liver inflammation in South India and is considered a valuable nervine tonic. Leaf juice is employed as a remedy for hydrophobia, administered both internally and externally. A leaf ointment is applied to inflamed or itchy parts in Indo-China. Plant extract is given for epilepsy and nervous disorders and is also used in bronchitis, as well as an ointment for sores, old ulcers, and hemorrhoids. The root, pounded and macerated in water, is taken for urinary complaints by the Mundas of Chota Nagpur (Caius, 1989). Leaves are used for epilepsy in Arunachal Pradesh (Sharma *et al.,* 2002). Root decoction is used for fever in Andhra Pradesh (Venkata Raju, 2002). Root juice, mixed with *Eclipta prostrata* in coconut oil, is applied as a hair tonic in Andhra Pradesh (Venkata Raju, 2002). Plant paste is applied on warts (Singh, 2002). The root is used for wounds, and foul ulcers in Arunachal Pradesh (Saklani and Rao, 2002). Root powder is given for blood dysentery (Sen *et al.,* 1986).
I. trifoliata L.	Annual herb	The seeds are prescribed along with other mucilaginous drugs as a restorative (Caius, 1989). They are also used as an aphrodisiac in Arunachal Pradesh (Sharma *et al.,* 2002).
I. trita L.f.	Herb/shrub	The seeds are used as a nutritive tonic (Caius, 1989).
I. wightii Graham ex Wight & Arn.	Erect shrub	The leaves are used in treating dysentery in the Western Ghats (Henry *et al*., 1996).

grasslands. Similarly, *I. glabra* L., *I. trifoliata* L. and *I. prostrate* Willd. are important forage species and can be used for the improvement of grasslands/rangelands in Orissa and other parts of India (Subudhi and Dikshit, 2000).

Indigofera species exhibit toxic effects when consumed by animals, which is a limiting factor for their utilization as a forage resource. For instance, *I. hirsuta* has been reported to be toxic to cattle, leading to swollen legs that become scabby, cracked and may even bleed (Singh and Pandey, 1998). *I. linnaaei* has been associated with causing 'Birdsville disease' in horses in Australia (Henty, 1980), while *I. stricta* L.f. has been documented to induce abortion in cows. Animals feeding on *I. spicata* Forssk. have also displayed various disorders, resulting in weight loss and abortion (Henty, 1980).

Medicinal Value

Several species of *Indigofera* have traditionally been used to treat a variety of diseases and ailments (Table 3). *I. oblongifolia*, known as Raktpala in Ayurveda, is an important medicinal species in arid regions. *I. tinctoria* is also utilized for its medicinal properties. Various species of *Indigofera* exhibit confirmed biological activities. *I. cassioides* has antiviral properties, *I. mysorensis* Rottler ex DC. is anticancer, and *I. trita* L.f. ssp. *subulata* is hypotensive (Ved Prakash and Rajendran, 2002). *I. aspalathoides* Vahl ex DC. , known as the South Indian detergent, has plant ash that is used to remove dandruff and is a key ingredient in specific oils for treating syphilis and other skin diseases (Caius, 1989). Singh *et al*. (2023) provided an in-depth exploration of the medicinal aspect of *I. oblongifoilia*. Indigo is a natural substance that can be used as dyestuff, it is extracted from *Indigofera* species and can be used for the treatment of various diseases, such as epilepsy, bronchitis, liver disease and psychiatric illness (Anand *et al*., 1979).

Dye

Indigofera species have long been renowned in India for dye preparation, dating back to ancient times. Certain species such as *I. tinctoria* were historically the main sources of Indian indigo, crucial for blue dye production. Additional species like *I. caerulea* and *I. dosua* were also utilized for this purpose. Moreover, *I. oblongifolia* is employed as a dye plant in Mali and Zimbabwe (Mansfeld, 2001). The species like *I. atropurpurea* and *I. heteantha* are important floral dye-yielding plants in the Himalayan region (Gaur, 2008).

Green Manure

Indigofera species are traditionally used for green manure in India. *I. oblongifolia* is widely employed for this purpose. *I. teysmanni* Miq. is grown on field bunds to provide green manure on-site. *I. tinctoria* was once used as green manure for cotton and maize crops in Punjab and was also cultivated in rotation with tobacco and sugarcane.

Basket

Species such as *I. atropurpurea* and *I. hebepetala* are used for making baskets in Garhwal (Rana *et al*., 2002). Additionally, *I. caerulea* var. *caerulea* is used for both basket and rope making.

Fuel

The stem and branches of *I. cassioides* and *I. heterantha*. var. *gerardiana,* is also used as fuel in the Himalayas (Samant *et al*., 2007; Snehalatha *et al*., 2022).

Soil Conservation

Species such as *I. argentea, I. cassioides*, *I. heterantha* var. *gerardiana*, and *I. sessilioflora* DC. are effective soil binders. Additionally, *I. heterantha* var. *gerardiana* is used to provide shelter for seedlings.

Future Prospects

In India, the tropical pasture legumes currently cultivated are predominantly exotic species such as *Stylosanthes guianensis* (Aubl.) Sw., *S. hamata* (L.) Taub., *S. humilis* Kunth, *S. scabra* Vogel, *S. viscosa* (L.) Sw., *Macroptilium atropurpureum* (DC.) Urb., *M. lathyroides* (L.) Urb. etc., which are particularly suited to semi-arid conditions but may struggle in harsh arid environments (Singh and Beniwal, 2005). Hence, there is a necessity to explore and identify legume species adapted to hot arid conditions. Fortunately, the Thar Desert and Himalayan region harbor a vast array of naturally occurring wild *Indigofera,* ranging from prostrate annual herbs, to erect perennial herbs and shrubs, thriving across various landforms. *I. oblongifolia* and *I. cordifolia* are important fodder species for sheep, goats, camels and cattle in hot arid regions. The seed of *I. cordifolia* is the source of food during times of scarcity and famine. *I. oblongifolia* is one of the important traditional medicinal plant species in arid and semi-arid regions. The *Indigofera* species such as *I. tinctoria*, *I. caerulea*, and *I. dosua* are used as blue dye, but, *I. tinctoria* was once the main source of blue dye production in India. The species *I. atropurpurea*, *I. gerrardiana*, *I. heterantha* and *I. pulchella* are sources of good quality fodder, rich in protein in the Himalayan region. Therefore, attention should be given to suitable *Indigofera* species for arid and temperate regions. The comprehensive exploration and evaluation of important *Indigofera* species should receive high priority. Wild trailing species of *Indigofera* can enrich grazing land at a low cost. *Indigofera* seeds, which have high protein content, could serve as a rich source of animal feed concentrate. The genus also has potential as a source of gum. Additionally, many species have significant food, green manure, and medicinal value in rural and tribal areas, making them candidates for validation and eventual integration into farming systems for diversified agriculture. Woody perennials with medicinal value should be integrated with crop-based production systems to ensure sustained economic gains. However,

research is needed to assess their agronomic potential in both hot arid regions and the Himalayas.

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SHORT COMMUNICATION

Identification, Description, and Establishment of a Year-Round Fruiting Type in Jackfruit (*Artocarpus heterophyllus* Lam.) Germplasm

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Abstract

A unique jackfruit germplasm (IC-0650715) with year-round fruiting is identified, characterized and conserved at the ICAR-NBPGR regional station, Ranchi. The germplasm was collected from Domtoli, Simdega and grafted plants are conserved in the field gene bank. Jackfruit in Jharkhand is primarily used for vegetable purposes and is relevant for meeting consumer demands during off-seasons. IC-0650715 fetches a higher price during the offseason, offering a commercial advantage to farmers.

Keywords: Jackfruit, *Artocarpus heterophyllus* Lam., year-round fruiting, Germplasm conservation.

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Introduction

Jackfruit (*Artocarpus heterophyllus* Lam.) is a member of the Moraceae family and originates from India's south-western rainforests (Boning, 2006). It is cultivated in tropical regions such as India, Bangladesh, Nepal, Sri Lanka, Cambodia, Vietnam, Thailand, Malaysia, Indonesia, Myanmar, and the Philippines due to its numerous applications and nutritional benefits. Compared to other fruits, jackfruit has higher levels of protein, calcium, iron, vitamins, and other essential minerals (Prem *et al*., 2015). Despite its nutritional advantages, jackfruit is considered an «underutilized crop» due to ignorance, lack of post-harvest technology, and shortcomings in supply chain systems leading to the wastage of fruits (Nair *et al*., 2017).

Jackfruit is cultivated across India, with certain states, such as Jharkhand, being particularly popular for its cultivation (Choudhary *et al*., 2022). According to the annual report of the National Horticultural Board of India, for the year 2021-22, Jharkhand ranked sixth in jackfruit production. Jackfruit cultivation in Jharkhand is a significant agricultural practice with social and cultural importance. The Sanskrit name for jackfruit, «phanasa,» is thought to be borrowed from Mundari, the indigenous language of the Mundas, a tribal ethnic group of Jharkhand, Madhya Pradesh, Bihar, and Odisha (https://map-india.org/). The Mundas have a rich intangible folk tradition, reflected in activities like the Jackfruit game, also known as Kantara-Lnu. In Jharkhand, jackfruit is commonly found along roadsides and in tribal communities' homesteads. The jackfruit is a versatile species that provides food, timber, fuel, fodder, medicinal, and industrial products. The fruit is the primary

economic product, used both when mature and immature. The flakes obtained from ripened fruit are sweet and are used as a dessert or preserved in syrup. The fruits and seeds are also processed for food and other products. Jackfruit value-added products include chips, papads, pickles, ice cream, jelly, sweets, beverages like squash, nectar, wine, preserved flakes, etc. When unripe (green and tender), it is remarkably similar in texture to meat. Tender jackfruits are used for vegetable purposes and have gained popularity as a vegetarian alternative to meat due to their similar texture (APARI 2012, Stukin, 2016). Un-ripened jackfruit, preserved in brine and canned, is known as «vegetable meat.»

Using tender jackfruit for vegetable purposes is an age-old practice in India, especially in Bihar and Jharkhand. Usually, off-season jackfruit, available early or late in the season, is used for vegetable purposes. The jackfruit genotype that produces fruits throughout the year is quite popular in Jharkhand. It provides a consistent source of fruits that command higher prices in the off-season and is specifically used for vegetable purposes. Additionally, it is of great interest to researchers due to its unique biological characteristics and potential agricultural applications.

Based on the information obtained from the locals, we identified a prolific, year-round fruit producer in Domtoli, Simdega. The geo-coordinates collected during the collection are mapped using DIVA GIS (Figure 1). Simdega, situated in the south-western part of Jharkhand and part of the Netarhat-Ranchi plateau region, is at 22.62°N 84.52°E, with an average elevation of 418 m (1371 feet). The temperature varies from 3 to 38°C, and the average annual rainfall is around 1100 to 1200 mm.

Scions were collected from the source tree and propagated through grafting. The grafts were then allowed to grow for one year under net house conditions. This ensured the establishment of graft and the development of roots. Subsequently, the grafts were transferred to the field gene bank at ICAR-NBPGR regional station, Ranchi, for conservation. The passport data was deposited to ICAR-NBPGR, New Delhi, for IC number (Table 1). The IC number assigned to this accession is IC No-0650715.

IC No. 0650715 produces fruit round the year and is locally known as Barahmasi/Barahmoia kathal. The term is derived from two words «Barah,» which means twelve and «Mas/Mah» which means months.

The morphological traits of the source tree were recorded under field conditions (Figure 2). The observations were made according to the DUS descriptor developed by PPV & FR Authority India. The tree crown shape is spherical. Leaves are obovate with an acuminate apex, rounded base, and blisters on the upper surface. Leaf orientation is horizontal and the posture is flattened. Clavate-shaped fruits are born solitary throughout the tree, including roots. Stalk's attachment to fruit is depressed. Fruits are medium-sized and brown-coloured with sparse, pointed spines on the rind. The inner rind is thick, the core diameter is low and the latex exudate is high. Mature fruit bears soft, sweet,

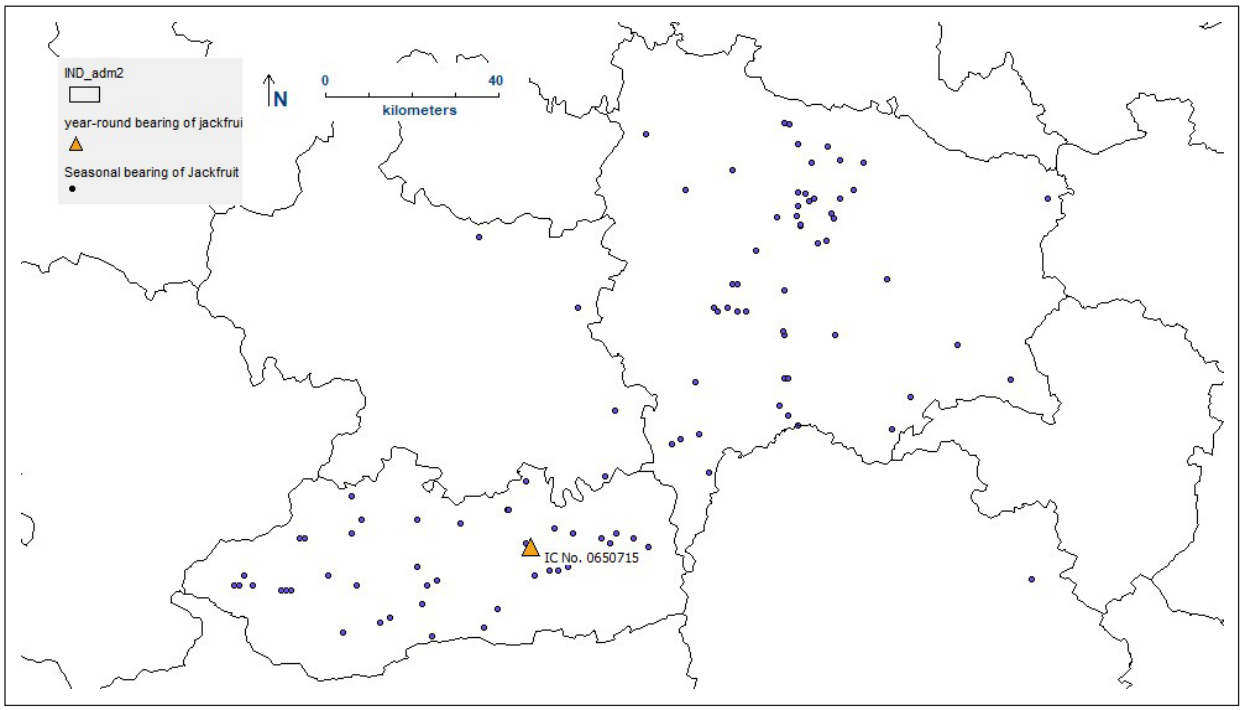

Figure 1: Mapping of coordinates indicating the area surveyed and place of germplasm collection

Table 1: Passport data of IC No 0650715

S.No.	*Items*	*Details*
1	Accession	IC-0650715
2	Alternate ID/Collectors No.	SA/NR/AK-2
3	Material Type	Grafts
4	Crop Species	*Artocarpus heterophyllus* Lam.
5	Common name	Jackfruit
6	Cultivar Name	Barahmasi Kathal
7	Bio-Status	Landrace/Traditional Cultivar
8	Sample Method	Selective
9	Sample type	Scion
10	Date of Collection	22 Nov 2021
11	Place (Village/Block/ District/State)	Domtoli/Kolebire/Simdega/ Jharkhand
12	Source	Private House
13	Frequency	Occasional
14	Habitat	Semi Cultivated
15	Pedigree	NA
16	Donor	Avinashi Baga
17	Trait	Year-round, prolific bearer
18	Latitude	22.62°N
19	Longitude	84.74°E
20	Altitude	497 AMSL

yellow-coloured flakes, which are thin, short, and irregular in shape. Flakes have oblong and white seeds.

Simdega is mainly a rural district, with 94% of its population inhabiting rural areas. The district is primarily settled by the Scheduled Tribes (ST), making up 70.2% of the population, possibly the highest in Jharkhand. The major tribal groups in the district are Munda, Oraon, and Kharia. A few families belonging to the primitive tribal group, such as Asur and Birhor, also live in the district. The relationship between the tribals and jackfruit is evident in a local game dedicated to this fruit called the Jackfruit game -Kantara-Lnu (Jena *et al*., 2020). The game is based on the social and cultural traditions of the Ho and Munda communities. It involves a dramatic presentation of stealing jackfruit, which is collected specifically for offering to the gods during pujas to ensure the well-being of society. It is believed that whoever steals the jackfruit will face the wrath of god. If the players cannot locate the stolen jackfruit, they call upon a religious performer to conduct a propitiation session, known as Sham Puja, to appease the gods. Immediately after the religious performance, the jackfruit is usually found, which is believed to be the result of divine intervention. This game symbolizes the folk behaviour and traditions of seeking the goodwill of the gods and spirits in the interest of society. It is also a way to protect the fruits from theft, a common practice in the village.

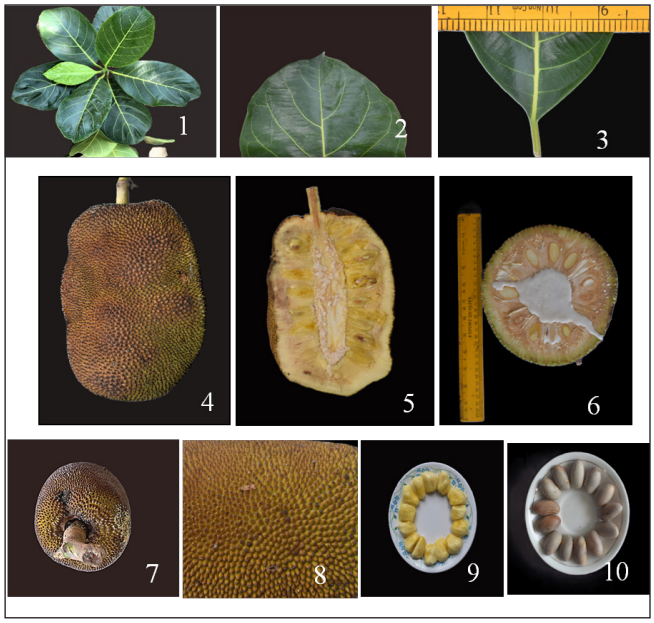

Figure 2: Morphological characteristics of IC No 0650715. 1. Leaf orientation (Horizontal), 2. Leaf apex (Acuminate) 3. Leaf base (rounded), 4. Fruit (Clavate shaped, medium-sized, brown in colour), 5. Vertical section, 6. Cross section, 7. Stalk attachment (depressed), 8. Spine shape (Pointed), 9. Flakes (irregular shape, thin, yellow), 10 seeds (oblong, white)

Apart from social and religious connotations, tender jackfruits are used for cooking, while mature fruits are used for table purposes. Flakes obtained from unripened but mature fruits and seeds from ripened fruits are also used for cooking purposes, and the inflorescence is used in salads. The leaves are preferred as fodder for goats. Additionally, the jackfruit has medicinal properties utilized by tribal and local Vaidya. The leaves can help with fever, boils, wounds, and skin diseases. The young fruits are acrid, astringent, and carminative, while the ripe fruits are sweet, cooling, laxative, aphrodisiac, and can also be used as a brain tonic. The seeds are diuretic and constipating. The wood is a nervine, anti-diabetic, and sedative and can be useful in convulsions. (Hembrom, 1996; John *et al*., 1999). The Jack tree wood is used as firewood, for making furniture, musical instruments, *etc*.

The rising popularity of tender jackfruit as a substitute for meat has led to a significant increase in its global market demand. This genotype holds immense potential in the market. The tribal community of Domtoli, Simdega, has been harnessing the versatility of jackfruit for culinary purposes, particularly as a vegetable. The custodian farmer of IC No 0650715 has revealed that the jackfruit she produces serves both her family's needs and is sold in the local market. Despite the prevalent theft of tender jackfruit, she harvests an impressive quantity—around 200 or more tender jackfruits, each weighing approximately 0.8 to 1.5 kg, from a single tree. This harvest is done in batches of 40 or more fruits at a time, allowing her to sell the produce at a

premium price during the off-season and use it for personal consumption during the main season when the price is less. She mentioned that she earns roughly 20 to 30 k per year by selling a portion of the produce during the off-season from a single tree that is over 100 years old. It provides a continuous supply of vegetables and a stable source of income for the native people in the region. Expanding the cultivation of this genotype on a large scale, supported by organized market initiatives, can fully realize its market potential.

In Jharkhand, jackfruit is primarily grown for vegetable purposes and is often seen growing by the roadside or practically in the backyard of every household. In addition to fetching a higher price, vegetable jackfruit minimizes post-harvest waste, allowing farmers to make more money. It is more expensive in the off-season and becomes the most popular alternative during India's festivities when most Hindus abstain from eating non-vegetarian cuisine. There is a global trend among people to choose vegetarian meals; jackfruit is the best choice under such circumstances. Consequently, it has gained worldwide traction among vegetarians. Considering this, a jackfruit cultivar with extended or year-round availability is highly sought after. It provides a consistent source of fruits that command higher prices in the off-season, enabling farmers to get a premium price for their produce. IC 0650715 produces fruits throughout the year and is suitable for cultivation for commercial gain. Besides commercial gain, this trait also has important scientific implications for researchers and jackfruit breeders. The jackfruit germplasm that bears fruit year-round can lead to insights into plant physiology, genetics, and agricultural practices, with potential benefits for crop improvement, food security, environmental conservation, and resilience to climate change, hence valuable germplasm for our field gene bank

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Data Availability

Data is included in the paper. Any further information is available on a request basis.

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Author Contribution

- Shephalika Amrapali: Survey, identification, and collection of germplasm, data recording, preparation, review, and editing of the manuscript
- S.B. Choudhary: Survey and identification of germplasm
- Raj Kumar Gautam: Review and editing of the manuscript
- Gyanendra Pratap Singh: Review and editing of the manuscript

Conflict of Interest

The authors declare no conflict of interest.

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Plant Germplasm Registration Notice*

A total of 187 proposals were received online for consideration of registration of germplasm for unique/superior traits. After preliminary screening by the member-secretary, Plant Germplasm Registration Committee (PGRC), 108 proposals complete in all respects were reviewed by the experts and were presented in the 49th meeting of the PGRC held in virtual mode on December 08, 2022. A total of 90 proposals with unique/novel features belonging to 35 species were finally recommended for registration. The information on registered germplasm is published to disseminate the information to crop breeders for the utilization of these genetic stocks in their crop improvement programmes. Description of 45 germplasm lines was published in IJPGR Vol 38 (Issue no. 2) 2025. Description of the remaining 45 germplasm lines registered by PGRC is given below:

1. SPV 2788 (IC618406; INGR22145), a sorghum germplasm with high brix content, high fresh stalk yield (4203 t/ha), and high juice content (14533 l/ha)

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Sweet sorghum [*Sorghum bicolor* (L.) Moench], a sugar crop with wider adaptation and high potential for bioenergy and ethanol production, is expected to meet food, feed, fodder, fuel, and fibre demands. Sweet sorghum offers a solution to the food versus fuel debate: the grain can be used as food or feed, the sugars extracted from the stem can be fermented directly, and the resulting bagasse can be used as fodder to generate heat from burning it, as a lingo-cellulosic feedstock for the production of second-generation biofuels, or as a source of biogas when it is used in an anaerobic digester (Molaverdi *et al*., 2013). The key traits for realising higher ethanol yields in sweet sorghum are higher biomass, fresh stalk yield, higher juice yields, and sugar content (Brix).

The sweet sorghum variety SPV 2788 is a derivative of (SSV F7-2)-1-1-1-1-1-1-2 which was developed through the pedigree method of breeding by crossing a *Kharif* sorghum genetic stock RSCN 2103 with the most popular sweet sorghum genetic stock SSV 74. This line recorded the superiority over checks for three important traits like brix (17.4 %), juice yield (14533 l/ha) and fresh stalk yield (42.3 t/ha) in the IAVHT-Sweet sorghum during *kharif* 2020 under AICRP trials (Table 1).

Table 1: Summary data for brix content (%), juice yield (l/ha) and fresh stalk yield (t/ha)- IAVHT-Sweet sorghum *kharif* 2020 (AICRP on Sorghum trial data)

Trait	*SPV 2788*	*CSV 19SS*	*CSV 24SS*	*CD%*
Brix (%)	17.4	15.9	16.1	1.3
Juice yield (l/ha)	14533	12264	14154	2867
Fresh stalk yield (t/ha)	42.3	39.4	38.7	5.4

Reference

M Molaverdi, K Karimi, M Khanahmadi and A Goshadrou (2013) Enhanced sweet sorghum stalk to ethanol by fungus Mucorindicus using solid state fermentation followed by simultaneous saccharification and fermentation. *Ind. Crops Prod*. 49: 580-585.

2. WCIJ-150-1 (IC340358; INGR22146), a wild jute germplasm with high resistance to stem rot caused by *Macrophomina phaseolina*

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Stem rot disease caused by *Macrophomina phaseolina* is a major biotic stress for commercial jute (*Corchorus* spp.) cultivation. However, jute cultivars are developed with a narrow genetic base (Kar *et al*., 2009) and most of these possess only moderate resistance against *M. phaseolina* (De and Mandal 2012; Meena *et al*., 2015). Identification of stable pest-resistant donor lines with wide genetic variation, especially from wild relatives, will play a greater role in widening the genetic base of present-day crop cultivars. Earlier, a highly stem rot-resistant source (WCIN-136-1 of *C. aestuans* L.) was identified in wild *Corchorus* germplasm.

To diversify the genetic background of stem rot resistance sources, wild *Corchorus* species was screened. WCIJ-150-1 (*Corchorus fascicularis* L.) was highly resistant against stem rot disease caused by *M. phaseolina*. The trait was found to be stable over the years under sick plot conditions. WCIJ-150-1 was developed through pureline selection from an exotic line WCIJ-150, collected from Thailand through the International Jute Organisation (IJO), Bangladesh. WCIJ-150-1 was screened under a sick plot condition for four consecutive years from 2015 to 2018 at ICAR-CRIJAF, Barrackpore, India along with recently registered stem rot-resistant line WCIN-136-1. AUDPC (Area Under Disease Progressive Curve) values were zero for both these lines. This indicates that like the check, WCIJ-150-1 possesses stable resistance against stem rot disease. An interspecific population was developed using WCIJ-150-1 as a donor parent to transfer the disease resistance trait to Tossa jute cultivar JRO 2407.

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Kar CS, A Kundu, D Sarkar, MK Sinha and BS Mahaptra (2009) Genetic diversity in jute (*Corchorus s*pp.) and its utilization: A review. *Indian J. Agric. Sci*. 79(8): 575-86.

Meena PN, RK De, A Roy, BS Gotyal, S Satpathy and S Mitra (2015) Evaluation of stem rot disease in jute (*Corchorus olitorius* L.) germplasm caused by *Macrophomina phaseolina* (Tassi) Goid. *J. Appl. & Nat. Sci.* 7(2): 857-859.

3. WCIN-183A (IC646844; INGR22147), a wild jute germplasm highly resistant to stem rot, root knot nematode, and Bihar hairy caterpillar

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Wild relatives of crop plants play an important role in donating specific traits such as resistance against biotic and abiotic stresses. But, identification of multiple desirable traits in a single source is a boon for the breeders, as a single cross can effectively improve the recipient line with multiple benefits. In tossa jute (*Corchorus olitorius* L.) stem rot (caused by *Macrophomina phaseolina*), yellow mite (*Polyphagotarsonemus latus*) and Bihar hairy caterpillar (*Spilosoma obliqua*) are major biotic stressors. Jute is also affected by root-knot nematodes (*Meloidogyne arenaria*, *M. incognita*), which can create serious problems in combination with the stem rot pathogen. A wild jute germplasm WCIN-136-1 was reported with resistance against stem rot and Bihar hairy caterpillar.

In the present study, selected stem rot-resistant wild jute lines were evaluated for other biotic stressors (Bihar hairy caterpillar and root-knot nematode) for multiple seasons. WCIN-136-1 was included as a check. WCIN-183A, a *Corchorus aestuans* (L.) line, was identified as resistant against all these biotic stressors. It was developed through pure line selection from the indigenous germplasm line WCIN-183 collected from Lakshadweep, India. This wild jute line was first identified as a resistant source against stem rot disease by the artificial stem inoculation method. During the subsequent years, its resistance was verified under the sick plot conditions at ICAR-CRIJAF, Barrackpore. WCIN-183A was highly resistant against stem rot disease (zero AUDPC values, Area Under Disease Progressive Curve) during screening for five consecutive years (2015-2019) under sick plot conditions on par with resistant check WCIN-136-1. Subsequently, it was evaluated for resistance against root-knot nematodes (*M. incognita*) and recorded with very low galls, egg mass per plant and had root gall index about 2.0 over the locations (UBKV, Coochbehar and ICAR-NBPGR, RS-Hyderabad) and years (2018, 2019 and 2022). Apart from the above biotic stresses, this germplasm is also recorded to have high resistance reaction towards Bihar hairy caterpillar with 100% larval mortality and 0% pupation. The line is cross-compatible with cultivated tossa jute and an F2 population was developed by crossing with a susceptible line (OIN-456) for all biotic stresses.

4. IC530491 (INGR22148), a black gram germplasm for waterlogging tolerance

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Black gram (*Vigna mungo* L.) is a nutritious legume crop sown during *kharif* season in India. The crop faces waterlogging stress at different growth stages due to the onset of the monsoon. Black gram is highly sensitive to waterlogging stress. The present study was carried out to identify the waterlogging-tolerant black gram genotypes by screening 150 black gram lines in an augmented block design during *kharif* 2016 and 2017. The study led to the identification of waterlogging-tolerant black gram indigenous accession i.e. IC530491 (Annual Reports, 2018). Afterward, pot and field experiments were conducted to study the physiological mechanism and to validate the waterlogging tolerance in a randomized block design at ICAR-IARI, New Delhi, and ICAR-IIPR, Kanpur in 2020 and 2021 (Bansal *et al*., 2022). Physiological investigation showed that waterlogging tolerance was related to chlorophyll content and membrane integrity. Field study validated the presence of the waterlogging tolerance trait in the identified genotype. The stress susceptibility index (SSI) was calculated based on seed yield and was recorded as 0.43 for IC530491 (SSI <0.5). IC530491 can be used as a donor in the crossing program for waterlogging tolerance in black gram.

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5. IC519933 (INGR22149), a black gram germplasm with waterlogging tolerance

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Black gram (*Vigna mungo* L.) is an important legume crop that is very sensitive to waterlogged conditions. Waterlogging may reduce the crop yield drastically, depending on the stress intensity and duration. The present study was carried out to identify waterlogging-tolerant black gram genotypes by screening 150 black gram lines in an augmented block design during *kharif* 2016 and 2017. The study identified a waterlogging-tolerant indigenous black gram accession, IC519933. To revalidate the identified lines under field conditions, experiments were conducted in a randomized block design at ICAR-IARI, New Delhi, and ICAR-IIPR, Kanpur during 2020 and 2021 (Bansal 2021; Bansal *et al*., 2022). Stress was imposed after 30 days of sowing by maintaining the water level 5-6 cm above the soil surface. The study validated the waterlogging tolerance in black gram line IC519933. The stress susceptibility index was calculated based on seed yield and was recorded to be 0.32 for IC519933. Identified accession IC519933 can be used as a donor in the black gram breeding program.

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6. LGG 607 (IC639816; INGR22150), a photo insensitive mung bean germplasm with early and synchronous maturity and resistance to MYMV

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LGG 607 is an advanced green gram (*Vigna radiata* L.) breeding line selected from the cross MGG 295 × COGG 912 by following the pedigree method of breeding. The line was identified and developed at Acharya N.G. Ranga Agricultural University, Regional Agricultural Research Station, Lam, Guntur. The proposed entry was resistant to Mungbean Yellow Mosaic Virus (MYMV).

LGG 607 is a photo-insensitive, high-yielding, early-duration variety that matures in 60 to 65 days with synchronous maturing and shining seed. The line is suitable for upland conditions during *kharif* and *rabi* seasons and also for rice fallow situations, and is found resistant to Mungbean Yellow Mosaic Virus (MYMV). Plant type is erect with green foliage, ovate leaf, and pods with top-bearing habit. The proposed entry was found resistant to Mungbean Yellow Mosaic Virus (MYMV) during *kharif* 2017 under MULLaRP network centres organized by the Indian Institute of Pulses Research, Kanpur (as per the 9-point scale of MYMV disease scoring). It was again tested during spring/summer 2019 and *rice fallows* 2019, and further confirmed that the entry was resistant to MYMV in almost all the locations tested. These results were confirmed with the experiments conducted at RARS, Lam for two consecutive years during 2017-18 and 2018-19. The entry can be utilized as a donor parent for MYMV resistance and farmers have the chance to utilize this entry for commercial cultivation due to its short-duration nature as a preceding crop in paddy areas during *kharif,* and also as a summer crop.

7. LBG 884 (IC639815; INGR22151), a photo insensitive black gram germplasm with medium maturity and resistance to MYMV

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LBG 884, an advanced breeding blackgram (*Vigna mungo* L.) line selected from the cross LBG 709 × KU 96-3 by following the pedigree method of breeding. The line was identified and developed at Acharya N.G. Ranga Agricultural University, Regional Agricultural Research Station, Lam, Guntur. The proposed entry was resistant to Mungbean Yellow mosaic virus (MYMV).

LBG 884 is a photo-insensitive, high-yielding and medium maturing variety. The line was highly suitable for upland conditions during *kharif* and *rabi* seasons and highly resistant to Mungbean Yellow Mosaic Virus (MYMV). The seeds are medium bold, dark black in colour with shining seed lusture. It is an erect plant type with dark green foliage, lanceolate leaf, and densely hairy pods. LBG 884 also tested in IVT (for seed yield) during *rabi* 2017-18 in seven locations covering five states, and the results revealed that the genotype recorded a mean yield of 1034 kg/ha and had got 23.24% yield advantage over the best check LBG 787 (839 kg/ha).

The proposed entry was highly resistant to Mungbean Yellow mosaic virus (MYMV). The genotype was evaluated during *rabi* 2017-18 under MULLaRP network centres organized by the Indian Institute of Pulses Research, Kanpur over four locations covering four states (Andhra Pradesh, Telangana, Odisha and Tamil Nadu). It was again tested during spring/summer 2019 in five locations covering five states (Andhra Pradesh, Tamil Nadu, Uttar Pradesh, Punjab and Bihar). The genotype was tested in hot spots under field conditions in all the locations. The results revealed that LBG 884 was found resistant to Mungbean Yellow Mosaic Virus and scored 1 on 1 to 9 scale in all the tested locations. At Lam (Guntur), Warangal and Berhampur recorded 1 score and found resistant to Mungbean Yellow Mosaic Virus (MYMV), where the check entry recorded 9 score (susceptible to Mungbean Yellow Mosaic Virus).

8. IC251387 (INGR22152), a black gram germplasm for resistance against bruchid species *Callosobruchus chinensis* (L.)

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A study was carried out during 2019 and 2021 at the ICAR-Indian Institute of Pulses Research (IIPR), Kanpur, to evaluate a set of wild *Vigna* accessions for their resistance to the three aforementioned destructive bruchid species. Bruchid species used in the screening were properly characterized using both morphological keys (Kingsolver, 2004) and molecular approaches, with GenBank accession numbers MN120830 (*C. chinensis*), MN120833 (*C. maculatus*),

and MN120832 (*C. analis*). The seeds of accessions were obtained from the Germplasm Bank of IIPR and have been multiplied in the wild garden. Each accession was characterized for seed morphological traits following the descriptors set by NBPGR (Mahajan *et al.,* 2000), to explore possible associations between seed features and resistance response. Screening involved both 'Free-choice' and 'No-choice' assays as per the standard protocols (Aidbhavi *et al.,* 2021), with resistant accessions undergoing further validation in confirmatory no-choice screening. Reactions were assessed based on the SI index and seed damage scores for each bruchid species.

Notably, the accession IC251387 of *Vigna mungo* var. *mungo* displayed strong resistance to *C. chinensis*. This accession recorded 0%, 4.4%, and 0% seed damage under free-choice, no-choice, and confirmatory no-choice tests, respectively, despite receiving an average of 4.2, 2.5, and 1.41 eggs per seed. While the eggs successfully hatched, all larvae failed to survive beyond the early instar stage. In rare cases where adults emerged under no-choice conditions, they exhibited developmental abnormalities, implying the possible presence of toxic biochemical compounds in the seed coat or cotyledons that may interfere with insect growth. Correlation analysis further suggested that morphological seed traits had no significant role in the resistance response, reinforcing the idea of biochemical factors being responsible.

This finding reports IC251387 as a valuable genetic resource for breeding bruchid-resistant legume varieties. Its resistance, particularly to *C. chinensis*, can be harnessed through targeted breeding and introgression into cultivated mungbean, urdbean, and other *Vigna* crops. Its inclusion in hybridization programs holds significant promise for developing improved cultivars with enhanced resistance to storage pests, thus contributing to reduced post-harvest losses and better food security.

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9. NIL VC 35/5-4 (IC647176; INGR22153), a chickpea germplasm with high resistance to fusarium wilt and wider adaptability

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Fusarium wilt caused by *F. oxysporum* f. sp. c*iceri* causes extensive damage to Chickpea production in many parts of the world. In the central part of India, the major chickpea growing area, race *2* (*Foc* 2) of the pathogen causes heavy yield losses. Molecular marker-assisted backcross (MABC) breeding applying foreground selection (FGS) and background selection (BGS) using genome-wide SSR markers for recovery of recurrent parent genome is an environment-independent, precise and quick approach for the development of cultivars of the trait of interest (Varshney *et al*., 2010). Therefore, a *desi* cultivar, Vijay, was used as a donor to introgress resistance to race 2 (*Foc2)* in Pusa 256 (an elite and popular *desi* chickpea cultivar). To confirm introgression of resistance, FGS was undertaken using two SSR markers (TA 37 and TA110). BGS was executed using 45 SSR markers, which were accommodated in 8 multiplexes. The identity of F1 plants was confirmed with molecular markers and true F1s were backcrossed with Pusa 256. This was followed by 3 cycles of foreground and background selection at each stage to generate 161 plants in BC_3F_2 during the period 2009–2013. Similarly, 46 BC_3F_1 Plants were also generated in another set during the same period. On the basis of foreground selection, 46 plants were found to be homozygotes in BC_3F_2. 17 plants recorded >91% background recovery with the highest recovery percentage of 96%. In BC_3F_1, 14 hybrid plants recorded a background recovery of >85% with the highest background recovery percentage of >94%. The identified plants were selfed to obtain 1341 BC_3F_3 and 2198 BC_3F_2 seeds, which were screened phenotypically for resistance to Fusarium wilt (race 2), besides confirmation with markers. Finally, 17 BC_3F_4 and 11 BC_3F_3 lines were obtained which led to the identification of 5 highly resistant lines of Pusa 256 with *Foc* 2 gene introgressed in them. Among these, the NIL VC 35/5-4 was recorded to have the least (0-4.46%) disease incidence in natural field conditions as well as in the race-specific

wilt-sick micro plots (Pratap *et al.,* 2017). The developed line can be used in introgression breeding programs for genetic improvement of *desi* chickpea.

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10. IC117744 (INGR22154), a chickpea (*Cicer arietinum*) germplasm with Ascochyta blight resistance

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The ascochyta blight caused by *Ascochyta rabiei* (Pass.) Labr. is one of the major devastating diseases of chickpea. The pathogen *A. rabiei* is highly variable in nature, which often leads to the fast evolution of its new pathotypes. As a result, frequent breakdown of the blight disease resistance in existing chickpea varieties is observed. Therefore, the new sources of resistance need to be identified from time to time. Keeping this background in mind, this work was undertaken to evaluate the existing chickpea germplasm diversity conserved in the Indian National Genebank against the disease under artificial epiphytotic conditions. A total of 1970 chickpea accessions were screened at two natural hotspot locations *viz*., PAU, Ludhiana and HAREC, CSK HPKV, Dhaulakuan. Chickpea germplasm originated primarily from India (1,567 acc.) followed by Iran (155 acc.), Syria (75 acc.), Ethiopia (71 acc.), Mexico (26 acc.), and Turkey (21 acc.). The chickpea germplasm was screened, and promising accessions were validated against the disease over the years. To keep the minimum threshold level of the pathogen, inoculation of the pathogen spore suspension 4×10^4 spores/ml was done in the evening by spraying. The relative humidity (RH) was maintained above 85% by running the perfo-spray system during the daytime from 10.00 to 16.00 h for 21 days after the inoculation. The standard methods for inoculum preparation and artificial inoculation procedure were followed (Gayacharan *et al.,* 2020). The level of disease severity was recorded on 0–9 scale, modified from the method given by (Jan and Wiese *et al.,* 1991). As a result of screening and validation at the locations over the years, IC117744 was found to be resistant to the disease. The passport information of the accession indicated that it originated from the Maharashtra state of India, and it was collected in 1991. IC117744 was constantly resistant (disease severity score 2) during 2015-16, 2017-18, and 2018-19 at PAU, Ludhiana location. The accession was also found highly resistant at HAREC, CSK HPKV, Dhaulakuan during 2015-16 with the disease severity score of 1. In the screening of germplasm susceptible check varieties *viz.,* L550 and JG62 were used. The disease severity score for checks was constantly 9 (highly susceptible).

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Jan H and MV Wiese (1991). Virulence forms of *Ascocta rabiei (Pass.) Labr. in Algeria*

11. IPACliesto 10 (IC646849; INGR22155), a pigeonpea germplasm with closed flower structure until complete fertilization and absence of diadelphous condition

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Pigeonpea (*Cajanus cajan* L.) is unique among leguminous crops because, despite having a floral structure designed for self-fertilization, it permits a considerable degree of natural outcrossing aided by insect pollinators. The large range (25-70%) of natural outcrossing led to the rapid deterioration of genetic purity in the released varieties, donors, and mapping populations (Bhatia *et al*., 1981; Saxena *et al*., 1990). The inconvenience caused by the loss of genetic purity is incomparable for the recently developed varieties bred for targeted traits; generally, the circumstances knock out that variety from the seed chain due to difficulties in ensuring genetic purity and seed certification standards. Keeping this in view, the present donor genotype IPAcliesto 10, having modified flower structure under an indeterminate genetic background with high-yielding attributes recorded less than three percent out-crossing, would be the potential donor for a future line of breeding for a high percent selfing indeterminate pigeonpea breeding programme.

The donor genotype IPAcleisto 10 was a pure line selected from the cross of IPA 203/ICPL 87154. After successful hybridity confirmation, the generation was advanced to develop RILs through pedigree breeding. IPAcleisto 10 was evaluated for three years (2018 to 2021) for the extent of selfing by taking open and selfing net-covered seeds to confirm the percent outcrossing for plant type, flower color, and other morphological traits. The consistently lowest percent outcrossing was recorded in IPAcliesto 10 for all the years.

IPAcleisto 10 produces 1.3±0.5 cm long pale yellow-colored flowers with variable spar streaks on its standard petal. All three petals, namely, the Standard, keel, and wing petals are fused to form a cavity-like construction. All nine filaments of the anthers are independently arranged from the base; as a result, the diadelphous condition is absent in the donor genotype IPAcleisto 10. Therefore, having independent anther filaments could be another reason for selfing, which failed to create the sufficient exertion force to open the petals wide for outcrossing.

Further, the pod surface wax and stickiness help in fewer attacks by the pod borer and pod fly complex. The 100 dry seed weights range from 9.5 to 10.5 g. The dry seeds are oval-shaped, with a red tinge near the hilum. The color of the seed coat is creamy white. The genotype IPAcliesto 10 attains its 50% flowering in 135 to 140 days and matures in 210 to 225 days. This line has a good yield attributing to yield potential of 19-21 q/ha and high selfing trait holds immense scope for breeders during varietal development.

12. IC251439 (INGR22156), a rice bean (*Vigna umbellata*) germplasm resistant to bruchid species [*Callosobrochus maculatus* (F.)]

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Previous studies have shown success in transferring resistance to Bruchid beetles from wild *Vigna* species, known for their natural defense mechanisms (Pratap *et al.,* 2021). Additionally, resistance to *C. chinensis* has been identified in wild accessions like TC1966 (War *et al.,* 2017).

A study was conducted to assess 42 wild accessions from 13 *Vigna* species for resistance to *C. chinensis*, *C. maculatus*, and *C. analis*. The screening was done from 2019 to 2021 at the Storage Entomology Laboratory, ICAR-Indian Institute of Pulses Research (IIPR), Kanpur. Seed material was obtained from IIPR's Germplasm Bank. Bruchid species were identified using both morphological keys and molecular confirmation (accession numbers MN120830, MN120833, and MN120832). Seed morphological traits were recorded using NBPGR descriptors to investigate potential links with resistance.

A three-step screening approach was used, consisting of both 'free-choice' and 'no-choice' screening assays followed by confirmatory no-choice screening (Aidbhavi *et al.,* 2021). In the free-choice test, insects could choose among different accessions, while the no-choice test offered only one seed type, testing its inherent resistance. Key observations included egg-to-adult development parameters, seed damage, weight loss, and MDP. The resistance is classified based on the SI index and seed damage percentage.

Among all accessions, *Vigna umbellata* IC251439 showed the highest resistance, especially to *C. maculatus*. Seed damage was exceptionally low-1.1% or less-in all tests. Despite receiving an average of 3.9, 2.8, and 2.48 eggs per grain in the three respective screening conditions and good hatching, larval development was arrested early, with most larvae failing to survive past the first instar, indicating strong antibiosis. In rare cases, malformed adults emerged, suggesting that toxic or inhibitory compounds in the seeds interfered with development.

Given its consistent resistance across tests, IC251439 would serve as a valuable genetic resource for breeding programs targeting *C. maculatus* resistance.

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13. IC248326 (INGR22157), a wild bean (*Vigna vexillata*) germplasm with resistance to the bruchid species *Callosobruchus chinensis* (L.)

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Bruchids (Coleoptera: Bruchinae) are major pests of pulses both in the field and during storage, although most economic losses occur during storage. Identifying bruchid resistance in traditional cultivars or landraces through Host Plant Resistance (HPR) studies is recommended. Incorporating genes from wild crop relatives offers a promising way to broaden the genetic base of legume varieties (Pratap *et al.,* 2020). The identification of a wild mungbean accession (TC1966) resistant to *C. chinensis* has opened new possibilities to explore resistance in wild and cultivated *Vigna* relatives. The extensive diversity of *wild* Vigna species maintained in Indian gene banks remains largely unexplored for bruchid resistance. Thus, this study aimed to screen a wide range of endemic *Vigna* accessions to identify resistant donors against the three common bruchid species in India.

Between 2019 and 2021, 42 wild *Vigna* accessions from thirteen different Indian endemic species were evaluated for their resistance or susceptibility to three bruchid species (*C. chinensis, C. maculatus,* and *C. analis*) at the Storage Entomology Laboratory, Division of Crop Protection, ICAR-Indian Institute of Pulses Research (IIPR), Kanpur. The bruchid species were identified using taxonomic keys (Arora, 1977; Rees, 2004; Kingsolver, 2004) and confirmed molecularly (Accession Nos: MN120830, MN120833, respectively). *Vigna* seeds were sourced from the Germplasm Bank, Division of Crop Improvement, ICAR-IIPR, Kanpur, and maintained in the institute's wild garden for breeding purposes. Seed traits, both qualitative and quantitative, were recorded as per NBPGR minimum descriptors to assess their impact on bruchid infestation and identify traits linked to resistance. All accessions were subjected to screening against the three bruchid species using rigorous methods, including 'Free-choice', 'No-choice', and 'Confirmatory no-choice' screening tests. Accessions were classified based on SI index and seed damage scores (Aidbhavi *et al*., 2021) to better understand their reactions to the three bruchids. Notably, accession IC248326 of *V. vexillata* showed resistance to *C. chinensis*, with seed damage levels of 0%, 2.2%, and 0% in free-choice, no-choice, and confirmatory no-choice tests, despite receiving average egg counts of 0.6, 1.6, and 1.69 eggs per grain, respectively. Earlier, (Marconi *et al.,* 1997) reported *V. vexillata* accessions resistant to *C. maculatus*, where although eggs hatched, larvae failed to develop into adults, dying before the first instar. A few adults that did emerge showed abnormalities, likely due to biochemical compounds in seed coats and cotyledons that hinder insect development. Therefore, this accession represents a valuable genetic resource for breeding programs aiming to introgress multiple traits into mungbean and urdbean.

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14. DLS-161-1 (IC646850; INGR22158), a chilli germplasm that seta fruits at day temperatures above 40°C and night temperatures above 25°C

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Although chilli (*Capsicum annuum* L.) is a warm climate loving crop and shows luxuriant vegetative growth in high temperature, however when the temperature goes above 40°C, chilli crop fails to set fruits. Therefore, to have a spring-summer crop, especially in North India where farmers take chilli crop in the summer season, it is important that the varieties grown in this season are able to tolerate the high temperature during April to July (which may go even beyond 40^0C) (Aruna *et al*, 2021). The DLS-161-1, the heat-tolerant line, was identified from a population raised from red chilli fruits procured from the local market of Ghaziabad, New Delhi. We had identified nearly five plants which were fruiting in the month of May to June in the year 2014. These plants were phenotypically quite different, indicating that these were possibly heterozygous in nature. These plants were subsequently selfed for the next four years till Summer 2018 to stabilize them. DLS-161-1 has shown promise and has given consistent performance in terms of fruit set during May, June, and July since the year 2016 (Table 1). An in-depth comparative analysis of the morphological, physiological, biochemical and molecular responses of chilli genotypes with contrasting heat tolerance has also been carried out, displaying marked potential of DLS-161-1 to combat high temperatures for setting fruits. DLS-161-1 accumulated more amounts of protein as well as proline and displayed higher activities of antioxidant enzymes like SOD (superoxide dismutase) and POD (peroxidase) in comparison to susceptible genotypes. Out of seven Capsicum annuum specific heat shock proteins studied, three genes, namely CaHSP 832 (HSP 83), Ca HSP 703(HSP70), and Ca HSP 2272 (small HSP) showed significant differences in expression level between DLS-161-1 and susceptible genotypes thereby suggesting their utility in discriminating the genotypes for their tolerance to heat stress (Srivastava *et al*., 2022). Utilising this genotype to develop hybrids may help to develop heat-tolerant hybrids/varieties for commercial cultivation by farmers of especially north India who face severe heat during the summer season.

Table 1: Evaluation of DLS-161-1 for yield in May to July during 2016, 2017 and 2018

Genotype	*Total yield per plant(gm)*		
	2016	*2017*	*2018*
DLS -161-1	563.00	568.00	615.00
Kashi Anmol (National check)	95	80	68.33
Pusa Sadabahar	78	68	85.00
CD	45.78	48.87	59.29
CV	12.67	16.77	10.755

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15. DLS-152-1 (IC646851; INGR22159), a chilli germplasm that seta fruits at day temperatures above 40°C and night temperatures above 25°C

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Fruit setting in chilli (*Capsicum annuum* L.) is highly sensitive to night temperatures, with an optimal range of 15°C to 20°C. However, in Delhi, night temperatures during May and June often exceed 25°C, hindering fruit set in most chilli varieties. DLS-152-1, a heat-tolerant genotype developed at ICAR-IARI, New Delhi, has the unique ability to set fruits even during the extreme heat of May to July. This characteristic makes it a valuable resource for breeding heat-tolerant genotypes, enabling farmers to achieve additional harvests during this challenging period, when crops typically require care but yield little. The development of DLS-152-1 began with red chilli fruits procured from the Ghaziabad local market. In 2014, five plants capable of fruiting during May and June were identified from the population. These plants exhibited phenotypic diversity, suggesting a heterozygous nature. Through continuous selfing over four years, the line was stabilized by the summer of 2018. After stabilization, DLS-152-1 was characterized as an annual, herbaceous chilli genotype with an upright growth habit and a life span of approximately seven months. It has dark green, lanceolate leaves and a clustered, erect flowering habit, bearing 3–5 fruits per cluster. The medium green fruits have a rough texture, 5–6 cm length, 0.8–1 cm width, and an average weight of 2–3 g. Biochemical and molecular analyses revealed DLS-152-1's resilience to high-temperature stress. The genotype accumulated higher levels of protein and proline and exhibited greater activities of antioxidant enzymes such as superoxide dismutase (SOD) and peroxidase (POD) compared to susceptible genotypes. Additionally, among the seven Capsicum annuum-specific heat shock proteins studied, three-CaHSP 832 (HSP 83), CaHSP 703 (HSP 70), and CaHSP 2272 (small HSP)—showed significantly higher

Table 1: Evaluation of DLS-152-1 for yield in May to July during 2016, 2017, and 2018

Genotype	Total yield per plant (gm)		
	2016	2017	2018
DLS -152-1	445.00	389.00	470.67
Kashi Anmol (National check)	95	80	68.33
PusaSadabahar	78	68	85.00
CD	45.78	48.87	59.29
CV	12.67	16.77	10.755

expression in DLS-152-1, underscoring their potential as molecular markers for heat tolerance (Srivastava *et al*., 2022). In station trials conducted at IARI, New Delhi, DLS-152-1 produced two harvests during May and June, a period when the national check variety Kashi Anmol failed to yield any fruits (Table 1). This performance highlights its potential to address the challenges of heat stress and enhance chilli production during high-temperature months.

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16. VRCAR-214 (A-line) & Kashi Arun (B-line) [IC623137 & IC642958; INGR22160]: A red carrot petaloid-CMS line with better heterotic potential for root yield, uniformity & lycopene content

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On the basis of the presence of root pigments, carrot is broadly classified into two groups i.e. Carotenoid group (Orange, Red, and Yellow carrot) and the anthocyanin/purple group (Black and Rainbow carrot). The roots of red coloured tropical carrot are a very good source of lycopene and beta-carotene. The male sterility in carrot was discovered and analyzed by (Thompson *et al*., 1962) and in India, (Kalia *et al*., 2019) have transferred the CMS system in red, orange and purple coloured Asiatic carrots. The CMS system- a trait of commercial and proprietary importance was transferred in coloured tropical carrots at ICAR-IIVR, Varanasi, UP (Singh and Karmakar *et al*., 2021). The petaloid-CMS line was developed by crossing a naturally occurring CMS plant in an open-pollinated population having orangish-red coloured root with a red carrot variety Kashi Arun (VRCAR-186).

The newly developed CMS line VRCAR-214 (A-Line, IC642958) and its maintainer Kashi Arun (B-Line or Maintainer Line, IC623137) are uniform for red coloured root, self-coloured core, green coloured leaf, light-green coloured petiole, root shape of Danvers type (Tapering), bear green and white flowers in umbel and are ready for seed harvest in about 125 days after transplanting of stecklings. Moreover, the quantitative traits (pooled values for 2019-20 & 2020-21) of CMS line VRCAR-214 and its maintainer Kashi Arun is statistically at par for grass plant weight (161.3 & 154.2 g, respectively), root length (22.1 & 21.7 cm), root weight (118.7 & 114.2 g), shoulder diameter (3.65 & 3.58 cm), root yield potential (325 & 312 q/ha), days to 50% flowering (57.0 & 52.4 day), plant height at flowering (131.3 & 137.6 cm), lycopene content (7.45 & 7.36 mg/100 g FW), beta-carotene content (3.41 & 3.52 mg/100 g FW) and TSS (7.45 & 7.38 °Brix). The newly developed CMS line (VRCAR-214) and its maintainer (Kashi Arun) both is ideally synchronous in flowering/pollination activities, which facilitates proper pollination and maximum seed set in the CMS line. Furthermore, VRCAR-214 as a female parent in different cross-combinations performed very well for root yield (10-15% heterosis), lycopene content, and uniformity for growth, maturity & roots (8-10% heterosis); hence, it has potential to be used in the development of indigenous commercial hybrids of tropical carrot.

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17. IIHR 144-1 (IC642345; INGR22161), a bitter gourd germplasm with dark green, deeply lobed leaves, small fruit with discontinuous ridges and resistance to powdery mildew

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Bitter gourd (*Momordica charantia* var. muricata) is an economically important and nutritionally rich vegetable cultivated in Asia. India produces about 10.83 lakh metric tonnes of bitter gourd from an area of 0.96 lakh hectare (Saxena *et al*., 2016). Powdery mildew (*Podosphaera xanthii* U. Braun & Shishkoff) is one of the most important production constraints in bitter gourd growing areas of India and incidence occurs throughout the year with a crop loss of up to 50 per cent (Dhillon *et al.,* 2016). Though the disease can be controlled with fungicides, genetic resistance provides a more economically sound and environmentally safe approach. Hence, through germplasm screening and single plant selection, IIHR-144-1 has been developed, which is resistant to powdery mildew. IIHR 144-1 belongs to *Momodica charantia* var. *muricata*. Dark green, deeply lobed leaves. Fruit is small, dark green with discontinuous ridges. Resistant to powdery mildew (PDI=1.81 Natural Screening, 4.28 Artificial Screening) (Table 1). Yield is 8.8t/ha. This line can be used for developing powdery mildew-resistant bitter gourd varieties/hybrids.

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Table 1: Powdery mildew disease reaction under natural and artificial screening conditions in bitter gourd advanced breeding lines

	Natural screening			*Artificial screening*			
Genotype	*Mean PDI*	*AUDPC*	*DR*	*Mean PDI*	*AUDPC*	*DR*	*Mean 'r'*
Arka Harit	86.67 (68.75)	5629	HS	63.6 (52.64)	2720	HS	1.29
IIHR-144-1	1.81 (7.70)	88	R	4.28 (13.95)	172	R	0.08
IIHR-80-1-2	0.00 (0.29)	0	R	0.00 (0.29)	0	R	0.00
Pusa Vishesh	79.49 (63.07)	5170	HS	60.16 (50.86)	2583	HS	1.26
Pusa Do Mausami	74.67 (59.81)	4852	HS	63.28 (52.70)	2719	HS	1.24
Phule Ujwala	72.44 (58.36)	4710	HS	58.84 (50.09)	2535	HS	1.23
IIHR -40-1	31.06 (33.86)	1921	MR	39.49 (38.92)	1680	MS	0.83
IIHR -80-1-3	0.00 (0.29)	0	R	0.00 (0.29)	0	R	0.00
CD at 5%	3.22	-	-	2.01			
CV (%)	3.76	-	-	2.64			

18. DRMRCI-125 (IC646857; INGR22162), an Indian mustard germplasm with resistance to white rust disease caused by *Albugo candida*

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An Indian mustard [*Brassica juncea* (L.) Czern and Coss] genetic stock DRMRCI-125 was developed through Marker Assisted Backcross Breeding of a popular Indian mustard variety (NRCDR-02) and whiter rust resistant donor (Donskaja). The F1 was back-crossed to NRCDR-02 till BC_3. After that BC_3 generation was selfed up to BC_3F_5. During the generation advancement, phenotypic selection for WR-resistant plants was done, which was validated under lab conditions using the WR-specific marker (AcB1-A5.1). For the recovery of the genome of the female parent (NRCDR-02),

background selection using SSR markers was done.

The proposed genotype was tested under AICRP-RM during 2019-20 to 2021-22 for three years at six locations (MOR: Morena, PNT: Pantnagar, HSR: Hisar, LDH: Ludhiana, JGP: Jagdalpur, BPR: Bharatpur) for whiter rust disease under natural and artificial conditions. The presence of the white rust resistance allele was validated using WR-specific markers (AcB1-A5.1) at Bharatpur. Under pooled analysis (Table 1), it showed less White Rust disease (3.9%) as compared to the resistant check Bio-YSR (12.3%) and the susceptible check (38.2%). Genotype DRMRCI-125 showed less incidence of stagheads (6.1%) as compared to the resistant check (Bio-YSR: 14.9%) and the susceptible check Rohini (27.5%). The genotype DRMRCI-125 can be used as a donor for the development of white rust-resistant Indian mustard varieties with less effort.

Table 1: WR incidence (%) at 100 DAS of DRMRCI 125 (Pooled over 3 years) under artificial inoculations

Entry	*% WR at 100 DAS*				*Stageheads (%)*			
	2019-20	*2020-21*	*2021-22*	*Mean*	*2019-20*	*2020-21*	*2021-22*	*Mean*
DRMRCI125	7.9	3.4	0.4	3.9	10.0	8.4	0.0	6.1
BioYSR (Resistant check)	4.4	27.9	4.6	12.3	11.2	33.4	0.0	14.9
Rohini (Susceptible check)	35.2	35.1	44.4	38.2	13.6	33.8	35	27.5

(Source: AICRP-RM reports, 2020 to 2022)

19. DRMR 1191-2 (IC646856; INGR22163), an Indian mustard germplasm with high temperature tolerance in seedling stage

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The genotype DRMR 1191-2 derived from EC564647 × (NUDHYJ 3 × PCR 7) through pedigree selection was tested under AICRP-RM plant physiological trials during 2014-15 and 2015-16 at 5 locations. Pooled data of seedling mortality and dry weight of seedlings over the years and locations indicate that DRMR 1191-2 recorded the lowest seedling mortality as compared to checks (Table 1). DRMR 1191-2 also recorded the maximum dry weight of 10 seedlings as compared to checks.

Table 1: Pooled summary of physiological parameters for proposed genotype DRMR 1191-2 and checks in physiological trials during 2014-15 and 2015-16 of Indian mustard (*Brassica juncea*)

Parameter	*No of locations*	*DRMR 1191-2*	*Check varieties*		
			PM 25	*JD 6*	*Urvashi*
Seedling mortality (%)	5	24.8	31.9	27.9	36.8
Dry weight/10 seedlings (mg)	5	40.4	32.3	36.8	27.1

The heat-tolerant nature of DRMR 1191-2 was validated in laboratory (BOD) experiments at DRMR during 2017. Further, the genotype was physiologically and morphologically characterized at different developmental stages (Table 2). The conclusion from these studies indicated heat tolerance in different selected genotypes is in the following order: DRMR-1191-2> DRMR-1165-40, NPJ-124 >BPR-549-9.

Data generated over the locations and years in laboratory and field conditions indicate that the heat-tolerant characteristics of the promising genotype DRMR 1191-2 can be utilized in a breeding programme aimed at developing high-yielding heat-tolerant varieties. Hence, this line needs protection through germplasm registration.

Table 2: Classification of genotypes on the basis of decrease/ increase in physiological and biochemical parameters over 02 years (2016-17 & 2017-18)

Tolerant	DRMR 1191-2
Moderately tolerant	NPJ 124, DRMR 1165-40
Medium tolerant	BPR 549-9, JN 032

20. RIL87 (IC640189; INGR22164), an Indian mustard germplasm with high tolerance to soil sodicity stress

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Indian mustard [*Brassica juncea* (L.) Czern and Coss] is an important oil-seed crop in the world and is grown in more than 50 countries across the globe, which often experiences saline stress as it is grown extensively in the arid and semi-arid regions of the world. An area of nearly 6.73 million hectares is affected by these stresses in India (Singh *et al*., 2014). Hence, it becomes necessary to develop salt-tolerant genotypes that can serve as donors for breeding salt-tolerant crops in the future. 250 recombinant inbred lines were developed from a cross between CS 614-1-1-100-13×CS 56 to tag QTLs governing salt tolerance in Indian mustard. Out of the above-mentioned mapping population, RIL 87 has great potential for tolerance of sodic conditions.

Consistent performance of RIL87 was recorded among all test entries, including checks during three years of evaluations in the Uttar Pradesh State Adaptive Trial (salt-tolerant mustard) under Sodic conditions (pH_2 >9.3) during 2018-19 to 2020-21 at two locations, Hardoi and Etawah. On average, RIL87 provided a seed yield of 1959 kg/ha, which was 12.98% and 22.98% higher than the yields of two checks Kranti (1734 kg/ha) and CS 54 (1593 kg/ha), respectively, under sodic conditions. RIL87 also provided oil yield (734 kg/ha), which was 21% and 14% higher than the yields of checks CS 54 (605 kg/ha) and Kranti (641 kg/ha) under sodic conditions at ICAR-CSSRI Station trials. This strain matures in 133 days, which is almost at par with two checks. In spite of the same crop duration, the considerable superiority of RIL87 to check varieties highlights that the growth rate and daily productivity of this strain are higher than those of checks under sodic conditions. The strain RIL87 attains a height of approximately 165 cm and produces a high number of primary branches (6), secondary branches (8), main shoot length (68 cm), and 1000 seed weight (5.1 g) under sodic conditions (Table 1).

Looking at its seed and oil yields, RIL87 was proposed for registration as a national genetic stock/ donor for Sodic conditions (pH_2 up to 9.4) and could be used for the development of salt-tolerant mustard genotypes to soil sodicity conditions.

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Table 1: Performance (yield, adaptation and quality traits) under sodicity stress (pH_2 9.4)

*Parameter**	*Year of testing*	*No. of trials*	*Proposed variety*	*Check 1*	*Check 2*
			RIL87	*CS 54 (NC)*	*Kranti (NC)*
Mean seed yield (kg/ha)	2018-19	2	2166	1733	2000
	2019-20	2	2109	1753	1909
	2020-21	2	1378	1293	1293
	Mean	6	1884	1530	1734
Percent increase (+) or decrease (-) over checks	2018-19			+24.99	+8.30
	2019-20			+20.31	+10.48
	2020-21			+6.57	+6.57
	Mean			+18.27	+8.65

**Ref.– Uttar Pradesh State Level Research Advisory Committee Proceeding-2018-19 to 2021-22*

21. IC422166 (INGR22165), an Indian mustard germplasm with resistance against white rust disease

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Oilseed Brassica (*Brassica rapa* var. yellow sarson) plays a vital role in edible oil of the Indian Economy, and rapeseed-mustard is one of the most important oilseed crops. Rapeseed-mustard has been known to be severely damaged by some of the biotic stresses, especially white rust caused by *Albugo candida* (Pers. Ex. Lev.). Germplasm evaluation

Table 1: Phenotyping of mustard germplasm against White rust under natural conditions during rabi 2019-20 and 2020-21

Rating scale (0-9)			*Disease severity and response*		
PDI	*Disease response*	*No. of germplasm*	*Accession*	*Disease severity (%)*	*Response*
0%	Immune (I)	01	IC422166	0.00	I
>5%	Highly Resistant (HR)	Nil	Nil	-	-
5-10%	Resistant (R)	Nil	Nil	-	-
11-25%	Moderately Resistant (MR)	03	IC422157	18.67	MR
			IC422161	19.83	MR
			IC422176	16.33	MR
26-50%	Susceptible (S)	15	IC422156	47.50	S
			IC422158	36.44	S
			IC422162	50.00	S
			IC422168	48.79	S
			IC422169	47.89	S
			IC422177	49.74	S
			IC422184	38.39	S
			IC422186	45.61	S
			IC422187	29.77	S
			IC422194	35.74	S
			IC422188	37.98	S
			IC422181	45.48	S
			IC422173	43.54	S
			IC422164	46.81	S
			IC422160	44.88	S
>50%	Highly Susceptible (HS)	15	IC422159	70.05	HS
			IC422163	68.71	HS
			IC422167	69.11	HS
			IC422170	73.75	HS
			IC422171	71.33	HS
			IC422174	69.47	HS
			IC422175	71.11	HS
			IC422178	67.11	HS
			IC422179	76.17	HS
			IC422180	67.11	HS
			IC422182	61.78	HS
			IC422183	59.47	HS
			IC422185	73.21	HS
			IC422189	66.65	HS
			IC398234	52.05	HS
			PCR 7	79.55	HS
			BIO 902	74.41	HS
CD (P=0.05)				2.55	
SE(m)				0.90	
CV				3.00	

against biotic stresses has been a continuing programme at ICAR-NBPGR Regional Station, Jodhpur. During *rabi* 2019-20 and 2020-21, 34 accessions of indigenous germplasm were evaluated under natural conditions to identify sources of resistance, and scoring of white rust was recorded using a 0-9 scale as adopted by AICRP on rapeseed-mustard (Proceeding of AICRP R&M, 2011) to calculate plant disease index (PDI) at 20 days before the maturity of crop. One accession, i.e. IC422166, was found immune, whereas other accessions showed either susceptible or highly susceptible reactions (Table 1). IC422166 was further validated for its resistance using an artificial inoculation technique at the National Phytotron Facility, ICAR-IARI, New Delhi, against various isolates of *Albugo candida* collected from various mustard growing regions of the country (Ludhiana, SK Nagar, Bharatpur and Wellington). Highly susceptible variety Pusa Jai Kisan was used as a susceptible check. Observation was recorded after one week of inoculation at the cotyledonary stage, whereas disease severity was again recorded after disease development on the true leaf stage. The results showed that IC422166 is absolutely free of infection against the Ab-Ludhiana isolate at both stages. The identified accession can be utilized to develop a mapping population to map genomic regions conferring resistance to White rust. It is established that resistance is governed by one to three resistance genes. The reported genes/QTLs, such as LG A4, LG A5, and LG A6 can be validated in this newly identified accession and can be introgressed in the susceptible but otherwise agronomically superior *Brassica* germplasm through marker-assisted selection.

Reference

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22. RH 1222-28 (IC645775; INGR22166), an Indian mustard germplasm highly tolerant to *Sclerotinia* stem rot disease

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Sclerotinia stem rot of oilseed Brassica, caused by the phytopathogenic fungus *Sclerotinia sclerotiorum,* is a major impediment in the cultivation of Brassica, where it causes yield loss ranging from 5 to 100%. The germplasm of Indian mustard was screened to find a resistant/tolerant genotype for Sclerotinia stem rot. The genotype RH 1222-28 showed consistently tolerant/resistant reaction against stem rot disease when tested over a longer period, from 2014-15 to 2020-21, over three different locations (DRMR, Bharatpur; NRCPB, New Delhi; CCSHAU, Hisar) (Table 1).

Genotype RH 1222-28 was derived through hybridization between RH 0406 × RH 0401-B, procured from CCSHAU, Hisar, tested for Sclerotinia stem rot tolerance through artificial stem inoculation technique and growing under Sclerotinia sick plots at ICAR-DRMR, Bharatpur during 2014-15 and 2015-16 (Sharma *et al*., 2018). It showed a tolerant reaction (lesion size <3.0 cm, disease incidence <10%). It was again tested in 2016-17, 2017-18, and 2019-20 at DRMR, Bharatpur and found consistent in terms of tolerant reaction (lesion size <3.0 cm, disease incidence<10%). RH 1222-28 was also tested at NIPB, New Delhi, during 2016-17 to 2018-19 for three years, where it again showed a tolerant reaction (lesion size<3.0 cm, disease incidence<10%). RH 1222-28 was also tested for stem rot resistance at CCSHAU during

Table 1: Performance of RH 1222-28 against stem rot disease over six years (2014-15 to 2020-21) and three locations (Bharatpur, New Delhi, Hisar)

Year and location	*Method used*	*Genotype and Response*
2014-15, 2015-16 DRMR, Bharatpur	Stem inoculation and growing under a sick plot	RH 1222-28: Tolerant (Lesion size<3.0 cm, disease incidence<10%), Rohini (check): Susceptible, NRCYS (check): Susceptible
2015-16 to 2018-19 DRMR, Bharatpur	Stem inoculation and growing under a sick plot	RH 1222-28: Tolerant (Lesion size<3.0 cm, disease incidence<10%); Rohini (check): Susceptible, NRCYS (check): Susceptible
2016-17 to 2018-19 NRCPB, New Delhi	Stem inoculation and growing under a sick plot	RH 1222-28: Tolerant (Lesion size<3.0 cm, disease incidence<10%); Rohini (check): Susceptible
2019-20 CCSHAU, Hisar	Stem inoculation	RH 1222-28: Resistant (Lesion size 2.5-5 cm, AUDPC (32.28), High POX activity, Low CAT activity and High total soluble Phenolics)

Note: Resistant/tolerant scale used for scoring of RH 1222-28 was same (Lesion size <3 cm/ 2.5-5 cm).

2019-20 and found resistant to disease based on mean lesion length (2.5-5 cm), AUDPC (32.28), High POX activity, Low CAT activity, and High total soluble Phenolics (Singh *et al*., 2020). Therefore, this genetic stock can be used as a donor in Indian mustard (*Brassica juncea*) improvement programme to develop stem rot-resistant varieties.

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Singh M, R Avtar, A Pal, R Punia, VK Singh, M Bishnoi, A Singh, RR Choudhary and S Mandhania (2020) Genotype-specific antioxidant responses and assessment of resistance against *Sclerotinia sclerotiorum* causing *Sclerotinia* rot in Indian mustard. *Pathogens* 9(11): 892. doi: 10.3390/pathogens9110892

23. NIPB-Bnap114 (IC646858; INGR22167), a rapeseed germplasm with *Sclerotinia* stem rot disease resistance

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Stem rot disease caused by *Sclerotinia sclerotiorum* (Lib.) de Bary is one of the most devastating diseases of dicotyledonous species, including oilseed *Brassica*, and causes severe yield loss annually. The line NIPB-*Brassica napus* (L.) (NIPB-Bnap114) has been identified as resistant to the *Sclerotinia* stem rot disease in two different locations under both field and greenhouse conditions. This line has been found as self-compatible and crossable to *B. juncea*, *B. rapa*, and *B. carinata*. NIPB-Bnap114 line matures 145-155 days after sowing. The stem of this line is comparatively hardy, waxy, and richer in lignin content than other *B. juncea* or *B. rapa* lines. The height of the mature NIPB-Bnap114 line reaches up to 205 cm and the average diameter of its stem varies from 17-26 mm. The leaf of this line is slightly oval, smooth, and wider in shape, and the leaf margins are widely serrated. The inflorescence of the NIPB-Bnap114 line is typical of B. napus type i.e., racemes that form on the main and axillary branches. The siliques are thin and long and their arrangement is mainly at the right angle to the rachis and closely distributed over the floral axis.). The line was found moderately resistant to the stem rot disease and usually, a symptom like water-soaked lesions of 0.5-3 cm size appears 21 days post-infection at the site of S. sclerotiorum inoculation (Table 1). It has also been observed that the NIPB-Bnap114 line is compatible for crossing with *B. juncea*, *B. rapa*, and *B. carinata*, and no embryo rescue or bridge crossing was required for the trait transfer. Although the stem rot resistance trait has been considered a quantitative trait whereas the interspecific *hybrids* of the NIPB-Bnap114 with *B. juncea* elite cultivar (Pusa Jaikisan) in the F1 generation were found to inherit the stem rot resistance as a qualitative trait.

Table 1: The Field-based stem inoculation assay results after 21 days of infection of the 65-days-old NIPB-Bnap114 lines with *S. sclerotiorum* ESR-01 isolate

Location	*Trait*	*2017-18*	*2018-19*	*2019-20*	*2020-21*	*2021-22*
ICAR-IARI, New Delhi	Mean stem diameter (mm)	19.5	20.2	18.3	23.4	21.6
	Mean lesion length (cm)	2.5	1.5	3.2	0.	1.75
ICAR-DRMR, Bharatpur	Mean stem diameter (mm)	19.5	21.6	20.7	21.8	23.2
	Mean lesion length (cm)	0.5	1.5	0.5	0.7	0.5

24. NIPB-Bcar115 (IC646859; INGR22168), an african mustard germplasm with *Sclerotinia* stem rot disease resistance and dark purple stem

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Stem rot disease caused by *Sclerotinia sclerotiorum* (Lib.) de Bary is one of the most devastating diseases of dicotyledonous species, including oilseed *Brassica* and causes severe yield loss annually. The resistance breeding program for the stem rot disease is mainly hindered because of the non-availability of resistant sources, especially in *B. juncea,* or the presence of partial resistance/tolerance in very few germplasms of *B. napus, B. oleracia,* and *Sinapis alba*. The line NIPB-*Brassica Carinata* 115 (NIPB-Bcar115) has been identified as resistant to the *Sclerotinia* stem rot disease in two different locations namely IARI, New Delhi, and DRMR, Bharatpur, Rajasthan, under both field and greenhouse conditions. This line has been found as self-compatible and crossable to *B. juncea* and *B. napus*. NIPB-Bcar115 line matures 165-175 days after sowing. The stem of this line is very hardy and rich in lignin and anthocyanin, making its shoot purple. The pith diameter of the mature stem is comparatively less than the other *Brassica* species. The height of the mature Bcar115 line reaches up to 295 cm, and the diameter is ~24mm. The leaf of this line is smooth, slightly oval, and wider in shape, and the leaf margins are widely serrated. The silique arrangement is mainly adpressed and scantly distributed over the floral axis. The NIPB-*Brassica carinata* 115 (NIPB-Bcar115) has been screened for *Sclerotinia* stem rot disease thoroughly at two different locations namely IARI, New Delhi, and Bharatpur, Rajasthan, respectively, for five subsequent years (2017-2022) under both the field and glasshouse conditions. The line was found completely resistant to the stem rot disease and only black/brown scar marks were observed at the site of *S. sclerotiorum* inoculation after 21 days of infection (Table 1). It has also been observed that the NIPB-Bcar-115 line is compatible for crossing with *B. juncea* and *B. napus* and no embryo rescue or bridge crossing was required for the trait transfer. Although the stem rot resistance trait has been considered a quantitative trait, the interspecific hybrids of the NIPB-Bcar115 with *B. juncea* elite cultivar (Pusa Jaikisan) in the F1 generation were found to inherit the stem rot resistance as a qualitative trait.

Table 1: The Field-based stem inoculation assay results after 21 days of infection to the 65-days-old NIPB-Bcar 115 lines with *S. sclerotiorum* ESR-01 isolate

Location	*Trait*	*2017-18*	*2018-19*	*2019-20*	*2020-21*	*2021-22*
ICAR-IARI, New Delhi	Mean stem diameter (mm)	19.48	18.6	21.8	19.73	20.9
	Mean lesion length (cm)	Scar mark	Scar mark	Scar mark	Scar mark	Scar mark
ICAR-DRMR, Bharatpur	Mean stem diameter (mm)	21.3	20.7	21.2	19.4	22.3
	Mean lesion length (cm)	Scar mark	Scar mark	Scar mark	Scar mark	Scar mark

25. JS 20-38 (IC646860; INGR22169), a soybean germplasm for waterlogging tolerance

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Waterlogging stress is the main destructive abiotic stress affecting soybean (*Glycine max* L.) seed production worldwide. Soybean variety JS 97-52, a known source for waterlogging tolerance, was used as a check in the experiments conducted during 2013 to 2021 to identify a waterlogging-tolerant genotype. Controlled conditions were created by waterlogging for 15 days (12-15 cm water level at R_1 stage) and 10 days (10-12 cm water level at V_{2-3} stage) for reproductive and vegetative stages, respectively, as per standardized protocols (Chandra *et al.,* 2019). JS 20-38 {a breeding line (JS 96-63 × PK 768) developed by AICRP center, JNKVV, Jabalpur} performed better than JS 97-52 during *kharif* 2019 and *kharif* 2020 at Indore and *kharif* 2019 at Umiam (Annual Reports 2019 and 2021 ICAR-IISR, Indore. JS 20-38 also performed better in terms of root porosity, aerenchyma tissue development, and rapid growth of adventitious roots (Annual Report, ICAR-IISR, Indore 2014-15) and early maturity (88-90 days). JS 20-38 showed better performance than Check JS 97-52for different water logging parameters *i.e.* less reduction in grain yield, SCMR (SPAD chlorophyll meter reading), chlorophyll content, root nodule dry weight, foliar damage, higher stem elongation

Table 1: Summary of waterlogging tolerance parameters in JS 20-38 as compared to tolerant check JS 97-52

Waterlogging tolerance parameters as compared to ambient conditions	*JS 20-38*	*JS 97-52*
Reproductive phase		
% reduction in yield/plant (2018)	6.65%	9%
% reduction in SCMR (2018)	0.68%	12.63%
% reduction in total chlorophyll content (2018)	28.98%	32.12%
% changes in plant height (2014)	+10%	-5%
Vegetative Phase		
Waterlogging tolerance coefficient (2019 & 2020)	75.97 & 77.91	72.12 & 48.50
% reduction in root nodule dry weight (2019)	3.37%	5.92%
Stem elongation rate (2019)	120.55%	118.81%
Plant survival rate (2020)	92.73%	85.17%
Foliar damage score (2020)	2	2.5
% reduction in SCMR (2020)	22.94%	33.05%
Adventitious root ratings (2020)	5	5

rate, water logging tolerance coefficient, plant survival rate, adventitious root development, root porosity and aerenchyma development under waterlogging conditions during evaluation at different plant stages (Table 1) (Chandra *et al.,* 2020).

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26. IC628011 (INGR22170), a banana germplasm resistant to root lesion nematode

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Broadening the genetic base of the diploids is the primary step in the banana breeding program. To date, only three diploid accessions are being used as male parents for improving biotic resistance. In general, Pisang Awak group (ABB) is susceptible to root lesion nematode (*Pratylenchus coffeae*). A compatible male source is a prerequisite to incorporate resistance without a yield penalty in the commercial cultivars. Hence, efforts were taken to develop Pisang Awak-based nematode-resistant and polleniferous diploid using the Karpooravalli, ABB genome. The Pisang Awak sub-group was hybridized with nematode-resistant diploid male source Pisang Jajee. A total of ten diploid progenies were obtained, of which one AA diploid polleniferous progeny (NRCB/P-0115 (IC No. 628011). Hence, the Karpuravalli-based diploid pollen fertile progeny No 115 (Karpuravalli × Pisang Lilin) has been screened under pot culture and field to know its resistance reaction against *P. coffeae*. Host reaction for *P. coffeae* was studied based on root lesion index with 1 to 5 scales as described by Pinochet (1988). This study revealed that NRCB/P-0115 (IC No. 628011) is resistant to root lesion nematode with a scale of 1 and is considered resistant.

Pseudostem is green with black brown blotches at the base of the petiole. The Pseudostem height is 1-5 to 1.7m with a 45 to 50 cm circumference at the base. Leaves are erect, green, and dull on both upper and lower surfaces. Peduncle is 20 to 25 cm long, dense in pubescent nature and green in colour, slightly angular in position. There are 4 to 6 hands of fruit in a bunch, 14 to 15 fruits in each hand. Rachis is 50-60 cm in length, completely barren and the bract scars are very predominant with maximum interspaces. Pendulous in position. Male bud is purple brown in colour,

top in shape with a pointed tip. Male flowers are orange-yellow in colour. Fruits are green in colour, 9 to 11 cm in length, with a gradual tip. Pulp is creamy with a sweet taste. Pedicel is very short with below 1 cm length.

Being a male fertile nature, this could be successfully utilized in the banana breeding programme to incorporate *P. coffeae* resistance in the Pisang Awak-based commercial cultivars.

27. IC628037 (INGR22171), a banana germplasm with resistance to root lesion nematode

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Plant parasitic nematodes are important production constraints of banana and plantain (*Musa* sp.) cultivation, causing yield loss of 19.7% globally. Among parasitic nematodes, the root-lesion nematode (*Pratylenchus coffeae*) is a polyphagous, migratory endoparsitic nematode pest causing 'Toppling Disease'. Among the land races of banana, Nendran belongs to AAB plantain subgroup, is grown in South India and it is suitable for multifarious use such as dessert, culinary, chips and baby powder preparation. But highly susceptible to root lesion nematode and a yield reduction was observed 44% and also hampering the ratoon cultivation. Cultivation of nematode-resistant Nendran is a promising strategy for low-cost, environmentally safe (Speijer and De Waele *et al.,* 1997). By keeping this in view, efforts were taken to develop *P. coffeae* resistance in Nendran through conventional breeding. Nematode-resistant diploid sources namely Pisang Lilin and cv. Roses were hybridized with cv. Nendran and twelve progenies were obtained. All the progenies were screened against *P. coffeae* at field conditions. Root samples were collected from different banana hybrids grown in nematode nematode-sick field. The nematode population was estimated and the root lesion index was calculated (Pinochet *et al.,* 1988) from the functional, cord roots (15 cm length), which were collected from the progenies during the flowering stage of the plant (Table 1). Among the 12 progenies NPL 30 (Nendran × Pisang Lilin) was recorded with an average root lesion index of 1. This resistant progeny was subjected to pot culture screening and showed a similar resistant reaction.

As the bunch and fruit characters of NPL 30 are similar to those of AAB genomic and pome sub-group of hill banana type, it can be substituted for Hill banana and recommended to the farmer to grow this resistant progeny in place of Hill banana varieties.

Table 1: Root lesion nematode resistant reaction of Nendran progenies based on root lesion index.

Hybrid no.	*Average nematode population /g root*	*Root-lesion Index*	*Reaction*
NPL 28	103	5	Susceptible
NPL 30	6	1	Resistant
NPL 33	152	5	Susceptible
NPL 34	106	5	Susceptible
NPL 36	105	5	Susceptible
NOP 43	111	5	Susceptible
NCR 2	139	5	Susceptible
NCR 5	45	4	Susceptible
NCR 8	114	5	Susceptible
NCR 10	81	5	Susceptible
NCR 18	39	4	Susceptible
NCR 21	38	4	Susceptible

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28 IC438858 (INGR22172), a jackfruit germplasm with high number of fruits (107 fruits/tree)

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Jack (*Artocarpus heterophyllus* Lam.) is a multipurpose tree. Every part of the tree can be put to use in various ways, with the majority of them being of commercial or nutritional value. ICAR-National Bureau of Plant Genetic

Resources, Regional Station Ranchi, collected a Jackfruit (*Artocarpus heterophyllus*) accession from Gumla district, Jharkhand, in 2003. Collected accession established in the Field Gene Bank at the regional station in the same year and started fruiting in 2011. It is a vigorous plant bearing 104 to 113 fruits per plant from 2017 to 2021. Thus popularisation of its clone across the region could bring a paradigm shift in Jackfruit-based diversified industrial applications (Ranasinghe *et al*., 2019). Given the importance of profuse bearing genotypes in realising commercial prospects of fruit crops, the accession seems promising in realising the potential commercial prospects of Jackfruit by facilitating a commercially viable orchard.

Reference

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29. VKG 03/029 (IC24351; INGR22173), a jackfruit germplasm with dwarf canopy

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Jack (*Artocarpus heterophyllus* Lam.) is a multipurpose tree. Every part of the tree can be put to use in various ways with the majority of them being of commercial or nutritional value. For instance, immature fruits are used as a vegetable and serve as an excellent vegetarian substitute for meat (Narasimham *et al.,* 1990). With varied texture and pulp colour mature fruit is consumed fresh or preserved in myriad ways to harness nutritional richness, particularly protein, minerals (Mg, K, Fe), and vitamins (ascorbic acid, pyridoxine, niacin, riboflavin, and folic acid) (Swami *et al*., 2012). Mature fruit also yields several beneficial phytochemicals like carotenoids, flavonoids, volatile fatty acids, sterols, and tannins. Most of these phytochemicals offer health benefits by preventing chronic diseases like cancer, inflammation, cardiovascular malfunctioning, cataract, and age-related muscular degeneration (Ranasinghe *et al*., 2019). ICAR-National Bureau of Plant Genetic Resources, Regional Station Ranchi, collected a Jackfruit (*Artocarpus heterophyllus*) accession from Teliya block of East Singhbhum, Jharkhand, in 1995. Collected accession established in the Field Gene Bank at the regional stationin the same year and started fruiting in 2016. It is a slow-growing accession of Jackfruit with a plant height of 2.7 m only in contrast to 6-8 m normal plant height. Its leaf, petiole, peduncle, fruit, pulp and seed sizes are smaller than the normal. Despite the reduced growth habit, the accession bearing fruit consistently and could serve as a potential rootstock for dwarfism inthe crop. Given the importance of dwarf rootstock in realising the commercial prospects of fruit crops, the accession seems promising in realising the potential commercial prospects of Jackfruit by facilitating high-density orchard farming of the crop.

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30. VKG 03/047 (IC24369; INGR22174), a jackfruit germplasm with extra early fruit bearing

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Jack (*Artocarpus heterophyllus* Lam.) is a multipurpose tree. Every part of the tree can be put to use in various ways, with the majority of them being of commercial or nutritional value. With varied texture and pulp colour mature fruit is consumed fresh or preserved in myriad ways to harness nutritional richness, particularly protein, minerals (Mg, K, Fe), and vitamins (ascorbic acid, pyridoxine, niacin, riboflavin, and folic acid) (Swami *et al*., 2012). ICAR- National Bureau of Plant Genetic Resources, Regional Station Ranchi, collected a Jackfruit (*Artocarpus heterophyllus*) accession

from Keonjhar district of Odisha in 1995. Collected accession established in the Field Gene Bank at the regional station in the same year and started fruiting in 2014. It is average height accession of Jackfruit starts bearing tender fruits in December, when other Jackfruit accessions have just started flowering. Therefore, its tender fruit reaches the market at least 60 days before under the Ranchi condition. The time advance offers a premium price to the harvested fruit (15 times higher price realisation). Thus, the accession is an important genotype for realising commercial advantage; besides, it could be a potential source for understanding gene(s) underlying commercially important traits like flowering and fruiting (Lu *et al.,* 2022) in the species. Such a basic understanding of commercial attributes will pave the way to design Jackfruits with diversified harvesting time so that better prices in naturalised regions can be ensured for the farmers.

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31. IC641340 (INGR22175), a noni germplasm with cluster bearing habit; 4 to 5 obovate elongate fruits borne at each of the alternate nodes; Large sized fruits weighing 307 g each attain average maximum length of 11.70 cm and width 5.9 cm

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Noni (*Morinda citrifolia* L.; Family: Rubiaceae) is a source ingredient in traditional medicine systems such as Ayurveda, Chinese, Polynesian, Australian and Nicobari traditional medicinal systems for curing of various ailments, including arthritis, colds, diabetes, and inflammation. It appears to be a '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 purposes, but fruits are most commonly used for pulp and juice extraction (Singh *et al*., 2012). It is well well-suited potential crop for tropical coastal and island regions, and Noni juice has huge potential as a health supplement in national and international markets (West *et al*., 2018). To explore its commercial potential, it was required to develop genotype(s) that can grow well in open as well as under partial shade conditions (in coconut, arecanut plantations) and are suitable for high-density planting. Its intercropping in existing coconut and arecanut plantations will increase income from the same piece of land and fit well with 'zero land 'cultivation.

Cluster bearing noni genotype is vigorous (3-4 m tall), conical tree canopy, rough bark, medium length, and short. Leaves are elliptical, with an acute leaf apex, broad green leaves, and medium length (10-15 cm). A cluster of average 4 to 5 obovate, elongate fruits is borne at each of the alternate nodes. The yellowish green fruits were large in size, weighing 307 g each with a maximum length of 11.70 cm and a width of 5.9 cm, in addition to their smooth texture with green floral eye rings. The fruit yield ranged from 25 to 38 kg/ tree/year. No major disease or pest incidence was recorded. The fruit seed, pulp and pulp recovery were estimated and recorded *viz*., percentage of seed (10.77), percentage of pulp (91.90) and percentage of fruit pulp recovery (69.96). This cluster bearing type is considered unique owing to the higher yield potential in terms of number of fruits per tree, weight of fruits per tree, regular bearing and uniform-sized fruits makes them suitable for processing. The cluster bearing noni germplasm Distinctness, Uniformity and Stability (DUS) characterization *viz*., 12 tree morphology and 24 fruit characters, was documented. This characterization will be useful to release this as a variety for the benefit of the Island farmers to improve their farm income.

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32. DLB-7 (IC646862; INGR22176), a lemon basil germplasm with broadly ovate leaf shape and richness in Citral A and B content

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Lemon basil (*Ocimum* × *africanum* L.), also known as lebutulsi is a hybrid between *O. basilicum* L. and *O. americanum* L. The herb is believed to be native to Asia (South-Eastern Asia), Africa, and South America. Lemon-scented basil is known for its sweet citrus flavour and fragrance. The highest antimicrobial activities were demonstrated by lemon basil as compared to other *Ocimum* species. The leaves are a good source of vitamin A, C, K, magnesium, iron, copper and calcium. Aromatic grasses are perennial in nature and are grown commercially for up to 5-6 years after plantation; therefore, farmers are hesitant to grow such perennial grass in their fields to avoid long-term field blockage. Seasonal variations are observed in a very specific manner in a country like India. The lemon basil is a short-duration crop and harvested even in the same season (70-75 days after planting) compared to perennial aromatic grasses. Therefore, lemon basil elite 'DLB-7' was found suitable for total citral production and has citral A (40.59%) plus citral B (48.59%) with broadly ovate leaf shape at 3-5th node from top as a morphological marker. While Citral A (22.71%) and Citral B (25.84%) content were significantly reduced with ovate to lanceolate leaf shape in the farmer's cultivar (Table 1). Such (DLB-7) elites have potential for the aroma and food industry and can be promoted as a selection for commercial cultivation.

Table 1: Average (two years) phytochemical compounds in essential oil

Compound	*Retention time*	*Retention index*	*DLB-7*	*Check*
Linalool (%)	8.661	1099	2.7	16.05
Terpinen-4-ol (%)	10.159	1177	-	-
α-Terpineol (%)	10.389	1189	-	1.09
Methyl Chavicol (%)	10.532	1196	0.01	0.23
(-)-beta-Pinene (%)	11.021	1266	1.53	8.08
Citral A (%)	11.259	1240	40.59	22.71
cis-Geraniol (%)	11.471	1245	1.03	8.17
Citral B (%)	11.767	1276	48.59	25.84
Lavandulyl acetate (%)	11.942	1289	0.01	0.31
Caryophyllene (%)	14.383	1419	0.21	6.79
Trans α-Bergamotene (%)	14.566	1435	0.62	6.56
Humulene (%)	14.924	1454	-	0.51
Germacrene D (%)	15.351	1481	-	0.88
Cis-α-Bisabolene (%)	16.198	1504	1.26	4.09
Caryophyllene oxide (%)	16.926	1581	0.72	0.56

(-) means compound not detected in essential oils.

33. DOGr-2 (IC646864; INGR22177), a shrubby basil germplasm with narrow ovate leaf shape and richness in ß-Copaene and a-Bergamotene

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Ocimum gratissimum (L.) is an aromatic shrub popularly known as clove basil and used as a condiment in several food items, and also used for its therapeutic properties. Clove basil is a naturally thriving shrub in the tropics and semi-arid regions, ubiquitous in tropical and sub-tropical forests of India, Brazil, Vietnam, Nigeria, South Africa,

Australia, New Zealand, and China. Clove basil belongs to the Lamiaceae family and is perennial in nature, having big leaves as compared to *O. sanctum*, *O. basilicum*, *O. canum*, *O. africanum,* etc. In order to take advantage of this hardy species, big leaf size and chemical content can be accommodated in poor or degraded soils with minimum inputs for the herbal industry, especially for dry leaves production. This is indicative of the fact that for a year harvest, this can be grown commercially for multiple harvests as compared to holy and sweet basil. The species has been used as medicine for the treatment of several diseases for a long time. It is also serving as a source of new drugs, especially in traditional systems like Ayurveda and modern pharmacology. Clove basil has also been used as a juice, dried powder of aerial parts, as condiments, tea and leaves as a vegetable in fish and soup preparation in African and Asian countries. Among the evaluated accessions, DOGr-2 was proven to be unique in characters significantly for maximum internodal length (10.80 cm), petiole length (6.58 cm), and leaf length (12.1 cm). Therefore, accession DOGr-2 has been identified for having maximum leaf length (12.10 cm) during the vegetative phase with a narrow, ovate leaf shape. In addition to morphotype, DOGr-2, possessing the maximum share of aromatic compounds in essential oil *viz*., methyl chavicol (3.75%), copaene (1.83%), β-elemene (2.33%), methyl eugenol (9.72%), α-bergamotene (15.23%), β-copanene (20.48%) and δ-cadinene (0.96%) were recorded among available accessions (Table 1). The richness of chemotype DOGr-2 in α-bergamotene and β-copanene is a precursor for the standardization and preparation of several formulations for sanitization and disease management.

Table 1: Average phytochemical variations in essential oil of selected accessions of *Ocimum gratissimum* (two-year data)

Compound	*Retention time (Min.)*	*Retention index[a] (HP-5MS)*	*Retention index[b] (HP-5MS)*	*Accession (%)*		
				DOGr-1	*DOGr-2*	*DOGr-3*
α-Pinene	7.492	937	939	0.97	0.88	0.32
Linalool	8.67	1099	1098	0.87	0.64	0.5
Methyl Chevicol	10.543	1196	1203	2.63	3.75	1.4
Eugenol	13.271	1357	1359	64.35	54.27	75.45
Copaene	13.641	1376	1378	1.24	1.83	1.41
β-Elemene	13.882	1391	1389	0.72	2.33	1.77
Methyleugenol	14.012	1402	1399	0.01	9.72	1.5
Caryophyllene	14.383	1419	1423	3.26	9.52	11.7
α-Bergamotene	14.566	1435	1433	11.41	15.23	11.46
β-Copaene	15.348	1437	1436.9	7.34	20.48	5.3
α-(Z,E)-Farnesene	15.427	1491	1486	2.51	2.44	0.88
δ-Cadinene	15.956	1524	1528	0.83	0.96	0.5
Caryophyllene oxide	16.925	1581	1578	0.01	0.58	0.61

** Retention index[a] = Experimental, Retention index[b] = Literature*

34. Ktlis-1 (IC646867; INGR22178), a lisianthus germplasm with violet rose shaped double flower suitable for cut flower production with high number of flowers

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Driven by geographic expansion and advances in cultivation and logistics, the trade in cut flowers has witnessed significant growth in recent years (Rabobank *et al*., 2015). Among the major cut flower species, Lisianthus (*Eustoma grandiflora*) has seen increasing commercialization and popularity due to its rose-like appearance, long post-harvest life, and wide variety of cultivars offering an attractive spectrum of colours. Recently introduced to India as a specialty cut flower, Lisianthus cultivation has rapidly expanded. Currently, the planting material for Lisianthus is imported, but the species faces adaptation challenges at temperatures above 25°C (Roni *et al*., 2016). These

Table 1: Mean performance of lisianthus genotypes for growth and flowering traits

Genotype	*Plant height (cm)*	*No. of flowers /stem*	*No. of shoots/ plant*	*Bud length (cm)*	*Flower diameter (cm)*	*Leaf area cm²)*	*Total number of flowers/ plant*	*No. of petals /flower*
Ktlis-11	64.85	7.08	4.25	5.18	7.32	26.40	17.17	29.33
Ktlis-12	46.21	5.08	3.50	4.73	6.70	24.22	13.50	24.25
Ktlis-8	59.79	7.25	4.25	4.79	6.91	19.38	20.58	17.33
Ktlis-14	64.64	7.50	3.67	4.78	6.51	24.24	23.00	21.33
Ktlis-19	43.50	5.92	4.00	4.37	5.87	17.66	19.92	5.00
Ktlis-15	63.36	6.75	3.67	4.43	6.70	23.27	14.50	17.58
Ktlis-17	65.75	10.00	3.25	4.62	5.88	23.78	18.92	17.00
Ktlis-1	82.98	6.83	3.42	4.99	6.89	37.05	24.17	22.83
Ktlis-5	69.00	9.17	3.83	4.83	7.18	20.25	27.00	26.33
Ktlis-18	52.10	6.00	3.58	4.14	5.72	18.26	18.75	22.75
Ktlis-16	52.03	5.67	3.00	4.95	7.16	26.69	17.33	22.00
Ktlis-2	55.10	6.17	3.58	3.92	5.73	23.27	15.33	21.92
Ktlis-6	60.51	7.17	4.25	4.46	6.02	20.65	22.75	17.17
Ktlis-20	64.55	8.83	4.58	4.57	6.49	24.54	27.42	20.33
Ktlis-21	59.50	5.58	4.33	4.93	7.99	31.37	18.67	25.17
Ktlis-13	67.13	6.00	4.42	4.84	7.83	23.94	23.58	24.00
Ktlis-7	65.78	9.08	5.33	4.47	6.16	22.33	29.83	23.00
Ktlis-10	65.50	6.50	4.08	4.97	4.63	19.96	21.33	5.00
Ktlis-9	70.98	7.50	3.75	4.52	7.01	15.84	21.67	16.50
$CD_{(P=5\%)}$	6.43	2.15	NS	0.62	0.81	7.71	NS	4.05
SE(m)	2.23	0.75	0.46	0.22	0.28	2.68	3.40	1.41
SE(d)	3.16	1.06	0.65	0.30	0.40	3.79	4.80	1.99
C.V.	6.27	18.36	20.16	7.99	7.40	19.89	28.27	12.20

difficulties have spurred genetic improvement programs, as genetic variation is essential for populations to evolve and adapt. The development of an indigenous hybrid seed industry for lisianthus is vital. One notable achievement in this context is the breeding line *'Ktlis-1*,' an indigenously developed line. This line produces violet coloured with a brown base (RHS Colour Chart: 86B), double flowers with high-quality standard-grade stems, boasting over 24 flowers per plant (Table 1), making it ideal for commercial cut flower production. It also has a longer flowering period, good seed setting and tolerance to diseases, making it well-suited for cut flower production and a prime candidate for use in commercial hybrid breeding across India.

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35. Ktlis-17 (IC646868; INGR22179), a lisianthus germplasm with pure white rose shaped double flower suitable for cut flower production with high number of flowers

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Lisianthus (*Eustoma grandiflora*) is an important floricultural crop cultivated worldwide. Lisianthus is becoming one of the most highly ranked cut flowers in the international market due to its rose-like flowers, excellent post-harvest life and blue/purple colour (Halevy and Konfranek, 1984). Lisianthus has been recently introduced in India as a new specialty cut flower and its cultivation has rapidly expanded, becoming a popular alternative for growers. The planting material of this crop is imported from abroad. *Eustoma grandiflora* presents adaptation difficulties at temperatures above 25°C (Roni *et al*., 2016), which has led to studies on genetic improvement programmes. The genetic variation created is useful because it helps the population to survive and change over time. The lisianthus breeding line, Ktlis-17 is one indigenously developed line. It has snow white coloured double flowers with green bases (143B, RHS colour chart), produces a good quality stem of standard grade with more than 18 flowers per plant, and is suitable for cut flower production. Its height is 65.75 cm and bears 10.0 flowers per stem (Table 1). It flowers during July-September under protected conditions. This line also has a longer flowering duration, good seed setting and resistance to diseases. It can be used by breeders all over India for the development of indigenous hybrids with novel traits.

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Ktlis-12	46.21	5.08	3.50	4.73	6.70	24.22	13.50	24.25
Ktlis-8	59.79	7.25	4.25	4.79	6.91	19.38	20.58	17.33
Ktlis-14	64.64	7.50	3.67	4.78	6.51	24.24	23.00	21.33
Ktlis-19	43.50	5.92	4.00	4.37	5.87	17.66	19.92	5.00
Ktlis-15	63.36	6.75	3.67	4.43	6.70	23.27	14.50	17.58
Ktlis-17	65.75	10.00	3.25	4.62	5.88	23.78	18.92	17.00
Ktlis-1	82.98	6.83	3.42	4.99	6.89	37.05	24.17	22.83
Ktlis-5	69.00	9.17	3.83	4.83	7.18	20.25	27.00	26.33
Ktlis-18	52.10	6.00	3.58	4.14	5.72	18.26	18.75	22.75
Ktlis-16	52.03	5.67	3.00	4.95	7.16	26.69	17.33	22.00
Ktlis-2	55.10	6.17	3.58	3.92	5.73	23.27	15.33	21.92
Ktlis-6	60.51	7.17	4.25	4.46	6.02	20.65	22.75	17.17
Ktlis-20	64.55	8.83	4.58	4.57	6.49	24.54	27.42	20.33
Ktlis-21	59.50	5.58	4.33	4.93	7.99	31.37	18.67	25.17
Ktlis-13	67.13	6.00	4.42	4.84	7.83	23.94	23.58	24.00
Ktlis-7	65.78	9.08	5.33	4.47	6.16	22.33	29.83	23.00
Ktlis-10	65.50	6.50	4.08	4.97	4.63	19.96	21.33	5.00
Ktlis-9	70.98	7.50	3.75	4.52	7.01	15.84	21.67	16.50
$CD_{(P=5\%)}$	6.43	2.15	NS	0.62	0.81	7.71	NS	4.05
SE(m)	2.23	0.75	0.46	0.22	0.28	2.68	3.40	1.41
SE(d)	3.16	1.06	0.65	0.30	0.40	3.79	4.80	1.99
C.V.	6.27	18.36	20.16	7.99	7.40	19.89	28.27	12.20

36. IIHR2-16 (IC636418; INGR22180), a chrysanthemum germplasm with attractive flower colour coupled with dwarf stature and spreading plant growth and early flowering

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Chrysanthemum (*Chrysanthemum morifolium*), family Asteraceae, is a leading commercial flower crop ofversatile use such as loose flowers for garland making and cut-flower for flower arrangement, planted in the bed and cultured in the pot as pot mum.

The chrysanthemum selection IIHR2-16 (IC636418) is derived from cv. White Prolific through half-sib selection was evaluated for three years in an 8-inch plastic pot in CRD under open-grown conditions. The half-sib selection IIHR2-16 has spreading growth, dwarf plants, flowers are semi-double, pinkish white in colour (RHS colour: 3D, Yellow Group, Fan 1) and 3 to 4 rows of ray florets. It is suitable for pot culture and bedding. Plants are dwarf (20.27 cm), spreading growth habit, flowers are semi-double, pinkish white (RHS colour: 3D, Yellow Group, Fan 1). It has a plant spread (33.87 cm), number of sprays per plant (7.43), days to bud appearance (53.00) and flower opening (69.17), flower diameter (3.43 cm), number of flowers per plant (84.70) and duration offlowering (30.83 days) (Table 1). Thus, IIHR2-16 with its attractive flower colour coupled with dwarf stature and spreading plant growth and early flowering is an elite germplasm for commercial production.

Table 1: Morpho-agronomic description of chrysanthemum half-sib selection IIHR2-16 (IC636418)

Trait description	*Pooled data of three years*
Plant height (cm)	20.27
Plant spread (cm)	33.87
Number of sprays per plant	7.43
Days to bud appearance	53.00
Days to flower opening	69.17
Flower diameter (cm)	3.43
No. of flowers per plant	84.70
Duration of flowering (day)	30.83
Growth habit	Spreading
Flower colour (R.H.S. colour chart)	3D, Yellow Group, Fan 1
Type of flower	Semi-double

37. IIHR2-13 (IC645570; INGR22181), a chrysanthemum germplasm with attractive flower colour coupled with dwarf stature and Early flowering

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The chrysanthemum (*Chrysanthemum morifolium*) selection IIHR2-13 (IC645570) is derived from cv. Sunil through half-sib selection and is developed at ICAR-Indian Institute of Horticultural Research, Bengaluru, Karnataka (13° 58' N Latitude, 78°E Longitude and at an altitude of 890 meters above mean sea level), India. It was evaluated for three years in an 8-inch plastic pot in CRD under open-grown conditions. The half-sib selection IIHR2-13 has erect growth, dwarf plants, flowers are semi-double, deep pinkish purple in colour (RHS colour: 71B, Red-Purple Group, Fan 2) and 5 to 6 rows of ray florets. It is suitable for pot culture and bedding. Plants are dwarf (30.17 cm), spreading growth habit, flowers are semi-double, pinkish white (RHS colour: 3D, Yellow Group, Fan 1). It has a plant spread (28.83 cm), number of sprays per plant (8.77), days to bud appearance (56.54) and flower opening (66.52), flower diameter (3.53 cm), number of flowers per plant (78.67) and duration of flowering (28.77 days) (Table 1). Thus, IIHR2-13 with its attractive flower colour coupled with dwarf stature and early flowering is an elite germplasm for commercial production.

Table 1: Morpho-agronomic description of chrysanthemum half-sib selection IIHR2-13 (IC645570)

Trait description	*Pooled data of three years*
Plant height (cm)	30.17
Plant spread (cm)	28.83
Number of sprays per plant	8.77
Days to bud appearance	56.54
Days to flower opening	66.52
Flower diameter (cm)	3.53
No. of flowers per plant	78.67
Duration of flowering (day)	28.77
Growth habit	Erect
Flower colour (R.H.S. colour chart)	71B, Red-Purple Group, Fan 2
Type of flower	Semi-double

38. IIHR4-8 (IC636415; INGR22182), a chrysanthemum germplasm with attractive flower colour, dwarf stature, early flowering and resistant to White Rust

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The chrysanthemum (*Chrysanthemum morifolium*) selection IIHR4-8 (IC636415) is derived from cv. Lal Pari through half-sib selection and is developed at ICAR-Indian Institute of Horticultural Research, Bengaluru, Karnataka (13° 58' N Latitude, 78°E Longitude and at an altitude of 890 meters above mean sea level), India. It was evaluated for three years in an 8-inch plastic pot in CRD under open-grown conditions. The half-sib selection IIHR4-8 has semi-erect growth, dwarf plants, flowers are semi-double, maroon-yellow colour (RHS colour: 17A, Yellow Orange Group, Fan 1), 5 to 6 rows of ray florets, and is resistant to white rust disease (*Puccinia horiana*). It is suitable for pot culture and bedding.

Plants are dwarf (25.51 cm), spreading growth habit, flowers are semi-double, maroon-yellow colour (RHS colour: 17A, Yellow Orange Group, Fan 1). It has a plant spread (34.80 cm), number of sprays per plant (10.35), days to bud appearance (55.37) and flower opening (62.77), flower diameter (3.44 cm), number of flowers per plant (140.64), and duration of flowering (33.78 days) (Table 1).

Table 1: Morpho-agronomic description of chrysanthemum half-sib selection IIHR4-8 (IC0636415)

Trait description	*Pooled data of three years*
Plant height (cm)	25.51
Plant spread (cm)	34.80
Number of sprays per plant	10.35
Days to bud appearance	55.37
Days to flower opening	62.77
Flower diameter (cm)	3.44
No. of flowers per plant	140.64
Duration of flowering (days)	33.78
Growth habit	Erect
Flower colour (R.H.S. colour chart)	17A, Yellow Orange Group, Fan 1
Type of flower	Semi-double

39. Co 85019 (IC646869; INGR22183), a sugarcane germplasm with high cane and sugar yield (14.50 t/ha) under tillering phase drought stress

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Drought is one of the important abiotic stresses causing severe yield losses in sugarcane (*Saccharum* sp.). Drought tolerance is complicated with environmental interactions and the plant's response to drought varies with the mode, timing, and severity of the dehydration stress and its occurrence with other abiotic and biotic stress factors. Drought is one of the major production constraints of Maharashtra state, and its severity largely depends on the quantum and frequency of rainfall by the south-west monsoon. Vidarbha and Marathwada regions are more prone to drought stress and are rated as high-risk regions. Identification of sugarcane clones that can tolerate limited water conditions will boost sugarcane production in drought-prone regions of the state. Drought experiment (two plants and one ratoon) trials were conducted at three locations *viz.,* Koparagaon, Nanded, and Pune in Maharashtra by withholding the irrigation during the tillering phase. The results showed that the clones Co 85019 performed extremely well in all the locations under drought for cane and sugar yield. The clone Co 85019 recorded the highest cane yield of 113.04 t/ha compared to the best standard CoM 0265 (98.41 t/ha). It showed an improvement of 33.22% and 14.86% for cane yield over Co 86032 and CoM 0265, respectively. In the ratoon trial at VSI, Pune, the entry Co 85019 showed an improvement of 45.12% for cane yield over the check Co 86032 and 4.51% over CoM 0265 under drought conditions.

The clone Co 85019 also recorded the highest sugar yield of 14.50 t/ha compared to the best standard CoM 0265 (11.75 t/ha). It showed an improvement of 36.40% over Co 86032 and 2.40% over CoM 0265 for sugar yield. It is a mid-late maturing clone evolved through hybridization and selection involving two high-yielding proven parents, Co 7201 and Co 775. It has zigzag purple coloured stalks with heavy wax coating and splits. Based on high cane and sugar yield, it was identified as a drought-tolerant clone and would serve as a valuable parent for developing drought-tolerant varieties in sugarcane

40. Co 98017 (IC646870; INGR22184), a sugarcane germplasm with high millable canes and cane yield under tillering phase drought

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Drought is one of the most important abiotic stresses that influences sugarcane (*Saccharum* sp.) growth and development at all stages, adversely affecting yield and sugar content. Multi-location trials (two plant and one ratoon trials) were conducted under drought conditions in R&D Farms of sugar factories located in Maharashtra. The drought was imposed during tillering phase (60-120 DAP) by withholding the irrigation.

The results showed that the clone Co 98017 recorded a cane yield of 112.72 t/ha and showed an improvement of 32.90% and 14.54% for cane yield over Co 86032 and the drought-tolerant check CoM 0265, respectively. In the ratoon trial at Kopargaon, Co 98017 recorded the 118.30 t/ha of cane yield, significantly superior as compared to the checks namely Co 86032 (51.77 t/ha) and CoM 0265 (54.72 t/ha), respectively, under severe drought conditions. The clone Co 98017 recorded the highest NMC of 74.68 (000/ha) under drought compared to the standards Co 86032 (66.99) and CoM 0265 (62.22). At Kopagaon, it recorded a mean NMC of 79.85 ('000/ha), followed by VSI, Pune (74.68). In ratoon trials, the clone Co 98017 performed extremely well with 94.97 ('000/ha) of NMC compared to 70.91 ('000/ha) recorded by the best standard Co 86032. In ratoon trials, Co 98017 recorded a mean number of tillers of 230.33 ('000/ha) compared to 183.46 ('000/ha) in Co 86032. In both Kopargaon and VSI, Pune, it recorded very high tillers at 120 Days (Table 3). Co 98017 is a mid-late maturing clone for subtropical regions and showed wide adaptability in tropical regions. This clone was evolved through hybridization between the parents Co 8316 and × Co 8213, followed by selection among the progenies of the cross. It has orange-red canes with ovate buds, spines, broad leaves and tight clasping. It exhibited superior drought tolerance potential with high cane yield and NMC under drought and a potential parent for developing superior sugarcane clones with drought tolerance.

41. Co 17008 (IC646871; INGR22185), a sugarcane germplasm with high cane thickness

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Sugarcane (*Saccharum* sp.) is one of the important C4 crops, cultivated in more than 100 countries and contributing to more than 80% global sugar production. One of the major objectives of tropical cane breeding stations is to develop high-yielding, high-quality quality and thick cane varieties. Many studies have shown that in sugarcane, cane yield is determined by the number of other component traits *viz*., cane height, cane diameter, cane weight and no. of millable canes. It is important to identify parental genetic stocks with high thickness to develop high-yielding sugarcane varieties. Co 17008 is a mid-late maturing, high-quality thick cane clone developed through hybridization and selection from the cross involving two parents, Co 0240 and Co 0214, at ICAR-Sugarcane Breeding Institute (SBI), Coimbatore. Subsequent evaluation in the different clonal stages and in the pre-zonal varietal station trial (PZVT) resulted in the identification of Co 17008 during 2017. Co 17008 was promoted to zonal varietal trials under AICRP (Sugarcane), and it was tested in 14 locations in the Peninsular zone. The trials were conducted with 18 entries and three standards (Co 86032, CoC 671 & Co 09004) during 2020 -21.

The results showed that the clone Co 17008 recorded the highest mean cane diameter of 3.40 cm compared to 2.93 cm recorded in the best standard CoC 671 at harvest. In addition, the clone also recorded a mean sucrose of 19.34% compared to 19.62% in the tropical standard Co 86032. At the 10^{th} month, the clone recorded a mean thickness of 3.24 cm compared to 2.75 cm in Co 86032, 2.75 cm in Co 09004 and 2.77 cm in CoC 671. Co 17008 is resistant to red rot against CF 671. No major incidence of pests was noticed during evaluation at different locations. It has very thick purple canes with medium height and tumescent internodes, green leaf sheath, green with purple tinge, closed canopy with tip drooping leaves, deltoid ligule, narrow light brown dewlap, and short lanceolate ligular process. It exhibits late, sparse flowering at Coimbatore. Hence, it could be a potential parent for developing thick cane varieties.

42. Co 15002 (IC646872; INGR22186), a sugarcane germplasm with red rot resistance combined with smut and yellow leaf disease resistance

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Red rot, the cancer of sugarcane (*Saccharum* sp.), is the major disease affecting sugarcane production in India, where no variety can be released without red rot resistance. In the context of climate change, most of the resistance behaviour to prevailing biotic/abiotic stresses is expected from the variety, which is also considered an environmentally friendly option. Co 1148 was a promising mid-late variety that went out of cultivation because of its susceptibility to red rot.

A red rot resistance Co 15002 was developed at ICAR-Sugarcane Breeding Institute from the susceptible variety Co 1148. During 2014-15, a set of 129 clones was evaluated for cane yield and juice quality traits along with standard varieties CoC 671 and Co 86032. The results revealed that the clone PZVT 13-195 (Co 15002) recorded the highest cane yield of 143.52 t/ha, which was significantly superior to both standards.

Co 15002 was screened for red rot, smut, and yellow leaf diseases under natural and artificially inoculated conditions at Coimbatore, Pune, Kolhapur and Navsari. In natural conditions, no incidence of red rot, smut and yellow leaf diseases was reported. At all the test locations, this line was resistant or moderately resistant to red rot in both plug and nodal methods. Co 15002 showed resistance to moderate resistance reaction to smut.

The genotype flowers with 20 % intensity during the first fortnight of November and has low pollen fertility of 30% unlike the original parent which has the pollen fertility of more than 60% and hence can be used as a female parent. The clone is characterized by erect, medium-thick thick tall canes with long internodes and indicated bud grooves, big ovoid projecting buds, an erect and tip droopy closed green canopy with light green leaf sheath, short lanceolate ligular process, and yellow wax band.

43. CoA 16321 (2010A 229) (IC646873; INGR22187), a sugarcane germplasm with red rot resistance

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Table 1: Reaction of CoA 16321 to Red rot and YLD in ZVT entries (2019-20 to 2020-21)

Trial name	*Genotype*	*Anakapalle*			*Cuddalore*		
		Red rot (CF06)			*Red rot (CF06*		
		Plug	*Nodal*	*YLD*	*Plug*	*Nodal*	*YLD*
AVT- Early I Plant (2019-20)	CoA 16321	R	R	MS	MR	R	MS
	CoC 16336	MS	R	MR	MS	R	MS
	CoC16337	MS	R	MR	MR	R	MR
	CoV 16356	MR	-	MR	MR	R	MR
AVT- Early II Plant (2020-21)	CoA 16321	R	R	R	MR	R	MR
	CoC 16336	MS	R	R	MS	R	MR
	CoC16337	MS	R	R	MR	R	MR
	CoV 16356	MR	R	R	MR	R	R

Note: R – Resistant, S – Susceptible, MR- Moderately Resistant, MS – Moderately Susceptible, HS – Highly Susceptible

A promising early clone CoA 16321 was developed from 81V48 (CoV 89101) GC at the Regional Agricultural Research Station, Anakapalle. It was tested against the popular standards CoC 01061, CoA 92081 and Co or 03151 for its performance under yield trials from 2019-20 to 2020-21 in two sugarcane (*Saccharum officinarum*) plants and one ratoon crop under AICRP trials. The pre-release clone CoA 16321 recorded maximum number of millable canes (113.23 thousands/ha), cane yield (122.09 t/ha), sugar yield (15.68t/ha) and was found to be superior than the standard CoC 01061 (105.36 thousand/ha, 106.56 t/ha and 12.68t/ha), CoA 92081 (118.27 thousand/ha, 102.17 t/ha and 12.61 t/ha) and Co or 03151 (99.98 thousand/ha, 100.20 t/ha and 10.90 t/ha) respectively. The quality parameters *viz*., brix percent (20.11), per cent juice sucrose (18.36) and CCS per cent (12.84) in CoA 16321 were on par with the best standard CoC 01061 (19.01 %, 17.12% and 11.95%), respectively. With respect to the yield components, the clone has recorded higher cane length (2.22 m), cane diameter (2.21cm) and single cane weight (1.09 kg) when compared to the best standard CoC 01061 (2.68 m, 1.58 cm and 1.02 kg), respectively. The improved clone CoA 16321 was also resistant to red rot under the nodal and plug method of inoculation (Table 1). The clone recorded a resistant reaction by the plug method of inoculation consistently for three years when tested with susceptible check CoC 671**.** It has medium thick cane with an erect, tall growing habit and nonlodging nature. The clone can be distinguished by a purple cane (exposed) with straight alignment of internodes and a purple leaf sheath with green foliage and slightly droopy at tips.

44. NUE/15-23 (IC645767; INGR22188), a potato hybrid with high Nitrogen Use Efficiency traits and high tuber yield under low nitrogen fertilizer input

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Global agriculture faces problems of environmental pollution due to the excess application of nitrogen (N) fertilizers in potato (*Solanum tuberosum*). Hence, we aimed to develop potato genotypes with enhanced nitrogen use efficiency (NUE) and yield traits under limited N availability (*Trehan et al.,* 2013). Bi-parental population of 116 progenies was generated by crossing two contrasting varieties *viz*., Kufri Jyoti (N inefficient) and Kufri Gaurav (N efficient). An advanced-stage potato hybrid NUE/15-23 was developed by crossing Kufri Jyoti (N inefficient) and Kufri Gaurav (N efficient) potato varieties. Field evaluation of this hybrid during the last four years mean data (2017-18 to 2020-21) indicated that NUE/15-23 is a nitrogen use efficient and produces about 25% higher yield (50.09 t/ha) than the best control Kufri Gaurav (39.09 t/ha) and more than 65% higher yield than the popular variety Kufri Jyoti (31.08 t/ha) at limited nitrogen (50 kg N/ha) fertilizer. The hybrid NUE/15-23 has significantly (P<0.05) higher Nitrogen Use Efficiency (NUE) parameters than the best control, such as NUE (3.27 vs. K. Gaurav: 2.23), Agronomic NUE (AgNUE) (13.21 vs. K. Gaurav: 9.17), and Nitrogen Utilization Efficiency (NUtE) (53.31 vs. K. Gaurav: 34.20), whereas at par for Nitrogen Uptake Efficiency (NUpE) (0.064 vs. K. Gaurav: 0.065). This hybrid has potential for cultivation for low-input agriculture, mainly with limited N fertilizer availability, and could be found effective in organic cultivation without N fertilizer application (Tiwari *et al.,* 2020). This hybrid has potential for cultivation for low-input agriculture, mainly under limited N and organic or natural farming.

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45. Mango Queen (IC646861; INGR22189), a mango (*Mangifera indica*) germplasm rich in Vitamin A and possessing high number of fruits per bunch and high fruit weight

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It is a 25 feet tall 16 years old tree, yielding 2000 fruits per year with upto 15 fruits per bunch. Each fruit has an average weight 232 grams (Table 1).

The quality of the fruit is unsurpassed as tested by Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar.

Table 1: Fruit characteristics of Mango Queen (Sima)

Average fruit length (mm)	86.5
Average fruit breadth (mm)	62
Average fruit weight (gms)	232.436
Average peel thickness (mm)	1.44
Average peel percentage (w/w)	15.1
Average pulp content (w/w)	64.4%
Pulp to stone ratio	4.69
TSS (degree Brix)	15.9
Acidity (w/w)	0.2
TSS/Acid (Ratio)	79.5
Reducing sugar (%)	3.006
RS/Acid (ratio)	15.03
Vitamin A (IU)	11338

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