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PGR NOTE

Collecting Vegetable Genetic Resources in India: Guidelines and Methods

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Collecting vegetable genetic resources (VGRs) is a cumbersome process hence requires specialised methods and knowledge on this group of crops. Successful collection of VGRs needs strategic approach in order to collect quality germplasm. To achieve the target, information on mode of propagation, plant part to be conserved and post-harvest processing of germplasm are pre-requisite to reduce mortality during transit. The international or national guidelines are available in public domain that do not much focus on collecting VGRs in general and crops of Indian origin in particular. Absence of which collecting bulky and perishable material is difficult for the explorers. It is therefore an attempt to put together the knowledge resources to guide the amateur collectors, PGR workers, and researchers. This manuscript provides maturity indices of major vegetable crops and specifications for some selected taxa having vegetative mode of propagation and future researchable aspects. Besides, methods of preparation of herbarium specimens of VGRs and their photographs are provided to support wild relatives and less-known vegetable taxa.

Introduction

Genetic diversity, a basic need for breeding to develop desirable genotypes can be assembled through exploration and germplasm collecting missions within the country in a cost-effective and time-efficient manner. A systematic approach is necessary to ensure the success of a collecting programme, which can be achieved only by devising a scientifically standardized collecting strategy prior to its execution. The strategy must take into account the eco-geographic considerations including present status of diversity, species prioritization, the planning and logistics followed by post-harvest handling of the germplasm (NBPGR, 2016).

In vegetable genetic resources (VGRs), germplasm collecting is generally followed by multiplication, characterization, evaluation and conservation for use. This essentially requires knowledge on characteristics of landraces/ primitive cultivars and wild relatives of various vegetable crops, prevalent in the area of collection. In order to minimize the resources, appropriate methods needs to be followed for sampling. Depending on the priority of crop to be collected, richness of diversity and areas to be explored, duration of survey, the number of visits and, sample type and sample size for collecting germplasm varies.

The VGRs constitute wide range of germplasm in the form of seeds, vegetative propagules, cuttings, suckers, etc. Majority of vegetable crops belong to different botanical families, and are propagated through seed and/ or vegetative means viz. potato, yams, taros, pointed gourd, cho-cho, etc. and a few are biennials (carrot, radish, onion, etc.); and others perennials (trees-drum stick). The methods of collecting the VGRs depends on bulkiness and fleshiness of fruit, mode of propagation and thus varies to a greater extent from other crop-groups such as cereals, millets, legumes, etc.

For collecting of germplasm, the national or international guidelines have already been published (Arora, 1991; Pareek *et al*., 2000; Dansi, 2011; NBPGR, 2014; Bhatt, 2018). However, these generally deal with collection methods and not the crop germplasm specifications and maturity at which the VGRs are to be collected (Dansi, 2011; ICAR-NBPGR, 2016). Hence based on data available and experience of the authors, this work focuses on collecting VGRs for the benefit of plant genetic resource workers, amateur explorers and others.

Among the total spectrum of genetic resources, landraces are probably the most important genetic resources adapted to an array of natural and cultural environments, making it very distinct (Harlan, 1975). Details on collecting of VGRs can be gathered from earlier field surveys reports, passport data and published literature especially in case of less-known and difficult

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to collect wild vegetables in general and wild relatives in particular before proceeding on an exploration of VGR (Pradheep *et a*l., 2015; Pandey *et al.*, 2018).

Knowledge on crop duration, local names, breeding system and population structure, regions of diversity and unique traits of the cultivars/landraces, maturity period of primitive types/ landraces/ local types in crops, wild relatives, and key morphological characteristics for identification of taxon are desired. While collecting landrace of any vegetables crop, local name(s), used in a prevailing area of occurrence are to be assured by repeated cross-checking from neighbouring areas/ regions to avoid the chances of duplicate collections under variable names.

Literature on horticultural crops/breeding vegetable crops throws light on propagation, and maturity of a species. During exploration, material is selected from farmers' field, handled for couple of weeks during transit till reaches the base camp or for conservation. Specific handling methods are to be followed during evaluation programme which are differently handled by breeders.

Less-known VGRs and crop wild relatives (CWR) are also important genetic resources and should be collected from kitchen gardens and wild, taxa like *Allium stracheyi* Baker, *A. roylei* Stearn, *Solanum nigrum* L., *Malva verticillata* L., *Rumex nepalensis* Spreng., etc. from their distributional range. Mode of propagation of most of the CWR is not known; hence explorer has to decide the type of material to be collected based on his knowledge and experience. Knowledge on method of propagation/seed multiplication and maturity indices of fruit/ seed/ propagule and the stage at which the crop is to be collected, especially in bulky vegetables/ cucurbits where seed extraction may reduce the bulk weight during transit but if the fruit is of less maturity and other samples are not available elsewhere, the collector has to decide based on maturity indices. Precautionary measures for post-harvest handling and transportation of germplasm to base camp/ Headquarters should be adopted.

Collecting vegetatively propagated material differs from that of collecting seeds material. In particular, it is more restrictive for the collecting timings of the mission. However, seed having good storability (except recalcitrant seeds), less bulky and easy to handle are preferred over the former (Fig. 1; Tyagi *et al*., 2016; Pandey *et al.*, 2019). To collect vegetative propagules in the form of roots and tubers (potato, cassava, yam, taro, sweet potato, etc.) and cuttings, suckers (pointed gourd, Malabar spinach), appropriate timing of the visit is the most important aspect of the planning. Vegetables such as onion, cho-cho, and drumstick are propagated by seed as well as vegetative means. Some of the crops may often produce very few seeds. A minimum of two separate visits can be made; in first visit the desirable genotype are marked as mother plant and second visit to the same site for collecting budwood. Hence, the decision is dependent on: a) the purpose of collecting; seeds of a new variety, vegetative samples for established varieties; b) site identification, and facilities available for multiplication; and c) breeding system of the species (monoecious/dioceous), seeds upon growing may turn up into male and female plants (pointed gourd).

Maturity Indices of Vegetable Crops

To enhance the efficiency of collecting mission, the aspects of seed/ fruit maturity indices for collecting are pre-requisite. An expert having physiology background can facilitate pinpointing the right stage of maturity prior to execution of collecting mission. A target should be set to extract maximum number of seed that qualify the gene bank standards (Tyagi *et al*., 2016). The collector should consider varietal differences, phyto-geographical areas, agronomic practices and temporal changes (season/ soil types/ irrigation/ stress). Information on seed maturity and right time of VGRs collecting especially for vegetatively propagated material are scarcely available in literature.

Due to asynchronized seed maturity or seed shattering (CWR/wild economic vegetables), care should be taken to collect seeds in case of *Allium*, brassicae, amaranths, *Luffa*, etc.. The inflorescence of amaranths and chenopods are harvested when 70 per cent of maturity (i.e. seed turns brown or black) or before the siliqua shatter in brassicae; gourds, cucumber, *Luffa* etc. are left to dry on the plants and harvested later when plant is completely dried. Others like melons, round gourd, ash gourd are harvested after drying of vine on fruit colour changes from green to yellow. In tuberous and bulbous vegetables, field-dry maturity is considered appropriate when bulb/ corm neck is completely dry/ papery or aerial part turns yellow.

In tuberous vegetable crops, like taros, propagules must be collected without causing damage to the material during harvesting or transportation. Most of the *Dioscorea*

Fig. 1. Collecting VGRs: a) pumpkin drying in sun for maturity; b) mature melon harvested for seed; c)farmer extracting seed from ash gourd fruit; d) wild onion packed for transportation; e) extracting pumpkin seeds in field; f) wild okra fully dried in field.

species are dioecious and bear male and female flowers on separate plants; flowering is erratic and seeds are seldom produced. *Dioscorea deltoidea*, a dioecious plant produces bulbils (small tubers from leaf axils) those can be used for propagation. For seed production male and female plants must be grown together. Vegetative propagation is done through the aerial and underground bulbils. Seed tuber weighing 125-100g optimum in lesser yam and its 200-250g pieces or whole tubers in white yam can be used for propagation. Giant *Alocasia* and the taros are propagated by rhizomes and needs to be cut between the upright stems to yield new plants. The information on maturity indices of VGR for seed/ vegetative propagule is given in Table 1 and 2.

Majority of bulbous vegetables *viz.* onion, leeks are propagated by seeds as well as vegetative propagules (bulbs, rhizomatous bases, bulbils). *Allium* species being sensitive to photoperiod do not flower under all ecological conditions may be collected as bulb/rhizomatous part. To collect fresh bulbs of *Allium sativum* var. *proliferum* ('tree onion', 'pearl onion') use of moss grass or by any soft organic packing material mix with moist soil can be used to protect bulb from losing moisture and damage during transportation. *Allium ampeloprasum* produce aerial bulbils in flower head or underground bulbils in the basal part can be air dried, stored in refrigerator or air stored in moist free open area and sown in the month of November (about 13°C). In general aerial and underground bulbils have longer shelf life and are easy to transport.

There are more than 120 VGRs listed for the Indian region (Pandey *et al*., 2018). Some of the less-known/ localized vegetables- winged bean, teasel gourd, purselane, agathi, bread fruit, vegetable banana or other – mestha, roselle, are not included in this manuscript. However, one may decide on the basis of similarity in physiology, followed by expert opinion and literature consultation.

Target Sources: Diversity in VGRs

A preliminary check-list of VGR diversity available in selected area can be prepared by the routine visits, feedback from the residents/ locals, farmers' information, previous exploration reports and published literature. For example if landraces/ farmers' varieties/folk variety are the target species, then the main objective would be to collect them from farmers' fields, kitchen gardens, farm stores and local seed shops (if not available with farmer). Markets are reliable source of information on vegetable crops, varieties available and preferred by the locals in the area, location of cultivation sites and time of crop availability. Generally cultivation of local vegetable is confined to marginal land kitchen garden for self-consumption and for sale in local market. Assembling requisite number of propagules germplasm in appropriate quantity is therefore not always possible in such cases.

In VGRs, especially the ucurbits viz. *Benincasa*, *Cucumis*, *Cucurbita*, *Lagenaria*, *Luffa*, *Momordica* and *Trichosanthes*, the number of plants and mature fruits in a farmer's field is usually limited, and the seeds are usually obtained from one or a few fruits. A total of 15-20 cuttings are sufficient for species propagated through the vegetative methods. Material with dubious identity or unknown identity, vernacular name should be recorded along with herbarium specimen and photographs (closeup of flower, pods) for authentication.

The pointed gourd is usually propagated through vine cuttings and root suckers. To propagate from root suckers, tuberous roots of pointed gourd are dug in the early spring, subdivided, and replanted. Both pre-rooted and fresh vine cuttings are used for propagation. Vegetative cutting should have 8-10 nodes per cutting and should be partially or fully defoliated to check transpiration. Vine cuttings made in previous year and rooted during winter are planted in the spring after danger from frost is over. The concept of female: male ratio of 10:1 is optimum for ensuring maximum fruit set should be kept in mind. Cho-cho is propagated vegetatively either from the old rootstock or reproduced from mature fruits (viviparous germination). *Momordica subangulata* subsp. *renigera* is propagated by seed as well as tubers. Seeds have difficulty in germination (segregation is 1:1 male female ratio). Some of the less-known vegetable species like West Indian arrowroot (*Maranta arundinacea*) and Queensland arrowroot (*Canna indica*) are propagated by suckers/rhizomes and also from seed. A less-known introduced vegetable, the tree tomato (*Cyphomandra batacea*) is propagated by stem cuttings with leaves intact (especially during transit) to yield better survival. Other vegetables such as *Momordica cymbalaria*, *Solena amplexicaulis*, *Momordica dioica* are propagated through seeds as well as perennial rootstocks tubers while wild leafy types- amaranths and chenopods are propagated through seeds.

Table 1. Maturity indices of vegetable crops [largely adopted from AVRDC- Asian Vegetable Research and Development Centre (1990), the feedback through explorers and validated from published sources]

Crop(s) $	Mode of propagation; maturity indices
Amaranths, chenopods	Seed; leaves and inflorescence turning brown, followed by seed shattering on touching
Ash gourd	Seed; fruit wall hard to pinch, skin turning greyish white; seed turning brown and hard
Asparagus	Vegetative; plant starts yellowing followed by leaf fall
Bitter gourd, spine gourd, sweet gourd, teasel gourd	Seed; fruit softening followed by turning to pale red to pale orange-brown, aril/ pulp turning red and slimy
Brassica (leafy types)	Seed; pods turning brown, cracking of the lower pods initiated
Brinjal	Seed; fruit beyond edible stage, fruit pericarp turn dull yellow and seed hard, tough walled
Cabbage, cauliflower, broccoli, other cole crops	Seed; head cracking followed by siliquae formation; seed turning dark brown
Carrot, radish, turnip	Seed; inflorescence turns brown and brittle; fruit formation, stem defoliate, wooden brown colour base-seed shatter on touching
Cassava	Stem cuttings# from 8-14 months old plants; seed
Chekkurmanis (*Souropus androgynus*)	Seed/stem cuttings; woody stem defoliated, leaves start drying
Chilli and other peppers	Seed; fruits turn red, pericarp lose moisture (start wrinkling)
Cho-Cho	Vegetative cuttings/ fruits; viviparous fruits detach from vine of mature plant (sprouting preferred)
Coriander and other members of Apiaceae	Seed; umbel turning brittle, flaccid/dry; seed dark brown and shedding
Cowpea	Seed; two third of pods turn brown; well-filled pods that snap readily, loose green colour, turgidity
Cucumber, long melons	Seed; vine drying, fruits with hard pericarp, pale yellow/golden-brown, seeds with hard skin; rattling in pepo
Curry leaf	Seed; fruit start ripening (hard/ shrivelled fruits are not viable); plant the fruit/remove the pulp; soft, loose pulp purple-violet colour
Drumstick	Seed-pod turning brown, hard and brittle; cuttings with brown hard wood (pencil thickness, 20-30cm)
Fenugreek	Seed; leaf/ pod turning brown followed by skin turning hard
Garden pea, French bean, dolichos bean, cluster bean, velvet bean, other beans	Seed; plant showing senescence, drying, pod turning darker-brown, dry and brittle; seed fully developed and hard
Onion, garlic, leek and other *Allium* species	Cloves/ bulbs- leaf turning complete brown; tops beginning to dry out and topple down (approximately 10 to 20 per cent fallen); bulbs scales drying, leaves drying and collapse of neck. For seed producing onions: seed#turning to brown-black
Indian round melon	Seeds; fruit wall soft, brown, seeds black
Malabar spinach	Seed/stem cuttings#; seed turning black, leaves turning brown on stem
Meetha karela (*Cyclanthera pedata*)	Seed; pod turning pale seed colour change to black, splitting of fruit
Okra	Seed; capsule turning hard, brown, drying, splitting; seeds mature to black colour
Onion	
Pointed gourd	Vegetative cuttings; fruit wall turning to pale orange, over mature if thumbnail cannot penetrate flesh readily; stem cutting from plant of mature bark
Potato	Mature tubers (seed tubers); aerial parts turning yellow, dried; seed extracted from fully mature fruits when fruit colour turning violet-brown
Pumpkin, bottle gourd, water melon, squashes	Seed; fruits wall turning hard to pinch, brownish yellow (beyond edible stage); colour of lower part turning darker, vines drying
Ridged gourd and sponge gourd	Seed; fruit hard, fibrous wall, opening on the tip clear and seeds black and dry (over mature if thumbnail cannot penetrate flesh readily)
Snake gourd	Seed; fruit wall turning to pale red (over mature if thumbnail cannot penetrate flesh readily)
Snapmelon	Seed; fruit wall cracking/degenerating, aroma on full ripening followed by seed maturity; colour change to pale brown
Spinach	Seed; spines of the seeds can be broken easily
Tomato	Seed; fruit fully ripe (90 per cent skin turn red, soft); seeds slipping when fruit is cut
West Indian arrowroot	Sucker/rhizome#, occasionally by seeds; leaves turn yellow, stem senescent
Yam and taro, giant taro, elephant foot yam and other edible aroids	Tuber; leaf turning complete brown; just rightly mature with initiation of sprouts; large enough (over-mature if tough and fibrous); senescence of the aerial parts
Yam bean	Seed# harvested from dry, yellow-brown pods; sprouted root tubers of previous year left in unirrigated soil, with hard periderm

#: more commonly used method for propagation; abstracted from Pandey *et al*. (2018); $: refer Pandey *et al.* (2018) for botanical names

Table 2. Specifications# for vegetative propagules of some selected VGR

Crop (s)	Details of material
Aerial yam	Aerial tubers (potato sized) borne in axil of leaf preferred over root tubers
Asparagus	Tuberous, mature roots (texture dry) of over one cm or more in diameter
Cassava	Basal midleaf/ stem cuttings# from 8-10 month old defoliated plant (seed germination only 50 per cent)
Chekkurmanis	Stem cuttings (woody stem 20-40cm)
Chinese chives	Rhizomatous underground rooted stem with apical bud
Chinese onion; chives	Bulbs with shining dry/ papery skin
Chinese potato	Tubers produced during storage period; sprouting tubers give better results
Chinese yam; white guinea yam	Big sized mature tubers (cut into pieces) or whole tuber if small
Cho-cho	Vegetative cuttings (15-20cm long and 0.5-0.8cm diameter); fruits (with sprouting preferred over non-sprouting) for good survival and rejuvenation
Coleus	Vine cuttings from 3-4 week old plants
Drumstick	Stem cuttings of 1.5-2.0cm sized diameter with mature brownish bark, preferably from old branches; seeds from fully mature pods (wild plants shatter seeds)
Elephant foot yam	Ripe fruit; cormlets (mortality is high); tuber pieces of 125-100g pieces or whole tuber if small, whole rhizome without injury
Garlic and leek	Cloves/ bulbs# from mature plants with skin shiny and dry; aerial bulbils from mature plants with shiny, dry skin (often seen under adverse conditions)
Giant Alocasia	Offsets [(in spring/ rainy season)/ stem cuttings (root readily)]; seeds (from mature dried cob along the spadix)
Greater yam	Tuber top with intact skin (125-100g optimum) or whole if small tubers/ aerial bulbils
Ivy gourd	Vegetative cuttings with 3-4 nodes of 10-20cm long x 1-0.5cm diameter from mature vine
Japanese bunching onion	Bulbs with thick, dry, papery skin
Malabar spinach	Leafy stem cutting from mature plant with 2-3 nodes, 30-40cm long x 2 cm diameter; fruit with juicy red pulp, dark black skin, and dried on plant (can be used for planting)
Onion and shallot	Bulb completely dry, shining papery tunic easily detachable; ideally bulbs to be collected when top leaves die down after flowering
Pointed gourd	Stem vine cuttings of female plants (with 8-10 nodes) from mature branch (cutting size: 10-15cm long and 0.5cm diameter wide), dark brown and hard stem preferably with roots
Potato	Mature tubers with skin peeling well set and healthy tuber# with at least two to three well developed eyes; (seeds formed in temperate areas used generally for research purpose)
Spine gourd	Terminal cuttings from 2-3 month old plants, taproot tubers of senescent plants of 2-3 year
Sweet potato	Fresh vine cuttings from senescent tubers
Tannia	Tops/suckers#, corms/cormels
Taro	Cuttings (stem of about 30-45x5-7cm) suckers/cormels/corm
Top onion	Bulb with outer skin completely dry, papery; mature aerial bulbils borne in flower head (shatter on touching)
Tree tomato	Stem cuttings from 1-2 year old tree (preferably of size 30-45x 1.5-2.5cm) with brownish-mature skin
West Indian arrowroot, Queensland arrow root	Suckers or rhizomes with two or more nodes each from 10-12 month old plants at senescence; occasionally propagated by seed

Pandey *et al*. (2018)

Herbarium Voucher Specimens and Photography

Herbarium specimens can serve as a useful resource for taxonomic identification of materials, especially those where identity or nomenclature is not accurately determined during the collecting, and for the less-known/ wild species of VGRs. The basic standard methods (Jain and Rao, 1977; Fuller and Barber, 1981; Rao and Sharma, 1990) along with modified methods developed by the National Herbarium of Cultivated Plants (NHCP), New Delhi (Pandey *et al*., 2017) for cultivated plants can be followed.

As a rule, voucher specimens should be prepared during collection, especially for the wild vegetables, and variants of vegetables (local cultivars) where taxonomic identity needs authentication and confirmation.

Density of spines in brinjal and non-spiny types can be depicted in the voucher with stem and leaf, besides the fruit/ calyx. If possible, fruit at young stage may be included to show the shape variation. In cucurbits, the processing of herbarium specimen needs initial drying in the field to avoid limping/overlapping of parts. In melons character differences are evident even at the initial

stage of fruit development and can be used to delineate intra-specific separation of taxa. A holistic understanding of the plant morphology can be re-ascertained if wet collection is supplemented to the germplasm.

Close-up of the plant stand and wide angle population, leaves and branching pattern, vegetative parts (in case of vegetatively propagated taxa- tubers, corm, suckers in whole and cross/longitudinal section), fruits/ pods/seeds, flower and flowering habit, the pistil, anther, open and closed/ bud as well as variability collected and may be photographed.

Guidelines and Research Thrust for VGRs

None of the national and international guidelines (Guirino *et al*., 1995, rev 2011; NBPGR, 2016) exclusively deal with vegetable group in general and also crops of Indian origin. Internationally, guidelines on collecting for vegetatively propagated crops focus more on roots and tubers donot sufficiently explain the methodology and specifications required while selecting material (Dansi, 2011). Following the general guidelines that are available in public domain are difficult for amateur collectors, PGR workers, researchers and information on specific group is insufficient. They may not have knowledge on specific VGRs. Present work is an exclusively attempted on specifications on the VGRs and their maturity index that an explorer needs while deciding to collect a bulky fruit that is available in a given time.

Information presented here is synthesized primarily from the field experience of the authors, and further validated from literature to facilitate explorers. Unavailability of such information in one place prompted the authors to compile the information for the use by young explorers who are dealing with crop specific explorations such as vegetables which are a difficult group mainly due to: 1) small number of seeds available during collection from kitchen garden, and small scale cultivation; 2) fleshy fruit that are bulky, tubers/ roots perishable and prone to fast damage. Table (3) below gives the comparison on guidelines for the two:

Information arising from this work is provided in this manuscript which emerges as de-novo approach through the experiences of the collectors and base knowledge by breeders for collecting quality germplasm material in VGRs. This would facilitate collecting of quality germplasm besides reducing loss of material during transit. Some of the research thrust areas are given below:

- Evaluation of per cent mortality using different methods during transit
- Methods on enhancing survival using different modes
- Selection of ambient conditions for storage
- Fruit maturity of crop *vis-a-vis* seed health

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Table 3. Comparison of general and VGRs collecting methods in PGR

Item	General collecting	Collecting VGRs
Guidelines	Cover general points; hardly cover VGR	Specialised for VGR
Source of availability of germplasm	Wider sources	Specific confinement in kitchen garden, small scale cultivation
Quantity of material gathered	Sufficient	Seed/propagule not sufficient; need multiplication before conservation
Mode of multiplication	Seed	Vegetative and seed both
Post-harvest methods	Not specific	Deal with perishable, bulky material
Maturity indices	Not focused	Species specific and specialized
Specifications for vegetative propagule	General and can applied widely	Very specific for different species

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RESEARCH ARTICLE

Phenotypic Characterization Reveals High Extent of Genetic Variation in Maize (*Zea mays* L.) Landraces of North-Eastern and North-Western Himalayan Regions of India

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Maize (*Zea mays* L.) is an important cereal crop with multiple uses as food, feed, fodder, fuel and biofuel. Phenotypic characterization of newly collected germplasm is essential for selecting genetically diverse lines for maize breeding. We characterized 99 landrace germplasm accessions collected from India's maize diversity hotspots located in North-Eastern Himalayan (NEH) and North-Western Himalayan (NWH) regions using 30 morphological traits. The high extent of phenotypic variation was observed both in plant and ear traits. Landrace accessions displayed desirable traits for earliness and yield contributing traits namely leaf length and width, ear length and width, kernel rows and kernels per row. Very high positive correlations estimated between flowering traits (days to tasseling and silking; r = 0.99) and maturity (0.78), and between plant height and ear height (0.90). However, kernels per row showed significantly negative correlations with flowering traits (-0.42) and maturity (-0.44). Cluster analysis grouped all 99 landrace accessions into eight clusters. Principal component biplot explained 88.24% variation. Boxplot analysis indicated that the maize landrace accessions from NWH region displayed comparatively high phenotypic diversity for plant phenology and architecture traits, while the accessions from NEH region showed the higher variation for tassel branching, ear number, ear width, kernels per row and 100-seed weight. Maize accessions expressed phenotypic variability for all qualitative traits with leaf colour, silk colour and ear shape showing the maximum variation. Shannon-Weaver diversity indices revealed the presence of high phenotypic variation for tassel branching (SDI = 0.99), ear shape, husk cover, ear width, kernel rows (0.95) and kernels per row (0.69). This genepool of landraces offers prospects for pre-breeding by providing new sources of allelic variation to enrich elite maize germplasm.

Key Words: Genetic variation, Himalayan region, India, Maize landraces, Phenotypic characterization

Introduction

Cereals are an important part of food resources for human beings and animals. Maize (*Zea mays* L.) being a main cereal crop has diverse uses as human food, animal feed and fodder, fuel and industrial by-products including biofuel production among all the cereals. It is considered the major staple food in the world and one of the most cultivated among the cereals (Shiferaw *et al*., 2011). It occupies an important position in the world economy and traded as a food, feed and industrial crop. With its higher content of carbohydrates, fats, proteins and some of the important vitamins and minerals, the crop has acquired a well-deserved status as a poor man's 'nutricereal' (Prasanna *et al*., 2001). Recently, global maize production has reached 1147.62 million tonnes (mt) from an area of 193.73 m ha and India produced 27.8 mt from 9.3 m ha (FAO Stat., 2018). This production and productivity needs to improve further to feed the burgeoning global population. Utilization of diverse maize germplasm to enhance the genetic base of donor lines is crucial for the development of new heterotic hybrids and to increase productivity level. Besides the use of new germplasm collections, exploitation of the existing genetic variation of global genebanks is fundamental for the success of crop breeding programmes (Tanksley and McCouch, 1997; McCouch *et al*., 2013).

Landraces constitute an important germplasm, not only well-adapted to the region in which these had

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evolved but also differ in details as to specific adaptation to the particular conditions within the environment (Harlan, 1975a, b). Popularly referred to as 'local varieties' or 'farmer's varieties', the landraces form genetically diverse and heterogeneous populations, which were typically selected by farmers for their adaptation to specific environments, variation in their characteristics like prolificacy, flowering behavior, yield, nutritive value and resistance to biotic and abiotic stresses (Zeven, 1998; Villa *et al*., 2005). They have evolved under subsistence agriculture and are still being cultivated by farmers in regions of crop domestication and diversity (Louette and Smale, 2000; Rojas-Barrera *et al*., 2019). Existence of a vast array of landraces in maize has provided ample opportunities for enriching the allelic diversity and to search for new resources of rare alleles (Prasanna and Sharma, 2005; Warburton *et al*., 2008; Hölker *et al*., 2019). However, genetic resources used in maize breeding programmes around the world represent ~10% of all landraces indicating that much of the genetic diversity remains to be efficiently and effectively explored and exploited. Further, the improved cultivars for higher yield, better quality, disease and pest resistance, and climate-resilience can be developed by using these genetic resources (Dowswell *et al*., 1996; McCouch *et al*., 2020).

The proper knowledge of agro-morphological traits and their genetics will be helpful for better conservation and utilization of such germplasm resources (Nass *et al*., 1993). Extensive variability in plant, ear and tassel characteristics is present in North-Eastern Himalayan (NEH) and North-Western Himalayan (NWH) regions as compared to relatively less varietal diversity existing in the plains of India (Prasanna, 2010). The social and cultural values of the tribes living in the NEH region played an important role in the conservation of maize landraces (Singh, 1977). The NEH region of India comprising states of Arunachal Pradesh, Assam, Nagaland, Manipur, Mizoram, Tripura, Meghalaya, Sikkim and areas in the Northern region of West Bengal possesses huge genetic diversity with respect to plant type, ear traits, quality and resistance to biotic and abiotic stresses (Prasanna and Sharma, 2005; Prasanna, 2012). The racial diversity of maize in India was studied by Grant and Wellhausen (1955) and they observed extensive variability in plant, tassel and ear characteristics in the NEH region and North-Western highlands in India. Extensive studies on the variability of maize in the NEH regions were carried out by Singh (1977). The presence of enormous maize diversity in this region had prompted Stonor and Anderson (1949) to propose an Asiatic origin of maize. For proper utilization of maize genetic resources in breeding programmes, morphological characterization is the first step for the assessment, description and classification of germplasm collections. The importance of phenotyping maize landraces has been highlighted by various studies across the globe (Hartings *et al*., 2008; Salazar *et al*., 2016; Belalia *et al*., 2019; Nelimor *et al*., 2019). Therefore, the aims of present study were i) to characterize maize landrace accessions from NEH and NWH regions of India for their morphological traits and ii) to analyze the extent of phenotypic variation present among the maize accessions for their exploitation in future crop improvement programmes.

Materials and Methods

Germplasm Materials and Field Experimentation

The materials comprising 99 maize landrace accessions and representing 10 different states of NEH and NWH regions of India, were used in the present study (Tables 1, 2). The seeds of maize accessions were obtained from working collection of National Gene Bank at Indian Council of Agricultural Research (ICAR)-National Bureau of Plant Genetic Resources (NBPGR), New Delhi. The geographical coordinates for individual accession were mapped using the information on village, district and state of collection sites through the software 'DIVA-GIS' (Hijmans *et al*. 2001) (Fig. 1). The experiment was laid out in Augmented Block Design (ABD) with four checks namely LM-13 (C-1; Inbred line), LM-14 (C-2; Inbred line), Prabhat (C-3; Composite variety) and PMH-1 (C-4; hybrid cultivar), replicated five times under normal sown irrigated conditions at NBPGR Experimental Farm, New Delhi over two years during *Kharif* seasons of 2017 and 2018. The experimental farm, with sandy loam soil and pH 7.8, is situated at 28°40' N latitude, 77°12' E longitude and 228 m altitude. The mean annual rainfall was 650 mm, of which more than 80% occurred during the south-west monsoon season (July-Sept.) with mean annual evaporation of 850 mm. Each block contained 20 germplasm accessions and four checks, which were randomized. All 99 germplasm accessions were grown in five ABD blocks with individual accession was planted in a two-row plot of 2.5 m length with row to row and plant to plant distances of 60 cm and 20 cm, respectively.

Table 1. Description of germplasm accessions - IC (Indigenous Collection) number, village, district and geographical coordinates of their collection sites located in 10 different states of India

S. No.	Accession Code	IC Number	Village and District of collection site	Geographical Coordinates (Latitude and Longitude)	State Name
1	MA201	IC077261	Sirmour, Sirmaur	30°30' N 77°12' E	Himachal Pradesh
2.	MA202	IC077390	Gool Udhampur, Jammu	32°43' N 74°51' E	Jammu and Kashmir
3.	MA203	IC077463	Jalna, Bageshwar	29°50' N 79°46' E	Uttarakhand
4.	MA204	IC077089	Dilsigiri,West Garo Hills	25°27' N 90°19' E	Meghalaya
5.	MA205	IC077126	Musubai, East Khasi Hills	25°22' N 91°45' E	Meghalaya
6.	MA206	IC077181	Thana, Bilaspur	31°22' N 76°42' E	Himachal Pradesh
7.	MA207	IC083103	Chotolongket, Dima Hasao	25°21' N 91°01' E	Assam
8.	MA208	IC083125	Singrib, West Kameng	27°13' N 92°13' E	Arunachal Pradesh
9.	MA209	IC083146	Chipiketa, Phek	25°41' N 94°27' E	Nagaland
10.	MA210	IC083153	Pikhbasa, Zunheboto	26°03' N 94°34' E	Nagaland
11.	MA211	IC097882	Asgarh, Pauri Garhwal	30°09' N 78°46' E	Uttarakhand
12.	MA212	IC097900	Paunti, Uttarkashi	30°52' N 78°05' E	Uttarakhand
13.	MA213	IC097918	Mashik, Dehradun	30°19' N 78°01' E	Uttarakhand
14.	MA214	IC097936	Barakat, Dehradun	30°19' N 78°01' E	Uttarakhand
15.	MA215	IC097965	Kotala, Hamirpur	31°41' N 76°31' E	Himachal Pradesh
16.	MA216	IC098131	Thambang, North Sikkim	27°31' N 88°30' E	Sikkim
17.	MA217	IC098133	Dhangkey, North Sikkim	27°34' N 88°28' E	Sikkim
18.	MA218	IC098274	Dhoot Patwa, Una	31°38' N 76°59' E	Himachal Pradesh
19.	MA219	IC098165	Moorang, Kinnaur	31°36' N 78°24' E	Himachal Pradesh
20.	MA220	IC109664	Mcleodganj, Kangra	32°13' N 76°19' E	Himachal Pradesh
21.	MA221	IC128762	Amrigok Hills, East Khasi Hills	25°22' N 91°45' E	Meghalaya
22.	MA222	IC128768	South Kolasib, Kolasib	24°13' N 92°40' E	Mizoram
23.	MA223	IC128788	Keithlamtur, Senapati	25°12' N 94°02' E	Manipur
24.	MA224	IC128792	Jaluki, Kohima	25°39' N 94°06' E	Nagaland
25.	MA225	IC128844	Kawnpui, Kolasib	24°03' N 92°39' E	Mizoram
26.	MA226	IC108162	Chamder, Mandi	31°35' N 76°55' E	Himachal Pradesh
27.	MA227	IC108164	Chet, Kullu	31°57' N 77°06' E	Himachal Pradesh
28.	MA228	IC109642	Banthu, Chamba	32°55' N 76°28' E	Himachal Pradesh
29.	MA229	IC130560	Hiralngomun, Aizawl	23°43' N 92°42' E	Mizoram
30.	MA230	IC130596	Sibilon, Tamenglong	24°59' N 93°30' E	Manipur
31.	MA231	IC130605	Bongjong, Chandel	24°18' N 93°35' E	Manipur
32.	MA232	IC130753	Pasighat, East Siang	28°03' N 95°19' E	Arunachal Pradesh
33.	MA233	IC130762	Rengging, East Siang	28°57' N 94°49' E	Arunachal Pradesh
34.	MA234	IC130786	Chododoke, West Siang	28°40' N 94°21' E	Arunachal Pradesh
35.	MA235	IC337348	Khuliya, Champawat	29°20' N 80°05' E	Uttarakhand
36.	MA236	IC278614	Larnoo, Anantnag	33°37' N 75°22' E	Jammu and Kashmir
37.	MA237	IC326557	Budhaal, Rajouri	33°22' N 74°19' E	Jammu and Kashmir
38.	MA238	IC326569	Lassana, Poonch	33°46' N 74°07' E	Jammu and Kashmir
39.	MA239	IC381423	Meleyal, Kupwara	34°07' N 74°10' E	Jammu and Kashmir
40.	MA240	IC393088	Janbila, Bageshwar	29°50' N 79°46' E	Uttarakhand
41.	MA241	IC411752	Tamina Chowkibal, Kupwara	34°10' N 74°03' E	Jammu and Kashmir
42.	MA242	IC411777	Tcharwan Kangan, Srinagar	34°04' N 74°49' E	Jammu and Kashmir
43.	MA243	IC413401	Lazura/Pomepore, Pulwama	33°52' N 74°54' E	Jammu and Kashmir
44.	MA244	IC413449	Zalus/Chadura , Budgam	34°01' N 74°43' E	Jammu and Kashmir
45.	MA245	IC315920	Mudeli, Lower Subansiri	27°49' N 93°31' E	Arunachal Pradesh
46.	MA246	IC281534	Tal, Chamoli	30°30' N 79°09' E	Uttarakhand
47.	MA247	IC281540	Chandhar, Tehri Garhwal	30°24' N 78°28' E	Uttarakhand
48.	MA248	IC338450	Ladflora, Nainital	29°22' N 79°27' E	Uttarakhand
49.	MA249	IC436982	Khaliyan, Rudraprayag	30°28' N 78°55' E	Uttarakhand

S. No.	Accession Code	IC Number	Village and District of collection site	Geographical Coordinates (Latitude and Longitude)	State Name
50.	MA250	IC556406	Bhaderwah, Doda	33°09' N 75°33' E	Jammu and Kashmir
51.	MA251	IC556417	Okuhlu, Ramban	33°25' N 74°28' E	Jammu and Kashmir
52.	MA252	IC556411	Ladder, Kistwar	34°01' N 74°19' E	Jammu and Kashmir
53.	MA253	IC556430	Harsi, Kangra	32°06' N 76°16' E	Himachal Pradesh
54.	MA254	IC556435	Kathla, Hamirpur	31°41' N 76°31' E	Himachal Pradesh
55.	MA255	IC568238	Dadal, Mandi	31°38' N 76°59' E	Himachal Pradesh
56.	MA256	IC568279	Chambi, Kangra	32°06' N 76°16' E	Himachal Pradesh
57.	MA257	IC556413	Ladder, Kistwar	34°12' N 74°21' E	Jammu and Kashmir
58.	MA258	IC556431	Harsi, Kangra	32°06' N 76°16' E	Himachal Pradesh
59.	MA259	IC556434	Kholi, Hamirpur	31°41' N 76°31' E	Himachal Pradesh
60.	MA260	IC550360	Suni, Shimla	31°31' N 77°48' E	Himachal Pradesh
61.	MA261	IC550365	Salooni, Chamba	32°34' N 76°07' E	Himachal Pradesh
62.	MA262	IC526764	Thaltlang, Saiha	22°21' N 93°03' E	Mizoram
63.	MA263	IC526436	Bangtalang, Serchhip	23°20' N 92°51' E	Mizoram
64.	MA264	IC540219	Mongsunyimti, Mokokchung	26°19' N 94°30' E	Nagaland
65.	MA265	IC557452	New Peren, Peren	25°31' N 93°39' E	Nagaland
66.	MA266	IC538895	Mur village, Kurung Kumey	27°34' N 93°12' E	Arunachal Pradesh
67.	MA267	IC526460	Phairuang, Lunglei	22°52' N 92°46' E	Mizoram
68.	MA268	IC524611	Bada Basti, Kohima	25°40' N 94°07' E	Nagaland
69.	MA269	IC524456	Tenyiphe, Dimapur	25°54' N 93°43' E	Nagaland
70.	MA270	IC419530	Pungro, Kiphire	25°49' N 94°51' E	Nagaland
71.	MA271	IC540242	Hakchang, Tuensang	26°18' N 94°51' E	Nagaland
72.	MA272	IC447091	Lower Bomdir, Tawang	27°34' N 91°52' E	Arunachal Pradesh
73.	MA273	IC524478	Sovima, Dimapur	25°54' N 93°43' E	Nagaland
74.	MA274	IC557462	Mokokchung, Mokokchung	26°22' N 94°37' E	Nagaland
75.	MA275	IC540223	Mopungchuket, Mokokchung	26°19' N 94°30' E	Nagaland
76.	MA276	IC527213	Daporijo, Upper Subansiri	27°58' N 94°30' E	Arunachal Pradesh
77.	MA277	IC538976	Pinegrove, Lower Subansiri	27°06' N 93°30' E	Arunachal Pradesh
78.	MA278	IC396886	Tingchim, North Sikkim	27°28' N 88°32' E	Sikkim
79.	MA279	IC412888	Majbat, Darrang	26°27' N 92°01' E	Assam
80.	MA280	IC447153	Kala Pahar, West Kameng	27°17' N 92°24' E	Arunachal Pradesh
81.	MA281	IC540252	Thronger, Tuensang	26°15' N 94°37' E	Nagaland
82.	MA282	IC526679	Sihtlangpui, Lawngtlai	22°18' N 92°42' E	Mizoram
83.	MA283	IC526702	Saiha, Saiha	22°21' N 93°03' E	Mizoram
84.	MA284	IC545332	Kengkhu, Changlang	27°44' N 96°38' E	Arunachal Pradesh
85.	MA285	IC524527	Bade, Dimapur	25°54' N 93°43' E	Nagaland
86.	MA286	IC447215	Merima, Kohima	25°40' N 94°07' E	Nagaland
87.	MA287	IC557454	Old Peren, Peren	25°31' N 93°39' E	Nagaland
88.	MA288	IC538939	Sangram, Kurung Kumey	27°34' N 93°12' E	Arunachal Pradesh
89.	MA289	IC526430	Bangtalang, Serchhip	23°20' N 92°51' E	Mizoram
90.	MA290	IC077124	Musubai, East Khasi Hills	25°22' N 91°45' E	Meghalaya
91.	MA291	IC077192	Daultpur, Una	31°46' N 75°59' E	Himachal Pradesh
92.	MA292	IC077160	Kitpal, Hamirpur	31°41' N 76°31' E	Himachal Pradesh
93.	MA293	IC130593	Sibilon, Tamenglong	24°59' N 93°30' E	Manipur
94.	MA294	IC469859	Sallachigiri, Pithoragarh	29°27' N 80°13' E	Uttarakhand
95.	MA295	IC282853	Bhattagaun, Dehradun	30°25' N 78°04' E	Uttarakhand
96.	MA296	IC469894	Sallalower, Pithoragarh	29°27' N 80°13' E	Uttarakhand
97.	MA297	IC538074	Narsinghdara, Champawat	29°20' N 80°05' E	Uttarakhand
98.	MA298	IC556415	Bankut, Ramban	33°04' N 74°55' E	Jammu and Kashmir
99.	MA299	IC556396	Naltee, Doda	33°09' N 75°55' E	Jammu and Kashmir

Table 2. Name of states, districts, and number of maize landrace germplasm collections characterized morphologically in present study

S. No.	Name of State	Name of District and Number of accessions	Number of Collections
A).	North-Western Himalayan Region (32 Districts)		49
1.	Jammu and Kashmir	Jammu (1), Anantnag (1), Rajouri (1), Poonch (1), Kupwara (2), Srinagar (1), Budgam (1), Pulwama (1), Doda (2), Ramban (2) and Kistwar (2) ; (11 Districts)	15
2.	Himachal Pradesh	Sirmaur (1), Bilaspur (1), Una (2), Kinnaur (1), Kangra (4), Hamirpur (4), Mandi (2), Kullu (1), Chamba (2) and Shimla (1); (10 Districts)	19
3.	Uttarakhand	Pauri Garhwal (1), Uttarkashi (1), Dehradun (3), Champawat (2), Bageshwar (2), Chamoli (1), Tehri Garhwal (1), Nainital (1), Rudraprayag (1) and Pithoragarh (2); (11 Districts)	15
B).	North-Eastern Himalayan Region (30 districts)		50
4.	Sikkim	North Sikkim (3); (1 District)	3
5.	Arunachal Pradesh	West Kameng (2), East Siang (2), West Siang (1), Kurung Kumey (2), Tawang (1), Upper Subansiri (1), Lower Subansiri (2) and Changlang (1); (8 Districts)	12
6.	Nagaland	Phek (1), Zunheboto (1), Kohima (3), Mokokchung (3), Tuensang (2), Dimapur (3), Kiphire (1) and Peren (2); (8 Districts)	16
7.	Manipur	Senapati (1), Tamenglong (2) and Chandel (1); (3 Districts)	4
8.	Mizoram	Kolasib (2), Aizawl (1), Saiha (2), Serchhip (2), Lunglei (1) and Lawngtlai (1); (6 Districts)	9
9.	Meghalaya	West Garo Hills (1), East Khasi Hills (3); (2 Districts)	4
10.	Assam	Dima Hasao (1), Darrang (1); (2 Districts)	2
Total	10	62 Districts	99

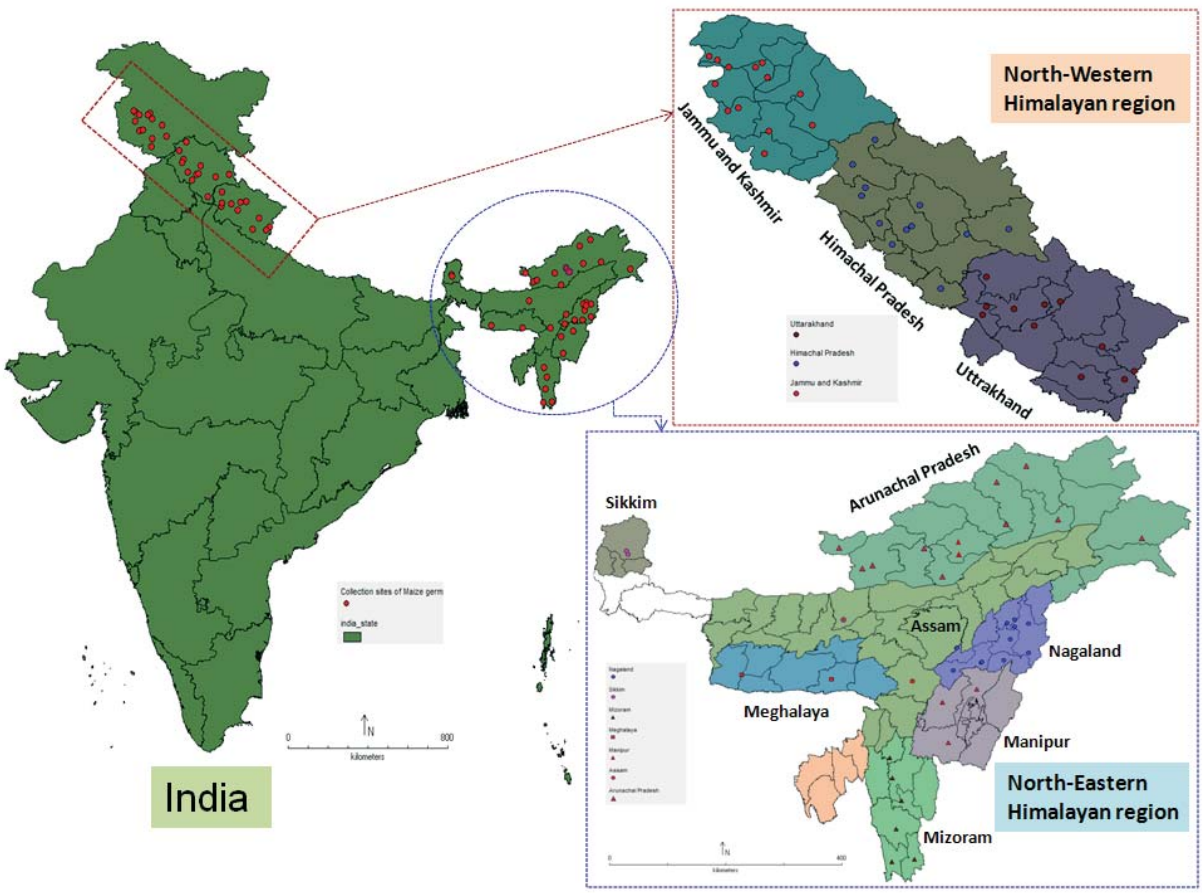

Fig. 1. Geo-referencing of maize landrace accession's collection sites located in 62 districts of 10 states of India's North-Eastern and North-Western Himalayan regions

Recommended cultural and agronomic practices were followed to raise a healthy crop.

Phenotypic Characterization

The data were recorded for 30 morphological traits following Bioversity International's descriptors for maize genetic resources (Alercia, 2011). These included 14 quantitative traits namely days to 50% tasseling (DT), days to 50% silking (DS), plant height (PH, cm), ear height (EH, cm), leaf length (LL, cm), leaf width (LW, cm), number of tassel branching (TB), number of ears per plant (EP), days to 80% maturity (DM), ear length (EL, cm), ear width (EW, cm), number of kernel rows (KR), number of kernels per row (KPR) and 100-seed weight (SW), and 16 qualitative traits *viz.*, early plant vigour, three tassel traits (texture, glume colour and glume-base colour), five leaf traits (colour, orientation, pubescence, texture and shape), silk colour, anthocyanin pigmentation, husk cover, ear shape and three kernel traits (colour, texture and shape). The morphological traits were measured on randomly chosen five plants per accession.

Statistical Analyses

Analysis of variance (ANOVA) for individual years and pooled data was carried out as per ABD using SAS 9.3 (SAS Institute, 2011) and then adjusted mean values were used for statistical analyses. The morphological traits were further analysed for mean, minimum, maximum, standard error, standard deviation, phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability (broad sense) and boxplot distribution. Pearson's correlation coefficients were calculated among all the quantitative variables using SPSS version 20 (IBM SPSS, 2020). The accessions were classified using hierarchical clustering analysis based on morphological traits. Euclidean distances were estimated and the matrix was further analyzed using Ward's method of minimum variance for generating constellation plot using JMP 14 software of SAS. Phenotypic diversity was also estimated using principal component analysis based on 14 quantitative traits. The biplot based on two principal components were also generated to depict the two-dimensional view of accession scores as well as trait variability. Shannon diversity indices (H') were calculated to study diversity in entire germplasm studied (Shannon and Weaver, 1948).

$$H' = \sum_{i=1}^{n} pi.\log_2 pi$$

where p_i is the proportion of accessions in the i^{th} class of an n-class character and n is the number of phenotypic classes for a character.

Results

Phenotypic Diversity for Quantitative Traits

Analysis of variance with pooled data for 14 different quantitative traits showed significant differences for all the traits except ear width and ear per plant (Supplementary Tables 1a-c) and revealed the presence of high extent of phenotypic variability in maize landraces accessions (Fig. 2). The germplasm characterization showed high range of variation for all the traits measured (Table 3.). Trait DT varied from 43.0 (IC077463) to 89.0 (IC130596) days after sowing with mean value of 55.9 days. DS varied from 47.7 (IC128844, IC108164) to 94.7 (IC130596) days with mean value of 58.9 days. PH ranged from 115.0 (LM-13) to 252.6 (IC447091) cm with mean value of 194.7 cm. EH varied from 59.3 (LM-14) to 159.2 (IC53885) cm with mean value

Fig. 2. Phenotypic variability in ear shape and size present in accessions from North-Western Himalayan (a-c) and North-Eastern Himalayan (d-f) regions of India

Table 3. Descriptive statistics for 14 quantitative traits analysed in 99 accessions of maize landraces and four checks based on two year's pooled data

Statistics / Trait	Range		Mean ± S.E.	Standard Deviation	Variance	PCV (%)	GCV (%)	H^2 % (broad sense)	SDI
	Min.	Maxi.							
DT	43.0	89.0	55.94 ± 0.69	7.01	49.11	12.54	11.59	53.40	0.390
DS	47.7	94.7	58.97 ± 0.71	7.25	52.59	12.31	11.43	54.62	0.370
PH	115.0	252.6	194.67 ± 2.81	28.52	813.30	14.65	13.24	55.90	0.397
EH	59.3	159.2	112.88 ± 2.10	21.34	455.47	18.92	14.13	47.21	0.411
LL	54.9	98.8	80.28 ± 0.98	9.96	99.14	12.40	9.11	46.33	0.104
LW	5.5	10.3	8.19 ± 0.09	0.88	5.20	10.64	7.73	44.19	0.470
TB	10.2	39.0	22.03 ± 0.59	6.00	36.00	27.23	15.94	54.21	0.986
EP	1.0	2.1	1.22 ± 0.03	0.28	0.08	22.67	15.59	44.26	0.779
DM	78.0	122.0	93.42 ± 0.58	5.90	34.75	6.31	4.90	53.89	0.263
EL	9.5	19.9	15.45 ± 0.17	1.68	2.82	10.87	8.98	73.86	0.385
EW	2.7	4.2	3.54 ± 0.03	0.27	0.07	7.60	5.50	70.76	0.691
KR	8.7	15.2	12.03 ± 0.12	1.23	1.52	10.24	6.71	64.93	0.950
KPR	17.0	39.3	28.23 ± 0.42	4.29	18.44	15.21	8.37	72.18	0.690
SW	12.8	31.0	23.22 ± 0.32	3.27	10.72	14.10	11.76	52.72	0.224

DT: Days to tasseling, DS: Days to silking, PH: Plant height (cm), EH: Ear height (cm), LL: Leaf length (cm), LW: Leaf width (cm), TB: Tassel branching, EP: Ears per plant, DM: Days to maturity, EL: Ear length (cm), EW: Ear width (cm), KR: Number of kernel rows, KPR: Number of kernel per row, SW: 100-Seed weight (g). PCV: Phenotypic Coefficient of Variance, GCV: Genotypic Coefficient of Variance, H^2: Heritability (broad sense), SDI: Shannon-Weaver Diversity Index

of 112.9 cm. LL varied from 54.9 (IC130596) to 98.8 (IC556413) cm with mean value of 80.3 cm, whereas LW ranged from 5.5 (IC097900) to 10.3 (PMH-1) cm with mean value of 8.2 cm. TB varied from 10.2 (IC130596) to 39.0 (IC524611) with mean value of 22.0. EP ranged from 1.0 (IC557452) to 2.1 (IC526430, IC077192) with mean value of 1.2. DM ranged from 78.0 (IC108164) to 122.0 (IC130596) days with a mean value of 93.4 days. EL showed high variation as it varied from 9.5 (IC338450) cm to 19.9 cm (IC538074) with a mean value of 15.5 cm, while EW ranged from 2.7 (IC338450) to 4.2 (IC282853) cm with mean value of 3.5 cm. KR varied from 8.7 (IC130596) to 15.2 (IC128788) with a mean value of 12.0, however, KPR displayed high range of variation from 17.0 (IC130596) to 39.3 (IC077390) with a mean value of 28.2. The seed weight ranged from 12.8 g (IC130596) to 31.0 g (PMH-1) with a mean value of 23.2 g.

The estimates of PCV and GCV with respect to 14 quantitative traits were obtained and heritability-broad sense was worked out. The estimated PCV values were higher than the GCV values for all the traits indicating greater influence of environment on the expression of traits. Higher estimates of phenotypic and genotypic coefficients were observed for tassel branching and ears per plant followed by ear height. However, low PCV and GCV were recorded for days to maturity and ear width followed by kernel rows. Broad-sense heritability estimates for assessed traits ranged between 44.19% (in leaf width) and 73.86 (in ear length) and are presented in Table 3. High heritability (>50%) were exhibited by flowering traits (days to 50% tasseling, days to 50% silking), plant height, tassel branching, days to maturity, ear length, ear width, kernel rows, kernels per row and 100-seed weight. Moderate to low (<50%) heritability estimates were shown by ear height, leaf length and width, and ear per plant. It is interesting to note that ear traits such as ear length and width, kernel rows, and kernels per row showed very high estimates of heritability. The higher values of standard deviation were found for plant height and ear height, and it was also reflected in very high estimates of variance for these traits (Table 3). Shannon-Weaver diversity indices were very high for tassel branching, ear per plant, ear width, kernels per row and kernel rows displaying the presence of very high phenotypic diversity for these traits in the landrace accessions analyzed in this study.

Boxplot Distribution of Genetic Variation

The boxplot analysis was performed to compare the variation in landrace accessions from NEH regions and NWH region for 14 quantitative traits (Fig. 3). The box showed the inter quartile range that is the box length to examine how the data was dispersed. The longer the box the more dispersed the data and the smaller the box less dispersed the data. The boxplot analysis revealed that the maize accessions from NEH region had more average values for most of the quantitative traits namely

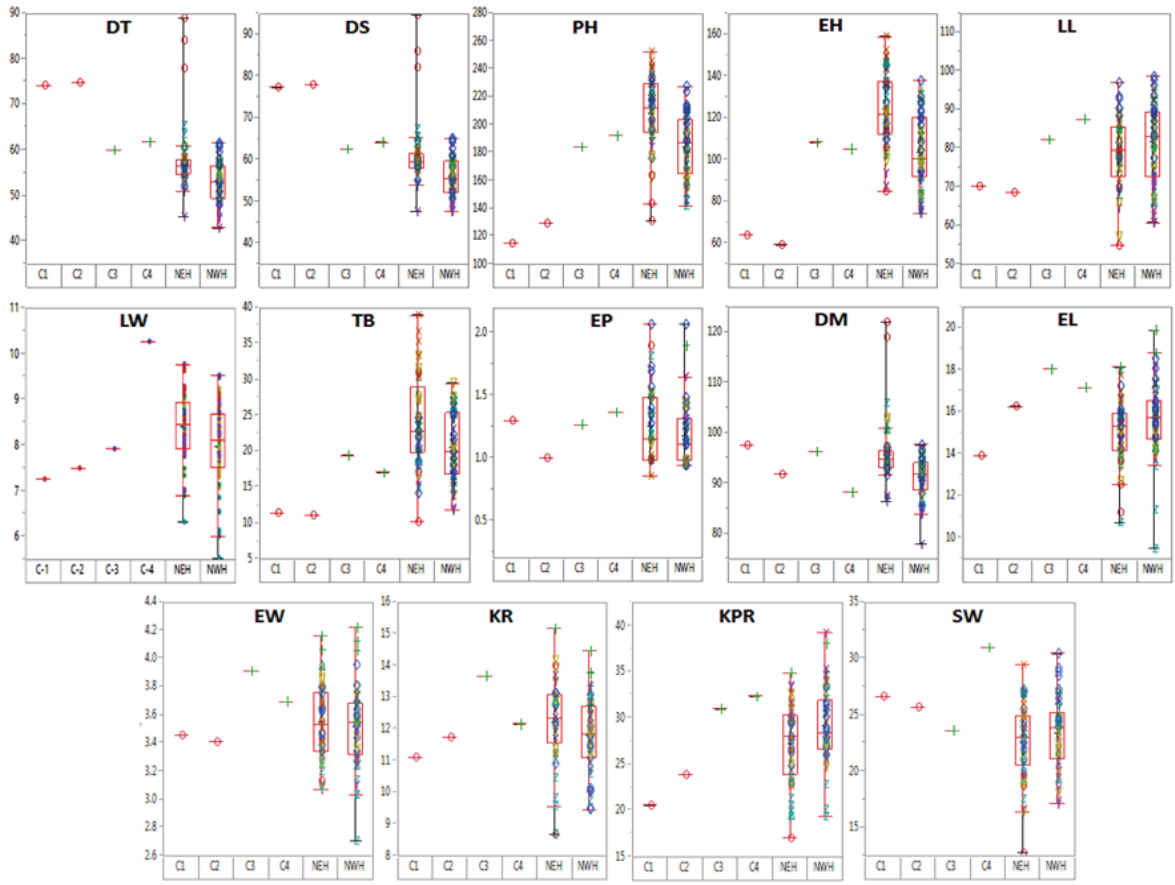

Fig. 3. Boxplot analysis depicting the differences in quartiles and mean values for 14 quantitative traits measured in 99 maize accessions from North-Eastern (50 accessions) and North-Western (49 accessions) Himalayan regions of India along with four checks

DT, DS, PH, EH, LW, TB, EP, DM and kernel KR. However, the inter quartile range, which showed the dispersal of the quantitative data, was comparatively less in most of these traits. The enter quartile range was higher for five traits namely TB, EP, EW, KPR and SW in accessions of NEH. The NWH accessions showed higher mean values for five traits such as LL, EL, EW, KPR and SW but the box length was higher for nine traits *viz*., DT, DS, PH, EH, LL, LW, EL, KR and DM. Thus, the maize landrace accessions from NWH region possessed greater morphological diversity in phenological and plant architecture traits as reflected in boxplot analysis (Fig. 3).

Associations among Quantitative Traits

The inter-relationships among 14 quantitative traits were estimated through Pearson's correlation coefficients (Supplementary Table 2). Days to tasseling (DT) had significantly positive correlations with DS (0.993) and DM (0.767). However, DT showed significantly negative correlations with EL (-0.219) and KPR (-0.399). The trait days to silking (DS) had significantly positive correlation with DM (0.779), and significantly negative correlation with KPR (-0.426). Similarly, plant height (PH) had significantly positive correlations with EH (0.895), LL (0.351), LW (0.265), TB (0.461), EL (0.453), (EW; 0.234) and SW (0.365). Ear height (EH) had significantly positive correlations with LL (0.360), LW (0.346), TB (0.447), DM (0.239), EL (0.288), EW (0.222) and SW (0.291). Leaf length (LL) showed significantly positive correlations with LW (0.533), EL (0.394), EW (0.365), KPR (0.198) and SW (0.467). However, leaf width (LW) exhibited significantly positive correlations with TB (0.227), ear per plant (EP; 0.270), EW (0.474) and kernel rows (KR; 0.251) and SW (0.285). Days to maturity (DM) showed significant negative correlations with EL (-0.217) and KPR (-0.435). Ear length (EL)

had significantly positive correlations with EW (0.440), KPR (0.524) and SW (0.435). Ear width (EW) had significantly positive correlations with KR (0.528), KPR (0.356) and SW (0.450). Kernel row (KR) had positive and significant correlation with KPR (0.439).

Principle Component and Cluster Analyses

The principal component analysis (PCA) based on 14 quantitative morphological traits was performed to reduce the data set. The PCA analysis determined Eigen values of 14 morphological traits in 99 maize landraces accessions along with four checks, explained variance and cumulative variance. *Eigen values* measure the amount of variation retained by each principal component. Ten principal components contributed the major share of variance (99.95%) in maize germplasm (Supplementary Table 3). The first principal component (PC1) accounted for 78.25% of variation (Supplementary Fig. 1). In this principal component the major contributing traits toward variation were plant height, ear height, ear length and tassel branching. Second principal component (PC2) accounted for 9.99% of variation with days to 50% tasseling, days to 50% silking, days to 80% maturity are the major contributing traits. However, PC3 showed 5.59% of variation and the main contributing traits were leaf length and width, and seed weight. PC4 accounted for 2.15% of variation and days to tasseling and days to silking were the major contributing traits. PC5 contributed 1.78% of variation and tassel branching was the major contributing trait.

A hierarchical cluster analysis (HCA) was performed based on 14 quantitative variables. In order to get the best possible classification, several analyses were tested with different agglomeration methods. The agglomeration method that produced the best result was the Ward method, which provided the constellation plot (Fig. 4). This plot arranges the individuals as endpoints and each cluster join as a new point, with lines drawn that represent membership. The longer lines represent greater distance between clusters. The plot divided the collections into 8 clusters with the membership of 3, 2, 8, 20, 8, 26, 27 and 9 accessions. Mean and standard deviation estimates for each cluster groups are presented in Supplementary Table 4. Among all 8 clusters, Cluster VII was the largest cluster and II was the smallest one. Cluster I comprised of germplasm accessions having small plant height and ear height. Cluster II had accessions having late maturity, less leaf length, less tassel branching, less ear per plant, less ear length, less kernels per row with

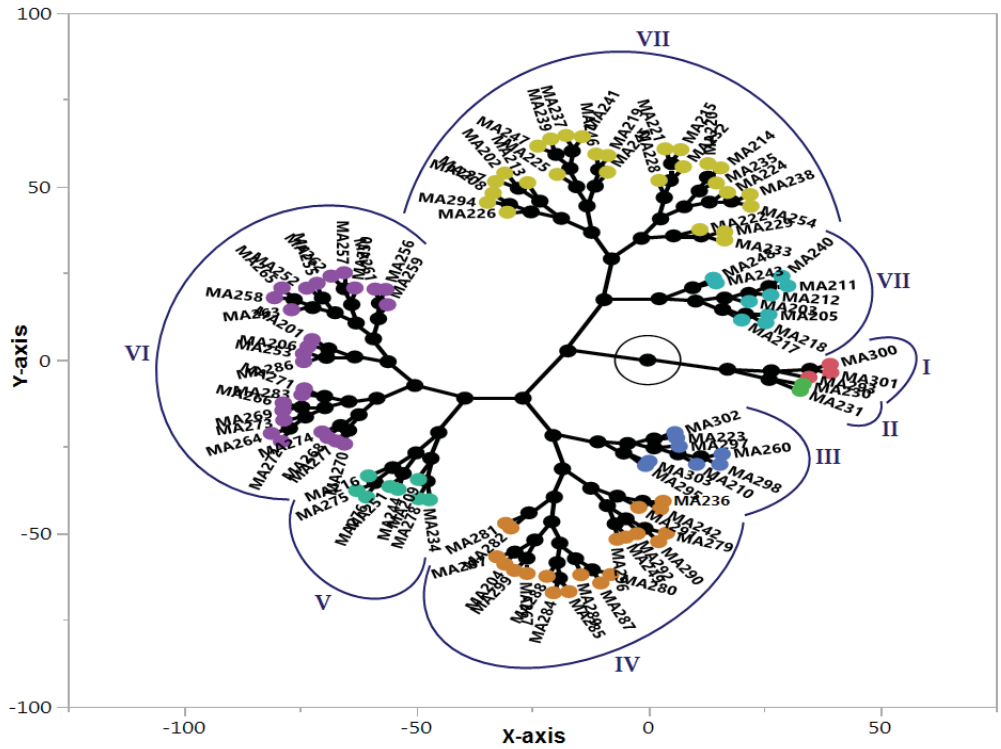

Fig. 4. Cluster analysis depicting constellation plot of maize accessions. Clusters I, II, III, IV, V, VI, VII and VIII represent different groups of maize collections

Table 4. Promising accessions selected for 14 quantitative traits based on two year's pooled data

Traits	Promising Accessions*
Day to 50% tasseling (DT)	IC077463 (MA203), IC108164 (MA227), IC128844 (MA225), IC108162 (MA226), IC077390 (MA202), IC098274 (MA218), IC381423 (MA239), IC393088 (MA240), IC278614 (MA236), IC411752 (MA241) ≤ 48 days
Day to 50% silking (DS)	IC128844 (MA225), IC108164 (MA227), IC077463 (MA203), IC108162 (MA226), IC077390 (MA202), IC098274 (MA218), IC278614 (MA236), IC381423 (MA239), IC393088 (MA240), IC411752 (MA241) ≤ 51 days
Plant height (cm) (PH)	IC447091 (MA272), IC538895 (MA266), IC524456 (MA269), IC557462 (MA274), IC083146 (MA209), IC526702 (MA283), IC538939 (MA288), IC524478 (MA273), IC447215 (MA286), IC557454 (MA287) ≥ 231 cm
Ear height (cm) (EH)	IC538895 (MA266), IC447091 (MA272), IC524456 (MA269), IC524478 (MA273), IC540252 (MA281), IC396886 (MA278), IC083146 (MA209), IC557454 (MA287), IC524611 (MA268), IC540219 (MA264) ≥ 143 cm
Leaf length (cm) (LL)	IC556413 (MA257), IC568238 (MA255), IC556430 (MA253), IC538939 (MA288), IC556406 (MA250), IC538074 (MA297), IC568279 (MA256), IC524527 (MA285), IC556415 (MA298), IC556411 (MA252) ≥ 93 cm
Leaf width (cm) (LW)	C-4(MA303), IC526764 (MA262), IC077124 (MA290), IC412888 (MA279), IC282853 (MA295), IC556415 (MA298), IC130593 (MA293), IC540252 (MA281), IC326569 (MA238), IC526436 (MA263) ≥ 9.24 cm
Tassel branching (TB)	IC524611 (MA268), IC557462 (MA274), IC419530 (MA270), IC538976 (MA277), IC524456 (MA269), IC526436 (MA263), IC128762 (MA221), IC130593 (MA293), IC526702 (MA283), IC540242 (MA271) ≥ 31
Ears per plant (EP)	IC526430 (MA289), IC077192 (MA291), IC130593 (MA293), IC282853 (MA295), IC130786 (MA234), IC545332 (MA284), IC557454 (MA287), IC526460 (MA267), IC447153 (MA280), IC077463 (MA203) ≥ 1.65
Days to 80% Maturity (DM)	IC108164 (MA227), IC077390 (MA202), IC077463 (MA203), IC469859 (MA294), IC098274 (MA218), IC338450 (MA248), IC077261 (MA201), IC108162 (MA226), IC281540 (MA247), IC077126 (MA205) ≤ 86.5 days
Ear length (cm) (EL)	IC538074 (MA297), IC097918 (MA213), IC077181 (MA206), IC077390 (MA202), IC083153 (MA210), IC556396 (MA299), C-3 (MA302), IC083146 (MA209), IC524456 (MA269), IC556430 (MA253) ≥ 17.6 cm
Ear width (cm) (EW)	IC282853 (MA295), IC083153 (MA210), IC538074 (MA297), IC128788 (MA223), IC556415 (MA298) IC556396 (MA299), IC540252 (MA281), IC538976 (MA277) C-3(MA302), IC128792 (MA224) ≥ 3.86 cm
Number of kernel rows (KR)	IC128788 (MA223), IC556415 (MA298), IC128768 (MA222), IC130605 (MA231), IC526679 (MA282), IC282853 (MA295), IC550360 (MA260), IC083125 (MA208), C-3 (MA302), IC128844 (MA225) ≥ 13.5
Number of kernels per row (KPR)	IC077390 (MA202), IC097918 (MA213), IC108162 (MA226), IC550360 (MA260), IC083153 (MA210), IC098274 (MA218), IC098165 (MA219), IC097965 (MA215), IC556415 (MA298), IC083125 (MA208) ≥ 33.6
100 seed weight (g) (SW)	C-4(MA303), IC550365 (MA261), IC526702 (MA283), IC077160 (MA292), IC077261 (MA201), IC568279 (MA256), IC077181 (MA206), IC077124 (MA290), IC083153 (MA210), IC538074 (MA297) ≥ 27.0 g

*Promising accessions were selected based on lowest 10 values for DT, DS and DM and for other traits it was top 10 entries.

high days to tasseling and silking. Cluster III comprised of accessions having high leaf length, leaf width, ear length, ear width with high kernel rows, kernels per row and medium maturity. Accessions from cluster IV having high ears per plant with medium seed weight. Cluster V had accessions having medium maturity and less kernel rows. Accessions from cluster VI having medium maturity with high plant height, ear height, tassel branching and high seed weight. Cluster VII comprised of accessions having medium plant height and medium ear height. Cluster VIII had accessions having early maturity, less leaf width, ear width along with lesser days to tasseling and silking.

Phenotypic Diversity in Qualitative Traits

Frequency distributions of 16 qualitative traits showing variability among maize landrace accessions from NEH and NWH regions of India are presented in Supplementary Fig. 2. The early plant vigour characterized the maize accessions into 3 groups namely poor (9), good (22) and very good (68) accessions. Most of the maize accessions were included in the category of very good plant vigour indicating that the majority of these accessions were selected by farmers for their robust growth habit. Most of the maize accessions (88) exhibited medium type of tassel texture (88.9%), whereas dense type was observed only in 11 accessions (11.1%). Tassel glume colour was light-purple in most of the maize accessions (51) followed by green (41) and purple (7). Tassel glume-base colour was present in 60 accessions of maize (60.6 %), while it was absent in rest. Leaf colour categorized the maize accessions into four groups and the green colored leaves were the most frequent (75.8%) followed by dark-green (18.2%) and light-green (5.1%). Yellow green leaves were present in only one landrace accession (IC393088). Most of the accessions showed the drooping type of leaf orientation (71.7%), however, 28.3% accessions showed the erect leaf type. Leaf pubescence was present in 57% accessions. It was leathery type of leaf texture present in mostly accessions (51), while the normal leaf texture was observed in 48 accessions (48.5%). Medium leaf shape was the dominant (86.9%), while broad leaves were observed in 9.09% accessions and only four accessions (4.04%) exhibited narrow leaves.

Silk colour in most of the accessions was green (87.9%), however, pink (7), purple (3) and red (2) colored silk were also recorded. Anthocyanin pigmentation was present in 51.5% accessions, whereas in 48 accessions (48.5%) the pigmentation was absent. Most of the accessions displayed yellow kernel colour (93.9%), whereas 6 accessions (6.1%) included in the other category. Maize accessions were categorized into three groups on the basis of husk cover namely poor, intermediate and good. The intermediate type of husk cover was the most frequent (64.7%) followed by poor (25.3%). In majority of maize accessions the shape of ear was observed as cylindrical (54.6% accessions), while it was conical in 38.4% accessions. Cylindrical-conical and round shaped ears were also observed in 5.05% and 2.02% accessions, respectively. Most of the accessions produced flint type of kernel texture (65.7%) followed by dent in 33.3% accessions. However, semi-flint type was present only in one accession (IC130596). Round shaped kernels were the most frequent kernel shape found in 58.6% accessions of maize, whereas the indented kernel shape was observed in 41 maize accessions (41.4%).

Discussion

Maize (*Z. mays*) is an important cereal crop with the highest genetic diversity in the form of landraces, open pollinated varieties, composites, hybrids and crop wild relatives including progenitor species. The maize genome (2.3 Gb) consisting of ~85% repeated sequences mainly of hundreds of families of transposable elements and duplicated genes, is thought to be an ancient segmental allotetraploid but functions as genetically diploid (Gaut and Doebley, 1997; Gaut *et al*., 2000; Schnable *et al*., 2009). Chromosomal behaviour analysis in monosomics revealed the presence of duplicated chromosome segments in maize genome (Yadav *et al*., 2009). Although maize was first domesticated in Mexico, its landraces are widely distributed across the continents (Sachan, 1991; Matsuoka *et al*., 2002).

Maize varieties being cultivated in Indian subcontinent are early in maturity with yellow to light yellow kernels. These varieties had undergone natural as well as farmer's selection during past five centuries. The landrace materials had adapted to local niches due to conservation and selection by local people's inhabiting different tribal regions inaccessible to urban communities (Singh, 1977). These man-made selections and conservation of landraces through religious customs and rituals had resulted in high genetic drift and consequently development of geographically isolated populations of landraces mainly in NEH and NWH high-lands of India. These man-made selections for centuries have resulted in the high extent of morphological variations in plant, tassel and ear traits in these maize landraces of NEH and NWH regions of India (Prasanna and Sharma, 2005). Numerous expeditions were undertaken for germplasm collections in the past (Grant and Wellhausen, 1955; Ono and Suzuki, 1956; Dhawan, 1964; Thapa, 1966) and reported wealth of information on the landraces and the presence of high extent of morphological diversity in the NEH region. They also compared the diversity of maize races present in NEH region with that of landrace germplasm found in plain areas of India. In contrast to the Indo-Gangetic plains, extensive variability for plant and ear characteristics (multiple cobs per plant) was reported in NEH region and NWH region (Anderson, 1945; Stonor and Anderson, 1949; Singh, 1977).

In the backdrop of such an extensive diversity of maize landraces in NEH and NWH regions of India, the plant exploration and germplasm collection teams of ICAR-NBPGR visited these regions and periodically collected diverse accessions of maize landraces (PGR portal, http://www.nbpgr. ernet.in:8080 /PGRPortal). In view of the available germplasm of maize landraces from NEH and NWH region in our national gene bank, we selected 99 accessions from 62 districts of 10 states located in maize biodiversity hotspots for phenotypic characterization of maize landraces. The high extent of morphological variation, as evident by high Shannon-Weaver diversity indices, was recorded for tassel branching (0.99), kernel rows (0.95), ear per plant (0.78), ear width (0.69), and kernel per row (0.69). However, moderate diversity was recorded in quantitative traits such as ear height (0.41) and leaf width (0.47). Most of the qualitative traits namely early plant vigour (0.79), tassel glume colour (0.89), tassel glume-base colour (0.67), Leaf colour (0.72), leaf orientation (0.75), leaf pubescence (0.68), leaf texture (0.69), anthocyanin pigmentation (0.69), ear shape (0.98), husk cover (0.85), kernel texture (0.69) and kernel shape (0.68) displayed very high level of phenotypic variation as revealed by Shannon-Weaver diversity indices.

Several studies had reported the existence of high phenotypic variability in maize landraces across the globe including Asia (Ilarslan *et al*., 2002; Wei *et al*., 2009; Sharma *et al*., 2010; Kumar *et al*., 2015; Kumari

et al., 2017), Africa (Belalia *et al*., 2018; Nelimor *et al*., 2019; Kasoma *et al*., 2020), Europe (Gouesnard *et al*., 1997; Rebourg *et al*., 2001; Hartings *et al*., 2008) and Central and South America (Bracco *et al*., 2009; Salazar *et al*., 2016; Osorio-Saenz *et al*., 2019). Ilarslan *et al.* (2002) assessed the genetic variability among 32 Turkish populations, mainly of flint and dent type races, based on 25 morphological and agronomic traits. They reported total variance ranged from 17.17% in kernel length to 82.75% in tassel length. Wei *et al.* (2009) evaluated phenotypic and genetic diversity of 102 maize landraces from Hubei province of South-West China based on 12 phenotypic characters. The landraces contained greater extent of variations for earliness, plant architecture, and ear and kernel characteristics. They suggested that the presence of abundant genetic diversity and favourable genes accumulated within these landraces should be used in maize breeding programmes.

In India, Sharma *et al.* (2010) reported phenotypically highly diverse maize landraces from NEH region and the majority of landraces analyzed were from Sikkim state including 'Sikkim primitive'. This study revealed that Sikkim primitive race, which had unique prolificacy (produced 5-9 ears on a single stalk), clearly separated from rest of the accessions. PCA showed two principal components describing 90% of total variation and 100 seed weight, ear length and diameter, kernels per ear and flowering behaviour were the most discriminating traits. However, in our study the Sikkim primitive and similar types of landraces were not included and hence number of ear per plant was less (1.0-2.1 ears/plant) as compared to Sharma *et al.* (2010). Kumar *et al.* (2015) evaluated 57 accessions of maize landraces from NWH region mainly from Himachal and Jammu and Kashmir using 17 agro-morphological and quality traits. In this study, cluster analysis revealed four groups and the accession from Jammu and Kashmir were grouped into cluster-I. PCA revealed that plant height, ear height, leaf length and quality traits (protein, oil, sugar and starch) were the major contributing traits towards diversity. They also selected three accessions based on quality trait for future maize breeding programme. Similarly, Thakur *et al.* (2017) reported high genetic diversity in 48 maize genotypes including 11 local maize germplasm from Himachal Pradesh and two germplasm lines from Sikkim based on 11 morphological traits. Kumari *et al.* (2017) characterized 75 diverse maize accessions collected from Nagaland and Manipur states of NEH region using 12 quantitative characters and reported significant morphological variability among these accessions. Cluster analysis grouped these accessions into five clusters and PCA showed that first two PCs contributed more than 50% of phenotypic variation. Plant height, ear height, ear width, number of kernels per row and kernel rows are major contributing traits towards phenotypic diversity.

The descriptive statistics revealed that our results on plant phenology (days to tasseling, days to silking, plant height, ear height, leaf length and leaf width) and ear traits (ear length, kernels per row and 100 seed weight) corroborate with the findings of Sharma *et al.* (2010), Salazar *et al.* (2016), Kumar *et al.* (2015) and Kumari *et al.* (2017) except minor variations in the range and means of ear height, plant height and seed weight. These differences in the quantitative traits could be explained due to presence of environmental, genotype × environmental effects, and the genetic makeup differences in the landraces materials analyzed in these studies. The presence of very high positive correlations between flowering (DT and DS, r=0.99) and maturity (DM and DT, r=0.77; DM and DS, r = 0.78) traits, and between plant and ear height (r=0.90) in our study indicates that the selection for one trait would simultaneously improve the correlated traits. Our results on correlation studies confirm the findings of Belalia *et al.* (2019) who reported highly positive correlations between PH and EH (r=0.95), and between days to anthesis and silking (r=0.94). Presence of linkage drag in traits showing significantly higher negative correlations would require breaking up of these negative correlations for simultaneous improvement in these traits for example kernels per row showed strong negative correlation with flowering traits (days to tasseling and silking) and maturity. Similarly, ear length and width, kernel rows and 100-seed weight also exhibited weak negative correlations with the flowering and maturity traits. The plant morphological traits namely leaf length, leaf width and tassel branching showed significant positive correlation with plant height and ear height indicating the presence of linked genes encoding for these different phenotypic traits (Cömertpay *et al*., 2012).

Moreover, our results are in good agreement with previous studies documenting the extensive variations for morphological traits in maize landraces collected from different parts of the world (Rebourg *et al*., 2001; Pressoir and Berthaud, 2004; Buckler *et al*., 2006;

Hartings *et al*., 2008; Warburton *et al*., 2008). The strong point of this research is the comparative study of phenotypic variability present in 49 accessions of maize landraces from NWH region with that of 50 accessions from NEH region of India using boxplot analysis. We found that the maize landrace accessions from NWH region possessed greater morphological diversity in plant phenology and architecture traits namely days to tasseling and silking, plant height, ear height, leaf length and width, ear length, kernel rows and days to maturity. However, the accessions of NEH region showed higher variability for tassel branching, ears per plant, ear width, kernels per row and 100-seed weight. Thus the maize accessions of NWH region possessed larger morphological diversity as compared to accessions from NEH region. Similar findings of presence of enormous diversity for plant, tassel and ear characteristics in the NEH and some parts of NWH region were also reported by earlier researchers (Dhawan, 1964; Thapa, 1966; Singh, 1977; Sharma and Prasanna, 2005; Prasanna 2010, 2012). Based on the range of variation in maize landrace accessions for different quantitative traits, we have identified 10 promising maize accessions for individual trait (Table 4). These promising accessions could be the valuable sources for genetic diversity of desirable traits for maize breeding programmes.

Conclusions

Genetic diversity is vital for genetic improvement to enhance production and productivity of maize crop. The crop genetic resources especially the landrace germplasm containing specific adaptation genes including desirable morphological and quality traits are invaluable sources of genetic diversity. In this study, we assessed morphological diversity in 99 maize landrace accessions collected from 10 different states of India located in biodiversity hotspots in NEH and NWH regions. The presence of high level of genetic variation for most of morphological traits in the landrace accessions provides an opportunity for their utilization in pre-breeding programmes for widening the primary genepool of maize and direct use as a resource for development of genetically diverse inbred lines for heterotic hybrid development in maize.

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*Supplementary Table or Figure mentioned in the article are available in the online version.

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RESEARCH ARTICLE

Cassava (*Manihot esculenta*) Synthetic Seed Production for Germplasm Exchange

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The bulkiness of the planting material and its association with cassava mosaic disease are the major constrains for cassava germplasm exchange. Hence, an efficient technique for short-term conservation and exchange of its germplasm by encapsulating non-embryogenic propagules was standardised. Among the media tested for sprouting of *in vitro* sown synthetic seeds, the maximum sprouting (91.30%) and satisfactory growth of the plants was observed in a semi-solid MS basal medium with 3% sucrose. The regeneration potential of cassava synthetic seeds stored at room temperature (25±1^{o}C) after sealing aseptically in sterile polythene bags were studied. An average of 70.50% plantlets were recovered from the synthetic seeds stored for 42 days and sprouting was reduced drastically after 49 days of storage. The recovered plantlets from the synthetic seeds were acclimatised in planting trays comprising of autoclaved sand and garden soil (1:1). Three weeks after acclimatization 97.0% survival with normal plant growth was observed.

Key Words: Artificial seed, Encapsulation, Room-temperature storage, Short-term conservation, Tapioca

Introduction

Cassava (*Manihot esculenta* Crantz) is the fourth most important source of dietary energy in tropical regions after rice, sugar, maize and the ninth important crop in the world (Bokanga, 2002). Its cultivation and processing provides employment opportunities and ensure food security for millions of people in Africa, Asia and the Americas (FAO, 2020). Cassava based industry is immensely contributing to food security, poverty alleviation, economic growth and finally, rural development. This crop yields satisfactorily in low fertility soil and wastelands. Historically cassava was cultivated as a famine reserve crop as it was a more reliable alternative source of food during drought periods. Thus, cassava has recognition as a preference crop in the context of climate change adaptation strategies. Recently, this crop emerged as food and a profitable commercial crop of industrial significance (Aerni, 2006).

Cassava is commercially propagated vegetatively by the planting stem cuttings. This mode of propagation spreads pest and diseases particularly viral diseases and makes it difficult to conserve the germplasm. Because of stem cuttings' association with the spread of pests and diseases (Cassava mosaic disease-CMD), this mode of propagation also leads to quarantine problems during germplasm exchange. In addition, the rate of planting material multiplication in cassava is extremely low (1:8) and also stems do not store well for longer periods. Moreover, due to bulkiness of the stem cuttings (planting material), it is very difficult in handling and transport to distant places, thus restricting the spread of the crop in non-traditional areas and exchange of cassava genetic resources (Rajendran *et al*., 2005).

To maintain the diversity and sustainable genetic improvement of the crop, it is crucial to collect, conserve and utilize the available germplasm of a particular crop. An alternative means of short-term conservation and exchanging of cassava germplasm via synthetic seeds by encapsulating micropropagules is investigated. Synthetic seeds are artificially encapsulated micropropagules such as somatic embryos, shoot tips, nodes, axillary buds or any other tissues, capable of developing into a plant under suitable conditions, like a true seed. During handling and storage, plant propagule is protected by encapsulation against mechanical damage and drying. Hence, synseeds have practical applications for germplasm conservation and exchange (Hasan and Takagi, 1995). In addition, synthetic seeds are relatively inexpensive to produce and easy to handle during transport (Rao *et al*., 1998). In cassava, the synthetic seed can be produced by encapsulating nodal cuttings and shoot tips from *in vitro* raised plants using 3.0% sodium alginate and 100 mM calcium chloride (Hegde *et al*., 2016).

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Additional benefits of synthetic seeds include low production costs, short and long-term storability, facilitation of germplasm exchange between the laboratories, easy handling, transportation of propagules to distant places and subsequent propagation (Parveen and Shahzad, 2014). These synthetic seeds of cassava would allow direct sowing under *in vitro* upon receipt during the exchange of the genetic material between the researchers. In the present study, investigations have been undertaken to assess vaiability of synthetic seeds of cassava stored under room temperature, for facilitating exchange of encapsulated cassava propagules.

Materials and Methods

Nodal segments and shoot tips of popular cassava variety H226, measuring 2 to 5 mm were excised from 30 to 35 days old *in vitro* propagated plants on Murashige and Skoog's (MS) basal medium (Murashige and Skoog, 1962). Protocol for synthetic seed production (Fig.1A) was similar as described by Hegde *et al*. (2016). The specially formulated standard purity chemicals used during the experiments were obtained from Hi-Media Laboratories Pvt. Ltd., India.

Sprouting and establishment of cassava synthetic seeds were studied under *in vitro* and *ex vitro* conditions with different media. For plant establishment from synthetic seeds under *in vitro* conditions, semi-solid as well as a liquid Hoagland (Hoagland and Arnon, 1950) and MS medium without as well as with 3.0% sucrose, were tested. The pH of the medium was adjusted to 5.8 and sterilization was done by autoclaving for 15 min at 121°C and 15 lb pressure. All cultures were incubated in the culture room at 25 ± 1°C under 16 h photoperiod provided by cool white LED lamps to observe the germination ability of the cassava synthetic seed in different media. For *ex vitro* germination, encapsulation solutions were supplemented with 0, 3, 6 and 9% sucrose with or without different concentrations and combinations of cytokinins (6-Benzylaminopurine – BAP @ 0.5, 1.0, 1.5 & 2.0 mg/L) and auxins (indole-3-butyric acid – IBA @ 0.5, 1.0, 1.5 & 2.0 mg/L) were tested. Synthetic seeds were sown *ex vitro* in pots containing different sterile (autoclaved) potting mixtures like cocopeat, vermiculite, sand with garden soil (1:1) and potting mixture readily available in the market (Agro-Bazar). These pots were covered with polythene bags to increase the relative humidity and kept in the nethouse for hardening.

Cassava synthetic seed storage study was conducted under room temperature and the germination ability of the stored synthetic seed was tested. Synthetic seeds were sealed aseptically under laminar flow in sterile, transparent and autoclavable polythene bags (HiDispo^{TM} Bag, HiMedia) (Fig.1B). These were stored at room temperature (25±1^{o}C) in dark up to 70 days. To study the germination ability, stored synthetic seeds were sown *in vitro* at seven days intervals on MS basal medium with 3% sucrose. The cassava plantlets derived from stored synthetic seeds were taken out from the medium after 42 days of incubation and washed with running tap water to remove the adhering medium. These plantlets were planted in protrays containing an autoclaved mixture of sand and garden soil (1:1) and acclimatised in a polyhouse after covering with perforated transparent polythene covers to maintain high relative humidity.

The experiments were carried out using a complete randomized design and repeated thrice with 25 synthetic seeds per treatment. Germination percentage, days taken for germination, number of shoots, shoot length (cm), number of leaves, number of roots and root length (cm) were recorded. The percent data was transformed using angular transformation before the analysis to test for statistical significance using OPSTAT (Sheoran *et al*., 1998).

Results and Discussion

In the present study, synthetic seeds of cassava variety H226 were produced by encapsulating nodal segments and shoot tips measuring 2 to 5 mm with 3.0% sodium alginate and 100 mM calcium chloride solutions with the complexing time fixed at 30 minutes. Encapsulation of explants with appropriate size, texture, stability and hardness is the key to producing synthetic seeds. Very hard beads restrict germination, while fragile beads make handling difficult. As reported by many researchers, 3% sodium alginate and 100 mM calcium chloride with 30 minutes complexing time is ideal for the production of synthetic seeds in several plant species (Ghanbarali *et al*. 2016; Baskaran *et al*., 2018; Kaminska *et al*., 2018 and Behera *et al*., 2020). *In vitro* produced somatic embryos are commonly used for the production of synthetic seeds since it behaves like a true botanical seed (Gantait *et al*., 2015). The rates of somatic embryo induction are very low in cassava and are highly dependant on the genotype, type of explant and growth regulators

concentration and combinations used (Anuradha *et al*., 2015). Production of synthetic seeds by encapsulating micro shoots with apical bud and nodes with axillary bud have been reported instead of somatic embryos (Gantait *et al*., 2015). Synthetic seeds produced by encapsulating these explants are economical and easier to handle as compared with somatic embryos. Hence, in the present work, cassava synthetic seeds were produced using nodal segments and shoot apices.

Effect of different semi-solid as well as liquid MS medium and Hoagland solution supplemented with or without 3.0% sucrose for the sprouting of synthetic seeds and establishment of complete cassava plant under *in vitro* condition is shown in Fig.1D-F. Sprouting of *in vitro* sown synthetic seeds were recorded when the encapsulation material splits open and the explants start growing. Among the treatments, maximum sprouting (91.30%) and growth was observed in semi-solid MS basal medium with 3% sucrose followed by liquid MS basal medium with 3% sucrose (90.48%). The difference in the sprouting of synthetic seeds between semi-solid and liquid MS basal medium supplemented with 3% sucrose was negligible. Drastic reduction in synthetic seed sprouting was observed in both liquid (42.86%) and semi-solid (57.14%) MS basal medium without sucrose. Similarly, 38.10% and 14.29% sprouting was recorded in Hoagland solution supplemented with or without sucrose, respectively (Fig. 2). Based on the observation, semi-solid MS basal medium supplemented with 3.0% sucrose is the best medium for the sprouting of cassava synthetic seed and establishment of the plant under *in vitro* condition. Similar to our results, high germination frequency of synthetic seeds was observed on full-strength MS medium in several plant species including *Solanum nigrum* (Verma *et al*., 2010), *Centella asiatica* (Prasad *et al*., 2014) and *Hedychium coronarium* (Behera *et al*., 2020).

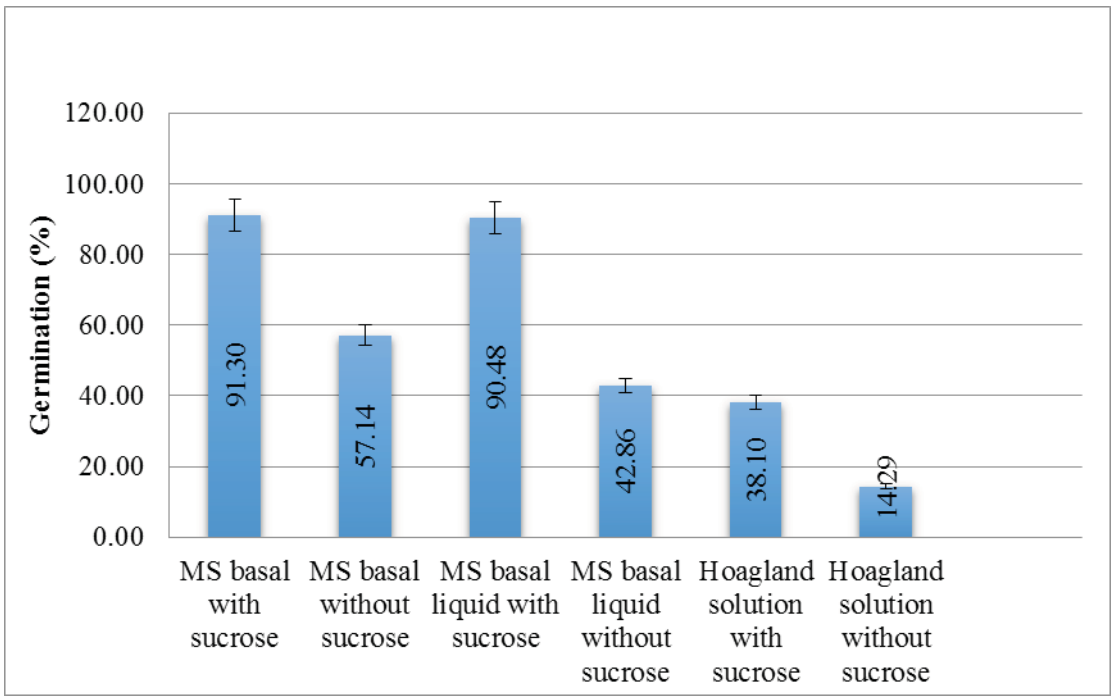
Synthetic seeds of the cassava in the present study failed to sprout and convert into plants under *ex vitro* conditions, despite supplementation of varied concentrations and combinations of sucrose and plant growth regulators to encapsulation solutions. Sterile potting mixtures including cocopeat, vermiculite, sand with garden soil (1:1) and potting mixture readily available in the market were unable to support the cassava synthetic seeds to produce a complete plant. Probably the nutrients in the micro-propagule as well as encapsulation material were not enough to support

Table 1. Germination of synthetic seeds of cassava after being stored at different durations under room temperature (25±1 ^{0}C)

Storage period (days)	Germination percentage, days after sowing								
	7	14	21	28	35	42	49	56	63
0	60.00 (7.80)	83.33 (9.18)	91.67 (9.63)	92.50 (9.67)	**92.50 (9.67)**	92.50 (9.67)	95.00 (9.80)	95.00 (9.80)	95.00 (9.80)
7	62.50 (7.91)	75.00 (8.72)	91.67 (9.63)	91.67 (9.63)	**91.67 (9.63)**	91.67 (9.63)	93.50 (9.72)	94.00 (9.75)	94.00 (9.75)
14	70.50 (8.45)	76.00 (8.78)	80.00 (9.00)	88.46 (9.46)	**88.46 (9.46)**	90.00 (9.54)	90.00 (9.54)	92.50 (9.67)	92.50 (9.67)
21	73.08 (8.61)	76.10 (8.78)	83.50 (9.19)	84.62 (9.25)	**85.00 (9.27)**	85.00 (9.27)	86.00 (9.33)	87.50 (9.41)	87.50 (9.41)
28	72.50 (8.56)	75.00 (8.71)	77.50 (8.85)	80.50 (9.02)	**83.50 (9.18)**	84.00 (9.21)	85.00 (9.26)	87.00 (9.37)	87.00 (9.37)
35	64.00 (8.06)	70.50 (8.45)	74.50 (8.68)	74.50 (8.68)	**75.50 (8.74)**	76.50 (8.80)	77.50 (8.85)	77.50 (8.85)	77.50 (8.85)
42	**55.50 (7.51)**	**61.50 (7.90)**	**65.00 (8.12)**	**67.50 (8.27)**	**70.50 (8.45)**	**70.50 (8.45)**	**74.50 (8.68)**	**74.50 (8.68)**	**74.50 (8.68)**
49	47.00 (6.93)	51.50 (7.24)	56.50 (7.58)	57.00 (7.61)	**58.00 (7.68)**	58.00 (7.68)	58.50 (7.71)	59.00 (7.75)	59.00 (7.75)
56	20.50 (4.64)	26.00 (5.19)	27.50 (5.34)	30.00 (5.57)	**30.00 (5.57)**	35.00 (5.99)	35.00 (5.99)	40.00 (6.40)	40.00 (6.40)
63	19.00 (4.47)	19.50 (4.53)	20.50 (4.64)	22.50 (4.85)	**22.50 (4.85)**	22.50 (4.85)	27.00 (5.29)	27.00 (5.29)	27.00 (5.29)
70	15.00 (4.00)	17.00 (4.24)	17.50 (4.30)	17.50 (4.30)	**21.50 (4.74)**	21.50 (4.74)	22.50 (4.85)	22.50 (4.85)	22.50 (4.85)
C.D.	0.426	0.445	0.458	0.464	**0.470**	0.472	0.471	0.474	0.474
SE (m)	0.144	0.151	0.155	0.157	**0.159**	0.160	0.160	0.161	0.161

(Figures in parenthesis are angular transformed values)

Fig. 1. Cassava synthetic seeds obtained by encapsulation of nodal segments and shoot tips (A); Cassava synthetic seeds sealed aseptically in sterile polythene cover for storage (B); Sprouting of cassava synthetic seeds during storage (C) and under *in vitro* (D); Cassava plant with well-developed shoot and roots obtained from synthetic seed (E&F); Acclimatized cassava plants with normal vegetative growth (G).

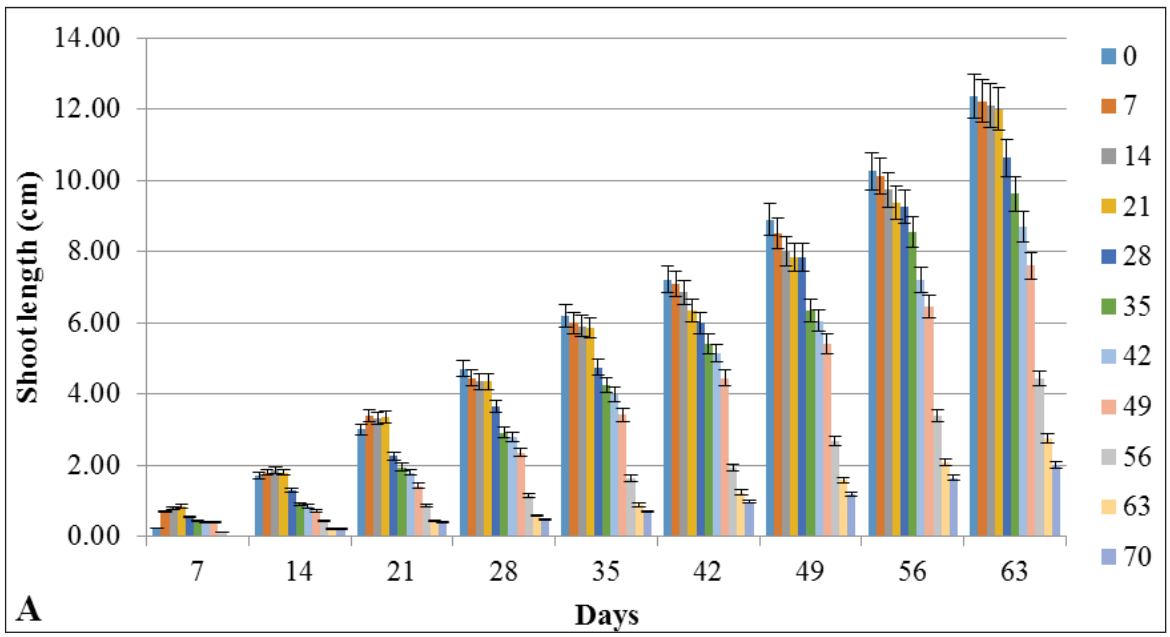


(Percentage error bar at 5%)

Fig. 2. Sprouting (germination) of cassava synthetic seeds on different culture media after 35 days of culture

the synthetic seeds for sprouting and establishment of plants under *ex vitro* conditions.

Sprouting percentage, shoot length and number of leaves were recorded at weekly intervals (Table 1, Fig. 3A & B). The highest sprouting/germination percentage (92.50%) was observed in freshly prepared cassava synthetic seeds (sown *in vitro* without storage). Sprouting decreased with increase in the duration of storage at room temperature. Drastic reduction of sprouting of encapsulated cassava explants was observed after 49 days of storage under room temperature. Stored synthetic seeds started sprouting after 14 days (Fig. 1C) and quick plant establishment was observed from them immediately after sowing *in vitro*. An average of 70.50% (35 days after



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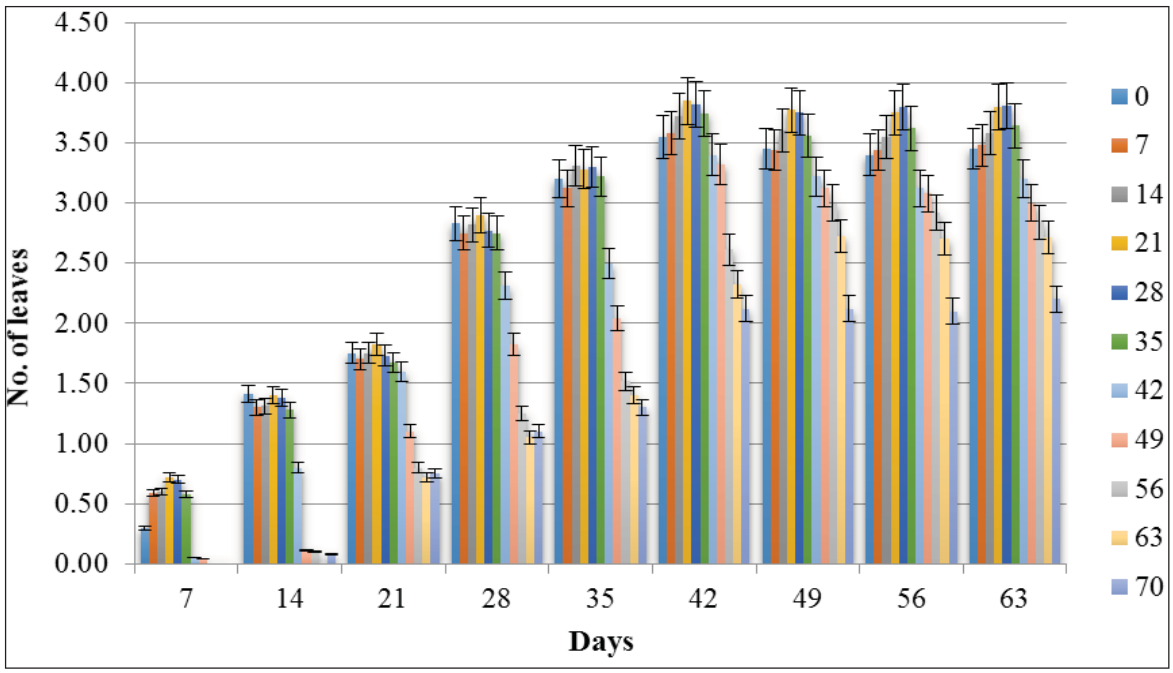

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Fig. 3. Growth and development of *in vitro* cassava plants raised from synthetic seeds being stored at different durations under room temperature (A) Shoot length (B) Number of leaves

sowing *in vitro*) sprouting and optimum growth of the plants were observed from the cassava synthetic seeds stored for 42 days (Table 1, Fig. 3A & B). Significant difference was not observed for the average shoot length (cm) of *in vitro* plants obtained from stored synthetic seeds for different durations up to 42 days and it was reduced drastically beyond 42 days of storage (Fig. 3A). Average numbers of leaves were reduced among the *in vitro* plants obtained from the synthetic seeds stored for 49 days and beyond (Fig. 3B).

Cassava synthetic seeds stored at room temperature were sown *in vitro* at seven days intervals on MS basal medium supplemented with 3.0% sucrose. After 42 days of incubation under the culture room, cassava plants were taken out from the medium (Fig.1E & F) and observations were recorded. The average number of shoots ranged from 1.10 to 1.45 and no significant difference were observed between the treatments, while a significant difference was observed in shoot length (cm), number of roots, number of leaves and root length (cm). The average shoot length was highest (16.23 cm) among the plants raised from fresh synthetic seeds. It decreased significantly among plants obtained from stored synthetic seeds beyond 42 days. A similar trend was observed for root length and number of leaves, while the root number was maximum (10.88) among the plants raised from synthetic seeds stored for 14 days (Fig. 4). The short-term storage of synthetic seeds under room temperature (25±1 ºC) with acceptable plant recovery rate is reported in very few plant species including *Sphagneticola calendulacea* (Kundu *et al*., 2018), *Cineraria maritima* (Srivastava *et al*., 2009) and *Hedychium coronarium* (Behera *et al*., 2020).

Survival of plantlets was about 93.0% - 97.0% (data not given) after three weeks from the date of acclimatization and normal growth of the plants were observed (Fig. 1G). The recovered plantlets from the synthetic seeds acclimatised in *ex vitro* conditions with high (>90%) survival of plants is reported by several earlier researchers in many plant species (Srivastava *et al*., 2009; Kundu *et al*., 2018; Behera *et al*., 2020)

The acceptable frequency of plant conversion (70.50%) was observed from the cassava synthetic seeds produced by encapsulating *in vitro*-derived non-embryogenic micro-propagules like shoot tips and nodal cuttings, aseptically stored for 42 days under room temperature (Table 1). The technique described here is quick and simple. This protocol can be used as a novel delivery system for exchange of genetic materials between the laboratories or countries.

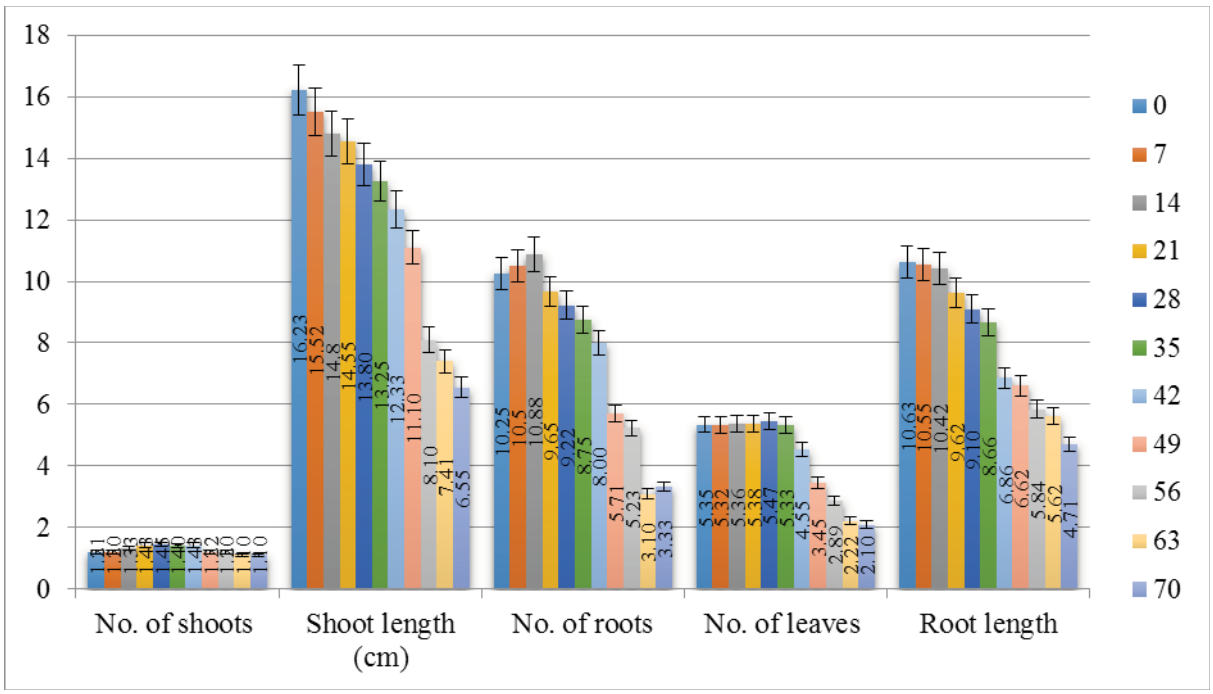

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Fig. 4. Effect of short-term storage of cassava synthetic seed at room temperature on growth and development of *in vitro* plants (42 days after sowing)

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RESEARCH ARTICLE

Genetic Diversity in Indian Landraces of Cucumber (*Cucumis sativus* L.) Based on Morpho-Horticultural Traits

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Until recently, the main focus of breeding programmes in cucumber (*Cucumis sativus* L.) was the development of varieties and hybrids for high yield. However, consumer preference of cucumber is mainly determined by the qualitative traits of the fruit namely colour, shape, size, tenderness, crispness, texture and organoleptic qualities. In this study, observations on 15 qualitative traits, three major quantitative traits, storage characters and organoleptic attributes were recorded in 53 cucumber accessions, collected from 12 Indian states. The skin colour of the tender fruits ranged from light green, green, dark green to cream and fruit shapes of elliptical elongate, oblong ellipsoid and globular were observed. Ten accessions exhibited orange coloured flesh during the ripe stage of the fruit, indicating carotenoid content in the flesh. Shelf life of 4-6 days was exhibited by IC331627, IC613481, IC613461, IC202058A, IC331619, IC613460, IC613477 and IC613478 with a weight loss of less than 10 g during storage under room temperature. Four accessions namely IC613480, IC613481, IC618084A and IC613484 exhibited organoleptic qualities better than the control variety, AAUC-2. Based on the multivariate analysis using PCA, fruit skin colour and texture were identified as the most discriminating qualitative traits explaining greater variability in cucumber. Elliptical elongate shape with green or light green colour of the fruits are the most common and preferred characters for commercial cucumber varieties. IC613481 and IC613480, both collections from West Bengal ranked superior with respect to organoleptic scoring, preferable storage qualities and promising yield attributes. Systematic characterization of the genotypes revealed that they are potential sources of genetic variability, worth harnessing through improvement programmes.

Key Words: Cucumber, *Cucumis sativus,* Consumer preference, Genetic diversity, Organoleptic quality

Introduction

Cucumber is an indigenous vegetable of India (Sebastian *et al*., 2010). In all probability, it originated in the foothills of the Himalayas. The two botanical varieties, namely the domesticated cucumber, *Cucumis sativus* var. *sativus* L. and the wild cucumber *C. sativus* var. *hardwickii* (Royle) Alef. are ubiquitously found distributed in this region (Lv *et al*., 2012). Wide genetic variability is likely to occur among cucumber genotypes from this region. The neighbouring China is considered as the secondary centre of diversity (Valcarcel *et al.*, 2018). The dissemination of cucumber from India to other regions afar occurred very early. The crop is presently being grown on a large-scale even in temperate zones under controlled environment like glass house.

Often, populations derived from adapted or improved breeding lines that possess a narrow genetic base have been used commonly in cucumber improvement programmes. Hence, selection as well as population improvement within the adapted material is unlikely to yield the expected return (Kumar *et al*., 2017). The landrace population with a relatively high degree of genetic variability, however is likely to be more advantageous than the improved varieties. A landrace is a locally adapted variety evolved from a variable population as a result of natural selection and shaped according to the choice of local people. Consumer preference in cucumber is mainly determined by the colour, shape, texture and organoleptic qualities of fruits. Although scarce, studies on inheritance pattern of these

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characters in cucumber have been reviewed long before by Poole (1944). These visually assessable morphological characters are the preferred choice for diversity studies, as these are among the easiest morphological markers used in germplasm management (Stanton *et al.,* 1994).

It is well-known that progenies derived from geographically and genetically diverse parents in the hybridization programme would prove advantageous in terms of their agronomic performance owing to the diversity in genetic makeup. The landraces constitute a valuable resource for cucumber breeders to increase variability concerning qualitative and quantitatively inherited characters and adaptation to specific growing conditions (Valcarcel *et al.*, 2018). Thus, inclusion of landraces, un-adapted and exotic lines in breeding population may result in improved varieties with desirable combination of characters.

North-eastern India characterized by the transition zone between the Indian, Indo-Malayan and Indo-Chinese biogeographic regions is enriched with diverse genetic resources, particularly of cucurbitaceous vegetables (Ranjan *et al*., 2019). In addition, these landraces being cultivated and maintained as open pollinated populations by the local farming community for generations, has contributed considerably to the variability observed in the population. Hence, the present study was undertaken to asses qualitative (15) and quantitative (3) traits in 53 accessions of cucumber collected from 12 states of India. Hence, systematic characterization and evaluation of these genotypes is essential to delineate their genetic potential.

Materials and Methods

The experimental materials consisted of 53 landraces of cucumber procured from the National Gene bank at ICAR-National Bureau of Plant Genetic Resources, New Delhi (Table 1). This collection largely comprised of local landraces grown by the tribal population of North-eastern states of India namely Tripura (8), Mizoram (14), Nagaland (1) and Arunachal Pradesh (4). It also included accessions from other agro-ecological zones *viz.,* Kerala (1), Karnataka (1), Maharashtra (2), West Bengal (12), Himachal Pradesh (1), Andaman and Nicobar Islands (3), Uttarakhand (2) and Odisha (1)

The experiment was laid out during June-Aug 2015 at the experimental farm of ICAR-NBPGR, Regional Station, Thrissur (10.5480^{o} N, 76.2830^{o} E) following an augmented block design with three control varieties, namely AAUC-2 (Assam Agricultural University Cucumber-2, a variety released from Assam Agricultural University, India), Swarna Agethi (variety released from ICAR-Research Complex for Eastern Region, Patna, Bihar, India) and Poinsette (an exotic collection). The mean annual temperature and rainfall in the region were 27.2^{o}C and 2639.4 mm, respectively. Each accession was represented by ten direct seeded plants raised at a spacing of 0.50 × 1.50 m in a row of 4.5 m length. The package of practice recommendations of KAU (2011) was followed for raising a good crop. As the soils were in the acidic pH range, lime was applied @ 600kg/ha as a pre-sowing treatment. The plots were hand weeded to avoid weed growth.

Observations on 15 qualitative characters pertaining to the leaf (3), plant habit (1), stem (1), flowers (2) and fruit (8) were recorded based on the minimal descriptor list developed by ICAR-NBPGR (2001) as illustrated in Table 2. Trait variability distribution was graphically depicted in pie diagrams. Principal component analysis was done using an online trial version of Minitab software. Single fruit weight, length and width, the shelf life attributes were the quantitative traits recorded. Shelf life of fruits was estimated by measuring the loss of weight of the fresh fruits and number of days of storage assessed as discolouration of the fruit skin upon storage at room temperature.

Sensory evaluation of the tender fruits from each accession was done immediately after harvest by a 15 member judging panel. Score-card for bitterness, crispness and flavour was prepared and each quality attribute was scored using a five point hedonic scale which ranged from zero to five. The mean score for each quality attribute for individual genotypes were computed over the data recorded by the judging panel. To represent the acceptability of the fruit in terms of bitterness, the complement (maximum score - average score) with respect to maximum score was taken. This exercise was done in order to maintain uniformity in reading the score in ascending order. Kendall's coefficient of concordance was employed to study the significance of perception of taste between the individuals as well as to help rank the accessions based on mean rank of different sensory attributes analysed. The hedonic scales were then converted to rank scores and rank analysis was done by Kendall's coefficient of concordance (Siegel, 1956).

Table 1. Landraces with their passport data used in the study

S. No	Collector No.	Accession No.	Genus	Species	State	Latitude	Longitude
1	JS/06-01	IC541367	*Cucumis*	*sativus*	A&N Islands	12.229	92.809
2	JS/06-25	IC541391	*Cucumis*	*sativus*	A&N Islands	12.508	92.932
3	MS/05-12	IC539818	*Cucumis*	*sativus*	A&N Islands	12.654	92.899
4	JB/12-203	IC613471	*Cucumis*	*sativus*	Arunachal Pradesh	27.158	95.569
5	JB/12-217	IC613472	*Cucumis*	*sativus*	Arunachal Pradesh	27.299	96.148
6	JB/12-236	IC613473	*Cucumis*	*sativus*	Arunachal Pradesh	27.086	95.442
7	JB-12-183A	IC618084A	*Cucumis*	*sativus*	Arunachal Pradesh	27.176	96.078
8	BB-11/2001A	IC331619	*Cucumis*	*sativus var. hardwickii*	Himachal Pradesh	30.905	77.097
9	JR-04-13	IC469517	*Cucumis*	*sativus*	Karnataka	12.763	76.060
10	JJK/10-601	IC595518	*Cucumis*	*sativus*	Kerala	9.684	76.337
11	BBL-67/2000	IC277048	*Cucumis*	*sativus var. hardwickii*	Maharashtra	17.248	73.371
12	BBL-49/2000	IC277030	*Cucumis*	*sativus var. hardwickii*	Maharashtra	18.516	73.182
13	JB-11/18	IC613457	*Cucumis*	*sativus*	Mizoram	23.545	92.442
14	JB-11/28	IC595504	*Cucumis*	*sativus*	Mizoram	24.150	92.384
15	JB-11/34	IC613458	*Cucumis*	*sativus*	Mizoram	24.150	92.384
16	JB-11/43	IC595505	*Cucumis*	*sativus*	Mizoram	24.102	92.342
17	JB-11/60	IC613459	*Cucumis*	*sativus*	Mizoram	24.145	92.405
18	JB-11/69	IC612081	*Cucumis*	*sativus*	Mizoram	24.185	92.422
19	JB-11/75	IC613461	*Cucumis*	*sativus*	Mizoram	24.236	92.431
20	JB-11/91	IC613462	*Cucumis*	*sativus*	Mizoram	24.224	92.431
21	JB-11/99	IC612082	*Cucumis*	*sativus*	Mizoram	24.011	92.403
22	JB-11/120	IC613465	*Cucumis*	*sativus*	Mizoram	23.145	92.405
23	JB-11/126	IC613466	*Cucumis*	*sativus*	Mizoram	23.154	92.384
24	JB-11/128	IC613467	*Cucumis*	*sativus*	Mizoram	23.154	92.384
25	JB-11/145	IC613488	*Cucumis*	*sativus*	Mizoram	23.224	92.431
26	JB/11-71	IC613460	*Cucumis*	*sativus*	Mizoram	24.185	92.422
27	KPAC-1494	IC613474	*Cucumis*	*sativus*	Nagaland	26.760	95.610
28	BBD-12/2001	IC331445	*Cucumis*	*sativus*	Odisha	18.856	82.735
29	JB/11-182-A	IC595508A	*Cucumis*	*sativus*	Tripura	23.084	91.364
30	JB/11-205	IC595510	*Cucumis*	*sativus*	Tripura	23.025	91.541
31	JB/11-206	IC618083	*Cucumis*	*sativus*	Tripura	23.025	91.541
32	JB/11-217	IC595512	*Cucumis*	*sativus*	Tripura	23.262	91.346
33	JB/11-229	IC595514	*Cucumis*	*sativus*	Tripura	24.012	91.381
34	JB/11-242	IC595515	*Cucumis*	*sativus*	Tripura	23.482	91.535
35	JB/11-262	IC595517	*Cucumis*	*sativus*	Tripura	23.545	91.502
36	JB/11-197	IC613470	*Cucumis*	*sativus*	Tripura	23.005	91.546
37	BB-19/2001A	IC331627	*Cucumis*	*sativus var. hardwickii*	Uttarakhand	30.317	78.032
38	U38/IS/AD/36	IC202058A	*Cucumis*	*sativus var. hardwickii*	Uttarakhand	30.317	78.032
39	SKYAC/239	IC613470	*Cucumis*	*sativus*	West Bengal	22.370	88.090
40	SKYAC/244	IC613475	*Cucumis*	*sativus*	West Bengal	22.900	88.300
41	SKYAC/247	IC613476	*Cucumis*	*sativus*	West Bengal	22.900	88.300
42	SKYAC/251	IC613477	*Cucumis*	*sativus*	West Bengal	22.900	88.300
43	SKYAC/253	IC613478	*Cucumis*	*sativus*	West Bengal	22.900	88.300
44	SKYAC/262	IC613479	*Cucumis*	*sativus*	West Bengal	22.940	88.230
45	SKYAC/265	IC613480	*Cucumis*	*sativus*	West Bengal	22.940	88.230
46	SKYAC/270	IC613481	*Cucumis*	*sativus*	West Bengal	23.070	88.590
47	SKYAC/295	IC613482	*Cucumis*	*sativus*	West Bengal	22.950	88.610
48	SKYAC/314	IC613483	*Cucumis*	*sativus*	West Bengal	23.050	88.700
49	SKYAC/316	IC613484	*Cucumis*	*sativus*	West Bengal	22.960	88.720
50	SKYAC/319	IC613485	*Cucumis*	*sativus*	West Bengal	22.990	88.820

Table 2. Descriptors and descriptor states for the 15 qualitative characters studied

Characters	Descriptor states and the respective scores								
Leaf shape	1-Cordate	2-Oblong	3-Ovate	4-Ovovate	5-Reniform	99-Others			
Leaf pubescence density	1-No hairs	2-Sparse	3-Intermediate	4-Dense	99-Others				
Leaf margin	1-Unifid	2-Bifid	3-Multifid	99-Others (Angular)					
Fruit skin colour	1-Cream	2-Yellow	3-Liht green	4-Green	5-Dark green	6-Orange	7-Pink	8-Brown	99-Others
Fruit shape	1-Elliptical elongate	2-Oblong ellipsoid	3-Globular (round)	4-Stem end tapered	5-Blossom end tapered	99-Others			
Fruit Flesh colour	1-White	2-Green	3-Yellow	4-Orange	99-Others (light green)				
Stem end fruit shape	1-Depressed	3-Flattened	5-Rounded	7-Pointed	99-Others				
Blossom end fruit shape	1-Depressed	3-Flattened	5-Rounded	7-Pointed	99-Others				
Fruit skin texture	1-Plain	2-Netted	3-Rough	99-Others					
Presence of seed cavity	0-Absent	1-Present							
Colour of tender fruits at 3 DAA	1-Cream	2-Yellow	3-Liht green	4-Green	5-Dark green	6-Orange	7-Pink	8-Brown	99-Others
Plant growth habit	3-Short viny	5-Medium viny	7-Long viny	99-Others					
Stem pubescence	0-Absent	1-Present							
Sex type	1-Monoecious	2-Gymno-monoecious	3-Andro-monoecious	4-Gyno Andro-monoecious	5- Androecious	6-Gynoecious	99-Others		
Flower colour	1-White	2-Cream	3-Yellow	4-Orange	99-Others				

Scoring for downy mildew disease caused by *Pseudoperonospora cubensis* was undertaken on the 60th day after sowing to assess the tolerance level of the accessions. The intensity of downy mildew disease was assessed with the score chart from 0 to 5 scale (0 - no infection; 1 - 0 to10 %; 2 - 10.1 to15 %; 3 - 15.1 to 25 %; 4 - 25.1 to 50 % and 5 - more than 50 percent of leaf area being covered with mildew growth) as described by Jamadar and Desai (1997). The per cent disease index of downy mildew was calculated using the formula given by McKinney (1923).

The seeds of these landraces were multiplied by selfing/ sibmating and conserved in National Genebank, New Delhi for long term storage and in Medium Term Storage (MTS) facility at Regional Station, Thrissur, for supply to farmers and breeder/researchers.

Results and Discussion

The scores and data recorded for both qualitative and quantitative traits respectively, are depicted in Table 3. The accessions exhibited no variability with regard to a few plant, leaf, floral and fruit characters. All accessions had a long viny growth habit with dense stem pubescence irrespective of their origin. Shape, pubescence density and margin of leaf, stem end and blossom end shape of the fruit also did not vary between accessions. The leaves were densely pubescent and the leaf shape was found to be cordate with angular margins in all the accessions. The vines produced yellow male and female flowers with majority of them being five petallate, while a few flowers possessed 6-8 petals. Fruits possessed flattened shape both at stem end and blossom end. Occasionally, fruits with depressed shape at stem end as well as blossom end were observed, but at a very low frequency. Esteras *et al*. (2008) had, however observed high variation among stem end and blossom end shape of cucumber fruits.

The characteristics that affect the marketability of cucumber are decided by four main attributes. They are colour and appearance, flavour (taste and aroma), texture and nutritional value in the order specified (Barrett *et al.*, 2010). Among the 53 genotypes, seven (IC613457, IC613475, IC277048, IC277030, IC202058A, IC613462 and IC595518) possessed cream/ivory coloured fruits (Fig. 1). The major fruit skin colour observed was light green followed by green. IC595505, IC613459 and Poinsette exhibited dark green colour. Rich diversity for exterior fruit colour (cream, pale green, dark green, yellow to vibrant orange with dark green streaks) among different cultivars in cucumber was also reported by

Table 3. Observations on qualitative and quantitative characters in 53 cucumber landraces

S.No.	Accessions	FSC	FS	FFC	BEFS	FST	PSC	CTF	FL/FD ratio	PDI (%)	SFW (g)	LWDS (g)	NDS
		Qualitative characters							Quantitative characters				
1	IC613457	1	2	1	3	2	0	1	2.97	74.5	268.04	19.04	6.39
2	IC595504	3	1	1	3	3	0	4	3.47	70.5	236.04	19.04	6.19
3	IC613458	4	1	99	3	3	0	5	3.85	48.5	303.04	16.04	5.99
4	IC595505	5	1	99	3	3	1	3	2.89	66.5	259.04	12.04	5.79
5	IC613459	5	2	2	3	3	0	5	2.08	80.5	180.04	12.04	6.19
6	IC612081	4	2	1	3	3	0	4	2.68	88.5	210.04	20.04	5.99
7	IC613461	3	2	99	3	3	0	3	2.95	78.5	256.04	9.04	4.19
8	IC613462	1	2	1	3	2	0	3	2.67	83.17	281.38	19.71	6.06
9	IC612082	3	2	1	3	2	0	3	2.16	89.17	234.38	11.71	4.26
10	IC613465	3	2	99	3	2	1	3	2.76	77.17	274.38	18.71	5.86
11	IC613466	3	1	1	3	3	1	3	3.58	79.17	273.38	20.71	5.46
12	IC613467	3	2	1	3	2	0	3	2.52	83.17	283.38	16.71	6.06
13	IC613488	3	2	1	3	2	0	3	2.59	63.17	292.38	13.71	6.21
14	IC595508A	4	2	1	3	3	0	3	2.20	59.17	176.38	11.71	4.39
15	IC613460	3	2	1	3	2	0	3	2.91	47.17	234.38	9.71	4.79
16	IC595510	4	1	99	3	3	0	4	4.21	69.17	282.38	16.71	5.59
17	IC618083	4	2	99	3	3	1	3	2.68	53.17	265.38	14.71	5.19
18	IC595512	4	2	1	3	3	0	3	3.06	65.17	168.38	20.71	5.79
19	IC595514	3	1	1	3	3	0	3	3.50	53.17	163.38	17.71	4.79
20	IC595515	4	2	1	3	3	0	3	1.85	55.17	273.38	13.71	5.99
21	IC595517	3	1	1	3	3	1	3	4.33	59.17	176.04	16.38	5.66
22	IC613471	3	2	1	5	2	0	4	2.77	69.17	304.04	15.38	6.06
23	IC613472	3	1	1	3	2	0	3	3.51	59.17	343.04	15.38	4.86
24	IC613473	3	1	1	3	3	0	3	3.89	41.17	282.04	20.38	5.46
25	IC624255	4	1	1	3	3	0	3	3.89	63.17	316.04	19.38	6.66
26	IC613475	1	1	1	3	3	0	4	4.00	55.17	223.04	12.38	4.66
27	IC613476	3	2	1	3	3	0	3	3.50	54.5	191.71	12.71	4.99
28	IC613477	4	1	1	3	3	0	3	3.16	22.5	219.71	9.71	5.39
29	IC613478	3	1	1	3	3	0	3	3.55	52.5	191.71	9.71	4.99
30	IC613479	4	1	1	3	3	0	3	3.84	34.5	269.71	4.71	3.99
31	IC613480	3	1	1	3	3	0	3	3.42	36.5	159.71	13.71	4.59
32	IC613481	3	1	1	3	3	0	3	3.42	48.5	238.71	8.71	4.79
33	IC613482	3	1	1	3	3	0	3	3.42	42.5	216.71	13.71	5.19
34	IC613483	4	1	1	3	3	1	3	3.66	30.5	205.38	16.04	4.66
35	IC613484	3	1	1	3	2	0	3	3.48	52.5	194.38	12.04	4.46
36	IC613485	3	1	1	3	3	0	3	3.08	62.5	225.38	10.04	4.66
37	IC613474	4	1	1	3	3	0	3	3.11	66.5	183.38	14.04	4.26
38	IC595518	1	2	1	3	3	0	1	2.73	18.5	321.38	17.04	4.46
39	IC618084A	4	1	1	3	3	0	3	3.35	62.5	238.38	12.04	3.66
40	IC331445	3	1	1	7	3	1	3	3.58	56.5	218.38	14.04	4.06
41	IC331627	4	3	2	3	1	0	3	1.20	61.17	32.97	8.38	6.06
42	IC277048	1	3	1	3	1	0	3	1.10	59.17	73.38	10.38	6.26
43	IC331619	3	2	99	3	1	0	3	1.58	45.17	81.38	9.38	6.26
44	IC541367	3	2	99	3	3	1	3	2.79	79.17	208.38	18.38	5.86
45	IC541391	4	1	2	3	3	0	3	4.00	71.17	174.38	19.38	5.26
46	IC469517	3	2	1	3	2	0	3	3.29	79.83	312.21	19.04	4.79
47	IC539818	4	1	1	3	2	1	3	3.50	43.83	261.71	15.04	4.19
48	IC277030	1	2	1	3	1	0	3	2.45	45.83	235.71	14.04	6.59
49	IC613470	3	1	1	3	3	0	3	2.69	75.83	198.71	11.04	4.19
50	IC202058-A	1	2	1	3	2	0	3	1.71	85.83	147.71	9.04	5.39
51	AAUC-2	3	1	1	3	3	0	3	3.57	69.5	241.25	15.38	5.45
52	Poinsette	5	1	1	3	2	0	4	3.17	72.75	241.63	14.88	5.13
53	Swarna Agethi	3	1	1	3	3	0	3	3.59	79.25	211.25	14.88	5.00

LS-Leaf shape; LPD-Leaf pubescence density; LM-Leaf margin; FSC-Fruit skin colour; FS-Fruit shape; FFC-Fruit flesh colour; SEFS- Stem end fruit shape; BEFS-Blossom end fruit shape; FST-Fruit skin texture; PSC-Presence of seed cavity; CTF-Colour of tender fruits at 3 DAA; PDI-Percent Disease Index; SFW-Single fruit weight, LWDS-Loss of weight during storage; NDS-Number of days of storage

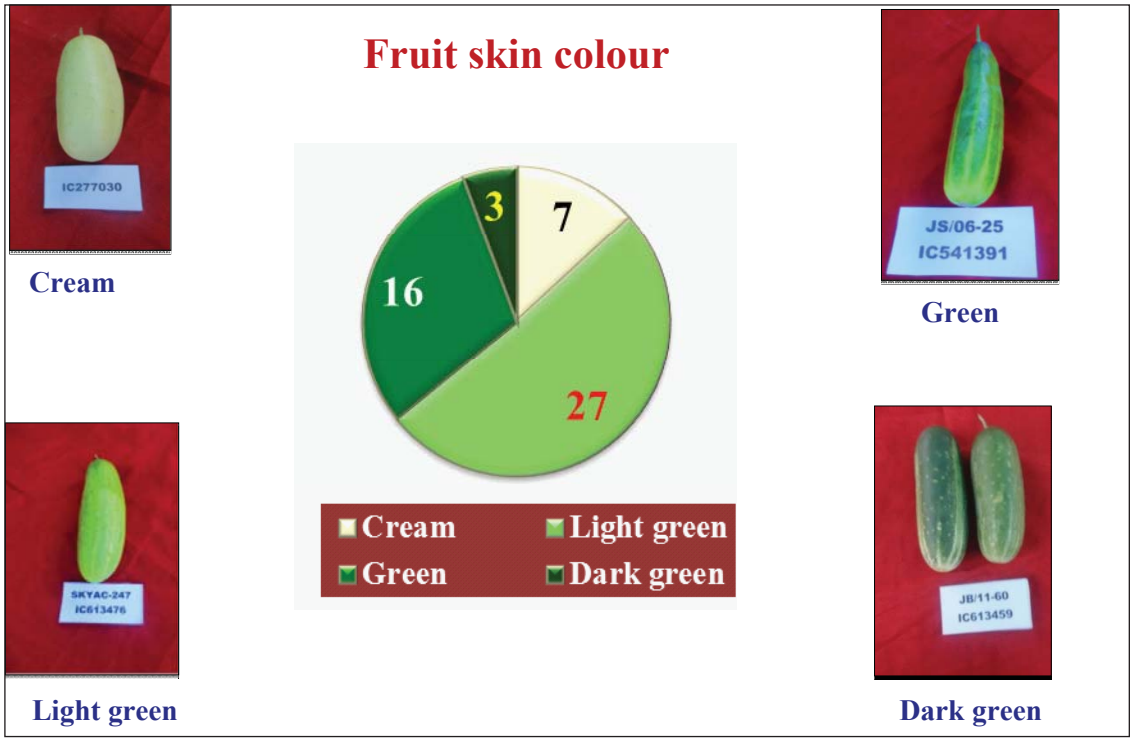


Fig. 1. Variability and frequency distribution of fruit skin colour

Ranjan *et al.* (2019). Liu *et al*. (2015) reported the desirability of white skinned cultivars of cucumber in the Gansu and Shandong province of China. Further, authors have observed that cream/ivory coloured slicing cucumber is the most preferred type in Hassan district and adjoining belt in Karnataka state of India. Cream coloured cucumbers will be a novel type, as most of the released varieties possess green to dark green skin colour. Hutchins (1940) reported that the fruit colour in cucumber is controlled by two genes *RC* versus *rc* with an inheritance ratio of 9 red fruit colour (*RC*): 3 orange (*Rc*): 3 yellow (*rC*): 1 cream (*rc*) colour. Similarly, white immature skin colour (*w*) was found to be recessive to the normal green (Cochran, 1938), and yellow green (*yg*) recessive to dark green and epistatic with light green (Youngner, 1952).

Fruit shapes of elliptical elongate, oblong ellipsoid and globular were observed. Elliptical elongate shape is the highly preferred one, as this shape gives maximum slices per fruit for salad purpose. Oblong ellipsoid shaped fruits were produced by 22 genotypes (41.05 per cent) whereas only two accessions (IC331627 and IC277048) exhibited globular/round shape (Fig. 2). Tiedjens (1928) in cucumber reported that environment has a profound effect on the shape and size of mature fruits in cucumber. Fruit length is also an important agronomic trait that affects yield and product appearance. It varied from 5 cm to 60 cm in different cucumber cultivars (Zhao *et al*, 2019). The length/diameter ratio (L/D) of pickling cucumber fruit is also important in the cucumber processing industry (Tolla, 1985). The length to diameter ratio of fruits determines the shape of the fruit. In the present study majority of the elliptical elongated fruits exhibited a length to breadth ratio greater than 3.00, the exception being IC613470 and IC595505. IC613470 produced comparatively small fruits with an average length of 12.67 cm, breadth of 4.71 cm and a length to diameter ratio of 2.69. Similarly, oblong ellipsoid fruits exhibited fruit length to breadth ratio of less than 3.00 except in IC613476 (3.50), IC469517 (3.29) and IC595512 (3.06). Single fruit weight ranged from 32.97 g (IC331627) to 343.04 g (IC613472). The fruit shape was round (length to breadth ratio: 1.20) in the former, while, it was elliptical elongate shape in the latter. Poinsette (241.63 g) recorded the highest value for the single fruit weight among checks. Fruit length, diameter and fruit weight are generally considered as

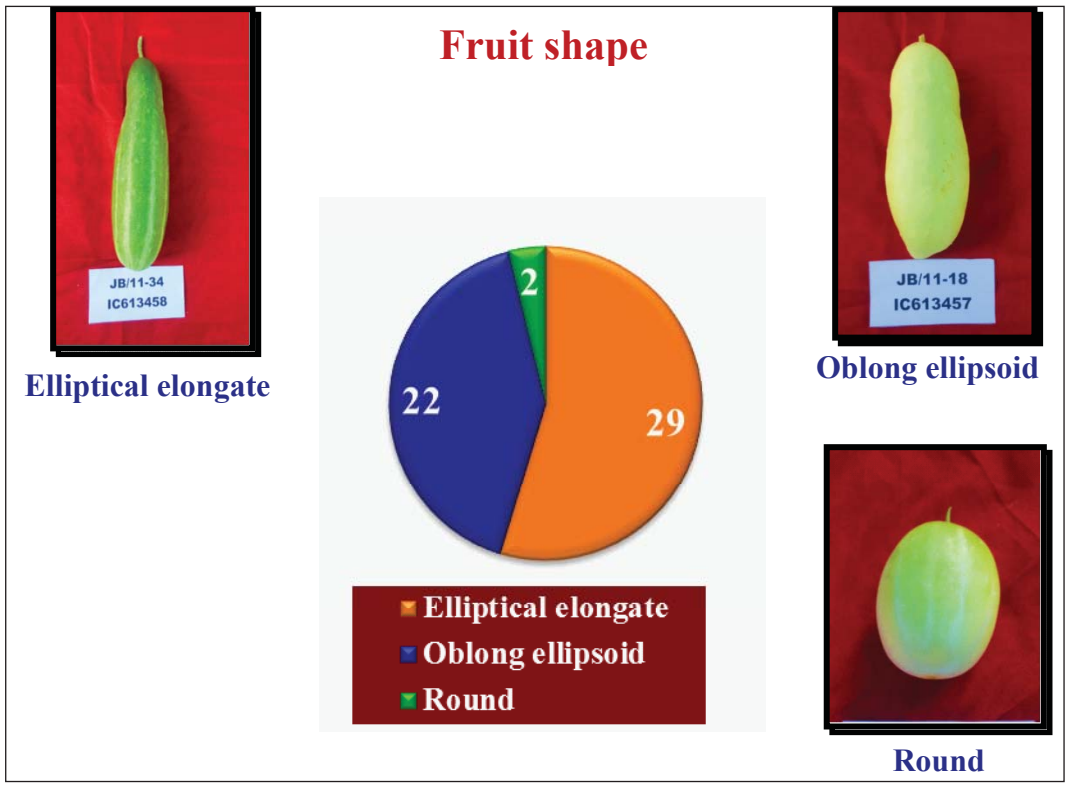


Fig. 2. Variability and frequency distribution of fruit shape

the most important yield contributing characters (Khan *et al*., 2015).

Forty two accessions (79%) registered white coloured flesh, whereas light green flesh was exhibited by eight (15%) accessions. Three genotypes possessed green fleshed fruits (IC331627, IC613459 and IC541391) (Fig. 3). Similar results of wide variability in flesh colour in cucumber (intense white, dingy white, light green, yellow to intense orange) were reported by Ranjan *et al*. (2019). Ten accessions namely IC595515, IC613467, IC613464, IC613471, IC613472, IC613461, IC595514, IC618084A, IC595508A and IC613463 exhibited orange coloured flesh of the fully ripe fruit, indicating the presence of carotenoids in the flesh (Fig. 4). Cochran (1938) reported that two alleles of a gene *viz*., cream (*W*) and white (*w*) control the flesh colour inheritance in cucumber. However, there are few reports on cucumber flesh colour at the molecular level. Shen (2009) identified three quantitative trait loci (QTLs) related to beta-carotene content (bCC4a, bCC4b, and bCC7) and two QTLs related to flesh colour (FCG5 and FCG7). Cucumbers generally lack the pigmentation of mesocarp (flesh) in contrast to other cucurbitaceous fruits. It was reported that a distinct group of cultivars namely Xishuangbanna gourd (*Cucumis sativus* var. *xishuangbannanesis*) from China is the source for high carotenoid content (Qi and Yuan, 1983) for introgression of β-carotene (orange flesh) genes to cucumber (Kumar *et al*., 2017). Lv *et al.* (2012) reported that Xishuangbanna gourd is closely related to cucumber germplasm of Indian origin. Moreover, Ranjan *et al.* (2019) reported the prevalence of diversity of orange fleshed cucumbers in North-eastern region of India. They observed that AZMC-1, a collection from Mizoram, India was found to contain total carotenoids content as high as 54.8 µg/g. These accessions with carotenoid content in the flesh of mature fruit have immense potential in nutraceutical preparations. Variation in skin colour, flesh colour and fruit shape was also reported by Pragathi (2014) in hybrid varieties of cucumber.

Smooth or plain skin is preferred over netted and rough skin of fruits and majority of the accessions exhibited fruits with tubercular skin texture. However, smooth skinned fruit texture was exhibited by four accessions (IC331627, IC277048, IC331619 and IC277030), netted by 14 and rough by 35 accessions (Fig. 5). The difference in skin texture of fruits was due to the persistence of tubercles or the remnant mass of tissues at the sites of tubercles. There are cultivars

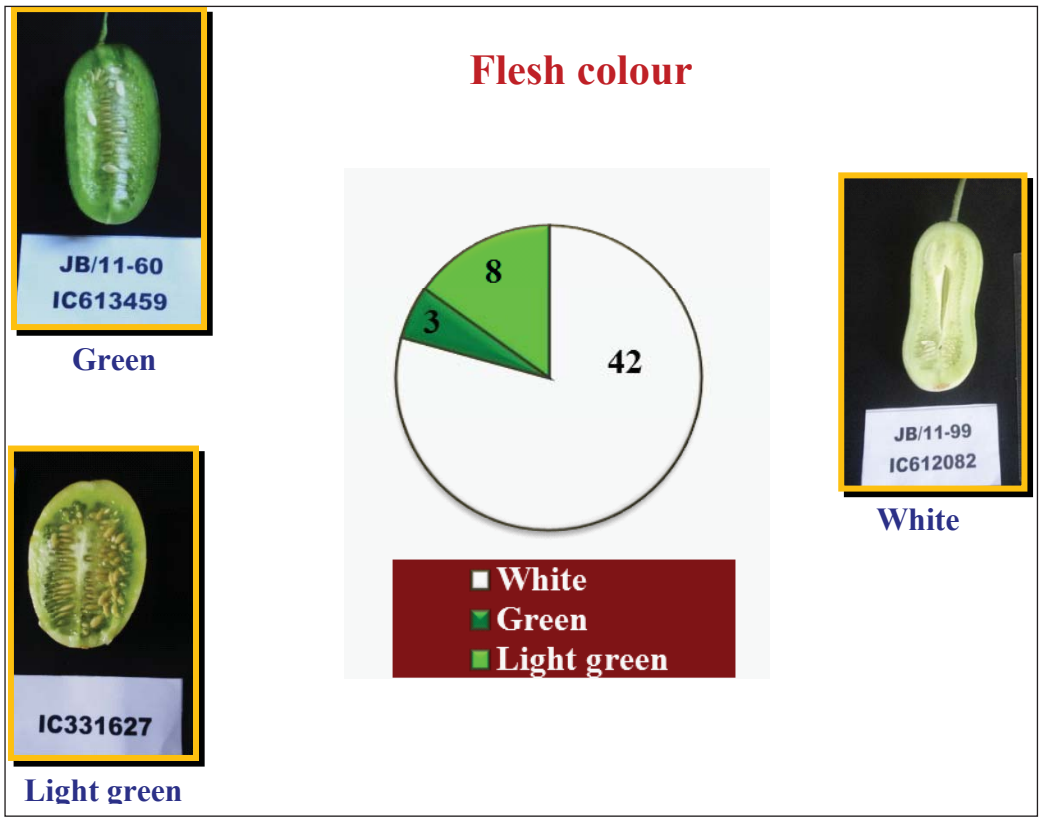


Fig. 3. Variability and frequency distribution of flesh colour

Fig. 4. Landraces with carotenoid content in flesh of ripe fruits

namely 'field cucumber' with prominent black or white spines and 'the greenhouse or forcing cucumber' also called 'English cucumber' with spineless fruit produced parthenocarpically as reported by Ranjan *et al*. (2019). Efforts should be directed to selecting fruits with smooth or plain or glossy skin texture. Thin and tender nature of the fruit skin contributes to its excellent eating quality but causes quick dehydration (Rubatzky and Yamaguchi, 1997). The accessions possessing plain fruit skin texture in the study were of bitter *Cucumis sativus* var. *hardwickii* types bearing globular fruits. Majority of the rough fruited types identified in the study were consumable

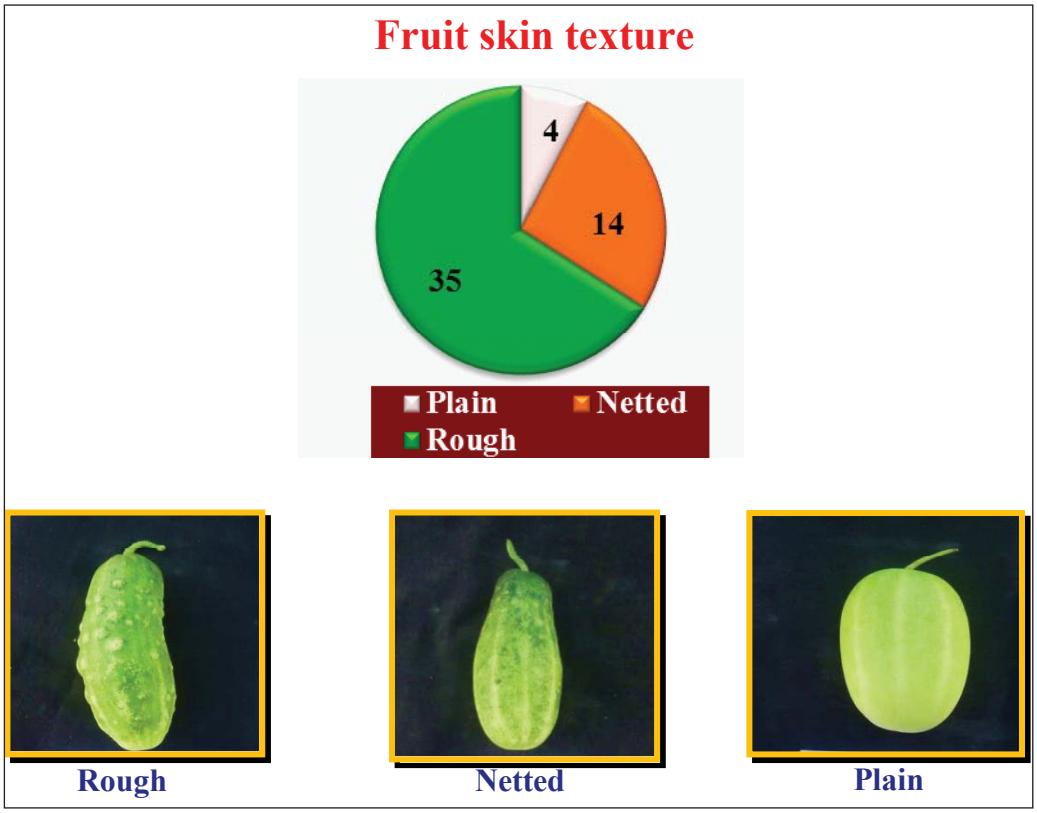


Fig. 5. Variability and frequency distribution of fruit skin

as the tubercles easily shed off during harvest. Rough and spiny fruit skin texture in cucumber genotypes were earlier reported by Zhang *et al.* (2012). In contrast to other qualitative traits, inheritance of characters related to fruit skin texture has been studied by many researchers. Those studies include the coarse spines (*F*) versus fine (*f*) nature of fruit (Hutchins, 1940), black (*B*) versus white (*r*) spines (Cochran, 1938; Hutchins, 1940; Tkachenko, 1935), few (*S*) versus numerous (*s*) spines (Hutchins, 1940), heavy netting (*H*) versus no netting (*h*) on fruits (Hutchins, 1940), spines (*A*) versus no spines (*a*) (Strong, 1931), mottled skin (*M*) versus self-coloured (*m*) (Strong, 1931), dull skin (*G*) versus glossy (*g*) (Strong, 1931), tough skin (*Te*) versus tender skin (*te*) (Strong, 1931), warty skin (*Tu*) versus non-warty (*tu*) (Strong, 1931) and smooth brown (*P*) versus rough yellow (*p*) fruit surface (Tkachenko, 1935).

Forty-four out of the 53 accessions (83%) lacked seed cavity in tender fruits while it was present in the remaining 9 accessions (17%). Seed cavity is not an appealing component as round slices without cavity is more preferred. However, it was noticed that the presence and absence of seed cavity were also influenced by the maturity stage of fruit at the time of harvest. Over maturing of fruits (by 2-3 days) led to development of seed cavity in some of the accessions.

There was wide variability in colour of tender fruits at 3 days after anthesis. The colour ranged from cream, light green, green to dark green. Two accessions *viz*., IC613457 and IC595518, possessed cream colour, whereas, it was green in six accessions *viz*., IC595504, IC612081, IC595510, IC613471, IC613475 and Poinsette and dark green in two accessions *viz*., IC613458 and IC613459. This trait was also influenced by environmental conditions prevailing during the growth period.

Storage studies are very important for maintenance of fresh and wholesome appearance of the fruits. After picking, cucumbers lose their quality gradually owing to the cell destruction and colour variations due to enzymatic reactions (Miano *et al*., 2016). The main deteriorating changes in cucumber during storage and distribution are yellowing of fruits, loss of moisture that leads to shrivelling and physiological injury caused by low temperature (Adamicki, 1985). Storage studies indicated that accessions differed significantly with respect to their storage potential in terms of loss of

weight owing to dehydration over storage as well as their period of marketability. Majority of the accessions developed shrivelled symptoms and discolouration at the stem end within 3-4 days during storage. Similar results were reported by Villata *et al.* (2003) in cucumber. The weight loss during storage ranged from 4.71 g (IC613479) to 20.71 g (IC613466). IC618084 recorded the least days of storage (3.66 days), with a weight loss of 12.04 g upon storage. On an average, the accessions could store up to 5.27 days with a mean weight loss of 14.34 g. It was observed that weight loss was not directly related to the number of days of storage. The accessions possessing less weight loss and more number of storage days are preferred. In the accessions studied, IC331627, IC613481, IC613461, IC202058A, IC331619, IC613460, IC613477 and IC613478 possessed good storability with weight loss of less than 10 g and storage days of 4-6 days.

Downy mildew is a major disease of cucumber in the open field as well as polyhouse conditions. Presently, very few cultivars are reported to be tolerant to this disease. In the present study, the incidence ranged from 18.50 per cent in IC595518 to 89.17 per cent in IC612082. Thirteen accessions recorded less than 50 per cent incidence. It is known that the multiplication and spread of fungus *Pseudoperonospora cubensis* causing downy mildew is highly dependent on the temperature and relative humidity prevailing in the area. As the crop was raised during monsoon season, chance of escape of disease infection was very less. Therefore, accessions that had exhibited tolerance reaction are to be screened further to confirm their tolerance to the pathogen infection.

Tender cucumbers are highly valued for their unique flavour as well as for the crispness of the flesh. Crispness is described as one of the most versatile single texture parameter universally liked by the consumers. In the present study, 29 accessions recorded less bitterness compared to the control variety Poinsette, which was the least bitter among the check varieties used (Table 4). Crispness was highest in the check variety Swarna Agethi. The fruits of three accessions were crispier than Swarna Agethi. The flavour was best expressed in fruits of AAUC-2 among check varieties and nine accessions recorded better values for flavour than AAUC-2. Based on the cumulative organoleptic score, IC613481 ranked first, followed by IC613480, both being collections from West Bengal. AAUC-2 was found to be the most organoleptically superior control variety registering a total score of 96.03. Four accessions namely IC613480, IC613481, IC618084A and IC613484 exhibited organoleptic qualities better than AAUC-2.

Principal component analysis was done using the six qualitative traits which were identified as variable among the accessions. The values of the Eigen-vectors and their contribution to variation are presented in Table 5. The five principal components accounted for 94.10 per cent of the total variance. The first three principal components having Eigen values more than 1.00 accounted for 76.40 per cent of variability. The first principal component (PC1) with an Eigen value of 2.084 corroborated for 34.70 per cent of total variance, and exhibited high contributing factor loadings from fruit skin colour (0.524), fruit skin texture (0.514), colour of tender fruits harvested at 3 days after anthesis (0.352), presence of seed cavity (0.253) and flesh colour (0.207), revealing the correlation of PC1 with these characters. Second principal component (PC2) with Eigen-value of 1.358 was contributed mainly by fruit shape (0.405), which contributed to 22.60 per cent of the total variation. The third principal component (PC3) accounted for 19.00 per cent of the total variation, with high contributions from colour of tender fruits harvested at 3 days after anthesis (0.701) and presence or absence of seed cavity (0.537). Loading of different variables based on the first two principal components (Fig. 6) indicated that fruit skin colour and texture contributed a greater proportion of the total variability. Similar results of fruit characters contributing significantly to the total divergence have also been reported by Manohar and Murthy (2011); Kumar *et al*. (2014) and Pal *et al*. (2018).

Conclusion

Attractive fruit skin colour like light green/green, uniformly long cylindrical fruits with crispy tender flesh, smooth surface without prominent spines, prickles and crook-neck are the primary objectives of breeding for the quality characters in cucumber (Kumar *et al*., 2017). As majority of the accessions studied were landraces, high variability was observed among them for most of the characters. Fruit skin colour, fruit shape, flesh colour, fruit skin texture and colour of the tender fruits at 3 days after anthesis, were a few of the most variable characters observed among the accessions studied. Sixteen accessions possessed elliptical elongate shape with light green fruit skin colour, whereas, 10 accessions possessed elliptical elongate fruits with green skin colour. IC613475 which possessed elliptical elongate shape

Table 4. Mean ranks recorded for organoleptic qualities in 53 cucumber landraces

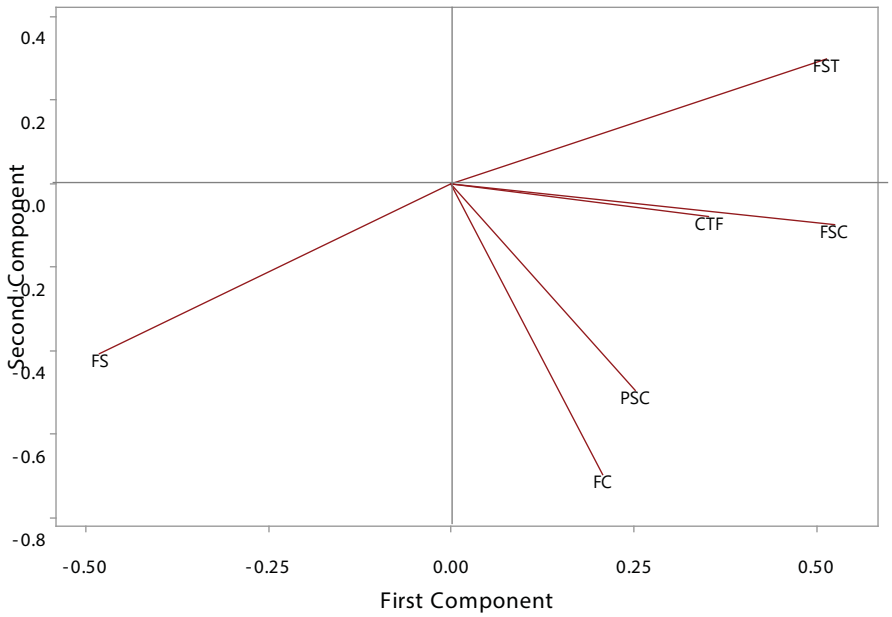
Accession No.	Bitterness	Crispness	Flavour	COS	Accession No.	Bitterness	Crispness	Flavour	COS
IC613457	26.03	27.54	23.68	77.25	IC613477	32.20	37.11	24.71	94.02
IC595504	30.07	39.21	25.43	94.71	IC613478	31.77	16.96	32.64	81.37
IC613458	30.93	19.50	25.04	75.47	IC613479	32.33	31.43	26.71	90.47
IC595505	29.67	25.00	24.43	79.10	IC613480	32.87	36.14	31.02	100.03
IC613459	3.97	18.07	18.93	40.97	IC613481	33.53	36.32	31.80	101.65
IC612081	25.10	14.86	27.50	67.46	IC613482	25.13	22.61	29.57	77.31
IC613461	25.00	13.32	31.79	70.11	IC613483	30.43	24.36	25.54	80.33
IC613462	30.27	25.68	26.43	82.38	IC613484	34.10	32.18	30.75	97.03
IC612082	28.50	18.61	25.93	73.04	IC613485	31.50	30.89	27.64	90.03
IC613465	26.97	8.32	30.29	65.58	IC613474	29.70	36.57	24.50	90.77
IC613466	27.97	12.61	32.32	72.90	IC595518	32.53	27.61	26.07	86.21
IC613467	30.57	26.07	25.00	81.64	IC618084A	28.17	42.18	28.93	99.28
IC613488	28.83	30.07	30.00	88.90	IC331445	33.07	34.46	26.29	93.82
IC595508A	28.20	29.04	26.89	84.13	IC331627	2.90	23.61	18.43	44.94
IC613460	29.87	15.14	25.57	70.58	IC277048	2.73	24.07	20.43	47.23
IC595510	32.70	32.96	24.07	89.73	IC331619	9.43	34.71	25.29	69.43
IC618083	29.47	23.14	28.86	81.47	IC541367	35.13	27.61	30.71	93.45
IC595512	33.53	27.50	33.96	94.99	IC541391	27.37	16.50	28.79	72.66
IC595514	15.40	8.57	34.64	58.61	IC469517	31.83	27.43	34.68	93.94
IC595515	29.90	26.07	25.75	81.72	IC539818	28.20	23.25	26.50	77.95
IC595517	30.60	33.68	22.11	86.39	IC277030	29.97	21.32	28.11	79.40
IC613471	29.90	41.21	23.64	94.75	IC613470	31.40	36.75	26.21	94.36
IC613472	27.47	22.93	24.18	74.58	IC202058A	3.53	16.79	21.79	42.11
IC613473	22.77	26.86	30.18	79.81	AAUC-2	27.43	38.14	30.46	96.03
SKYAC-239	29.70	24.71	23.86	78.27	Poinsette	29.13	30.89	29.71	89.73
IC613475	27.23	31.04	21.96	80.23	Swarna Agethi	15.07	39.57	25.32	79.96
IC613476	28.93	39.82	25.96	94.71	Minimum	2.73	8.32	18.43	40.97
					Maximum	35.13	42.18	34.68	101.65
COS: Cumulative organoleptic score Kendall's coefficient						**0.41**	**0.33**	**0.07**	

Table 5. Eigen value and eigen vectors of the first five principal components

Variables	PC1	PC2	PC3	PC4	PC5
Eigenvalue	2.084	1.358	1.140	0.566	0.497
Per cent variance	0.347	0.226	0.190	0.094	0.083
Cumulative variance (%)	0.347	0.574	0.764	0.858	0.941
Fruit skin colour	0.524	0.098	0.286	-0.502	0.546
Fruit shape	-0.480	0.405	0.254	-0.129	0.441
Flesh colour	0.207	-0.697	0.003	-0.625	0.112
Fruit skin texture	0.514	-0.300	0.270	-0.349	0.333
Presence of seed cavity	0.253	-0.495	0.537	0.468	0.343
Colour of tender fruits at 3 DAA	0.352	-0.079	0.701	-0.030	0.517

with cream coloured skin may be a promising genotype for breeders to develop white skinned varieties. Eight genotypes possessed good storability with weight loss of less than 10 g and storage days of 4-6 days. IC613481 and IC613480, collections from West Bengal ranked superior with respect to organoleptic scoring and other traits. IC613481 produced fruits with an average single fruit weight of 238 g, whereas it was 159 g in IC613480. The shelf life of fruits was more than 4 days with an average weight loss of 13.71g and 8.81g in IC613480 and IC613481, respectively. Both the accessions possessed an FL/FD ratio of 3.42 indicating the elliptical elongated fruit shape. Significantly high field tolerance to downy mildew disease was observed among the accessions. Furthermore, there is an urgent need to strengthen the orange fleshed cucumber germplasm, genome mapping for their utilization in breeding programme to develop carotenoid rich cucumber, which might play a significant role in contributing towards nutritional security (Ranjan *et al*, 2019)

Characterisation also allows identification of the most similar accessions that can be discarded by the breeders, this being the first step to the rationalization of the collection (Valcarcel *et al.*, 2018). Studies on genetic diversity analysis using the qualitative traits and their correlation with SSR markers have the



FSC-Fruit skin colour; FS-Fruit shape; FFC-Fruit flesh colour; FST-Fruit skin texture; PSC-Presence of seed cavity; CTF-Colour of tender fruits at 3 DAA

Fig. 6. Loading of different characters based on the first two principal components

potential to identify redundancy among the germplasm accessions (Yang *et al.*, 2015). Hence, a detailed study on inheritance of qualitative characters may be undertaken to identify unique morphological markers specific to various accessions. Further, molecular marker analysis to determine the genomic location via fine mapping, candidate gene analysis and dissection of closest flanking markers for marker assisted selection may help the breeders to investigate the genetic control of these traits. The genes from wild relatives namely *C. sativus* var. *hardwickii*, *C. setosus*, *C. hystrix* and *C. muriculatus* can also be exploited for cucumber improvement. Apart from the accessions included in this study, many high quality landraces still exist in the country, especially in North Eastern belt, which have been cultivated by farmers for centuries both for consumption and to meet the local market demand.

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RESEARCH ARTICLE

Phenotypic Variation in Black Pepper (*Piper nigrum* L.) Germplasm Grown under Arecanut Plantation in North East India

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The importance of local genotypes and landraces as a contributor to the gene pool of modern varieties with high economic value is well known. Though many varieties of black pepper were released for cultivation in different parts of India, only a few varieties are found suitable for North-East India, especially in the state of Assam where black pepper is grown as an important cash crop. This study was undertaken during 2013-2019 to evaluate 13 local black pepper genotypes for their growth, yield and yield attributes under Assam conditions of India. Significant differences were recorded among the accessions for most of the characters studied. Spike length varied from 9.57-13.45 cm. Compared to the popular check variety, 'Panniyur 1', the accession, IC-0599150 recorded 43.62% higher dry pepper yield indicating its superior yield potential. The result of this study indicates the importance of collection and characterization of local germplasm.

Key Words: Germplasm, Pepper, Yield, Yield attributes

Introduction

Pepper (*Piper nigrum* L.) is one of the important spices grown in India. In the major black pepper growing states of India, *viz*., Karnataka, Kerala and Assam, farmers are growing pepper as an intercrop in arecanut (*Areca catechu* L.) plantations (Thomas and Balasimha, 2011). In India, it is grown in an area of 1,38,929 ha with a production of 48,000 t (Spices Board, 2020). Kerala and Karnataka are the largest producers of black pepper in India accounting for 90% of the area and production (Tripathy *et al*., 2018). Indian pepper fetches a premium price in major international markets due to its preference and intrinsic qualities (Thomas, 2010). Black pepper, one of the oldest and most popular spices in the world, originated in the Western Ghats of India. Globally, it is cultivated in more than 25 countries in an area of 4,589,731 ha and total production of 523,000 t (IPC, 2017). Vietnam, Brazil, Indonesia, India, China, and Malaysia are the main black pepper growing and producing countries (FAOSTAT, 2019).

Black pepper is a perennial climbing vine grown mainly for its berries, an important ingredient used as spice and medicine (Anandaraj *et al*., 2013). In North East Region of India (NER), black pepper varieties 'Panniyur 1', 'Karimunda' and other local landraces are mostly grown in the farmers' field. Work on germplasm collection, conservation and characterization of black pepper has been carried out mainly in the southern states of India (Potty *et al*., 1979; Mathew *et al*., 1993). The main breeding objectives in black pepper are high yield, resistance to biotic and abiotic stresses, coupled with good quality traits. Therefore, germplasm exploration activities with local cultivars, wild relatives and related species followed by characterization could be useful for genetic improvement of black pepper (Prayogya *et al*., 2020). The ICAR-Indian Institute of Spices Research (IISR), at Kozhikode, Kerala, India maintains the world's largest collection of black pepper germplasm and related species collected from the centre of origin (Krishnamoorthy and Parthasarathy, 2009). Characterization and evaluation of the conserved germplasm for yield, quality and resistance to stresses has resulted in the release of promising landraces/cultivars as popular varieties (Sasikumar *et al*., 2004). Systematic research in India involving hybridization, clonal selection and open pollination from popular cultivars through the

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All India Coordinated Research Project (AICRP) on Spices and ICAR-IISR in the last four decades resulted in the release of superior lines of black pepper varieties (Thangaselvabal *et al*., 2008). Studies on assessment of released varieties of black pepper from NER has been carried out by Deka *et al*. (2016). Though, NER is delineated as suitable for black pepper cultivation in India, its production and productivity is constrained by the use of low yielding cultivars, non-availability of superior varieties, losses due to incidence of disease and pests, drought, non-adoption of suitable agronomic practices and lack of quality planting materials (Krishnamoorthy and Parthasarathy, 2009). So far, no systematic work has been done to enhance the varietal wealth of black pepper in NER, other than the released variety 'Panniyur 1' for intercrop in arecanut plantations prevalent in the region. To improve the varietal wealth for these regions and to develop production technologies that mitigate the risk of the black pepper producing farmers, work on collection, conservation, characterization of black pepper accessions has been initiated in the past few years by ICAR-Central Plantation Crops Research Institute (CPCRI), Research Centre, Kahikuchi, Guwahati, Assam. The present investigation was undertaken to evaluate the performance of 13 local black pepper accessions under arecanut plantations in the Kahikuchi farm along with 'Panniyur 1' as check variety for growth, yield and yield associated traits and to identify accessions suitable for cultivation in NER.

Materials and Methods

The present study was conducted for consecutive seven years from 2013 to 2019 at the research farm of ICAR-CPCRI, Research Centre, Kahikuchi, Guwahati, Assam, India. The research farm is situated at 20º18′ N latitude and 91º78′ E longitude with an altitude of 50 m above mean sea level. The mean maximum temperature varies from 15-32 ºC and the mean minimum temperature ranges between 8-22 ºC. The climate is sub-tropical with an annual rainfall of about 1,500 mm. The soil of the experimental site is alluvial clay loam with a pH range of 4.8-5.5. The study involved 13 black pepper collections bearing accessions number IC-0599138, IC-0599139, IC-0599140, IC-0599141, IC-0599142, IC-0599143, IC-0599144, IC-0599145, IC-0599146, IC-0599147, IC-0599148, IC-0599149 and IC-0599150 which are registered at ICAR-National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India. The 16 year old vines were collected from farmers' field of Kamrup district of Assam and supported on arecanut (*Areca catechu* L.) planted at a spacing of 2.7 m × 2.7 m. The experiment was laid out in Randomized Block Design (RBD) with three replications and under each replicate, 11 vines were selected for observations. Vegetative growth parameters like vine diameter and petiole length were recorded following the black pepper descriptors (IPGRI, 1995). A total of 50 spikes were taken randomly from each vine for recording spike length, peduncle length, length of berry and number of berries per spike. Number of spikes per lateral branch and the length of lateral branch were also recorded from individual vine. Number of spikes per kilogram and fresh yield was recorded from each vine at the time of harvesting during April to May. Dry berry recovery was recorded by drying the berries (one kilogram each) for one week from each vine. The dry recovery percentage was calculated using the formulae: dry weight / fresh weight × 100.

The mean values of all the characters on 13 accessions were subjected to statistical analysis. To estimate the association among the different characters observed, simple correlation coefficients were calculated (Panse and Sukhatme, 1961). Data were statistically analyzed using MSTATC software (Russel, 1995).

Results and Discussion

Vegetative growth characteristics

Vegetative growth parameters recorded for 13 accessions showed that vine diameter was found to be significantly different among the accessions varying between 75.00-119.32 cm (Table 1). Accession IC-0599145 collected from Panbari area of Kamrup district of Assam had the highest vine diameter (119.32 cm). Longer length of lateral branch and broader leaf area (104.63 m^2) in the accession IC-0599145 might have contributed to greater diameter of the vine. In North East region India, an experiment conducted at Horticultural Research Station, Assam Agricultural University, Kahikuchi, Guwahati, Assam, India, Deka *et al*. (2016) reported higher vine column diameter in the varieties 'Subhakara' and 'Karimunda' among 12 tested genotypes. Similarly, study conducted at ICAR-CPCRI, Kasaragod, on evaluation of 16 black pepper varieties and hybrids grown as mixed crop in coconut (*Cocos nucifera* L.) garden showed variation in vine column diameter after five years of planting, with higher vine column diameter in the variety 'Thevam' (Maheswarappa *et al*., 2012). Leaf area among

Table 1. Vegetative growth characters of black pepper accessions under Assam condition

Accessions	Vine diameter (cm)	Leaf area (m^2)	Petiole length (cm)	Length of lateral branch (cm)	No. of nodes/lateral branch
IC-0599138	75.00	66.91	1.67	37.91	21.83
IC-0599139	87.16	75.40	1.85	47.45	20.66
IC-0599140	91.67	76.71	1.76	45.67	18.33
IC-0599141	88.32	75.88	1.86	42.36	17.16
IC-0599142	93.33	72.34	1.88	44.20	18.83
IC-0599143	99.16	78.52	1.92	48.25	25.20
IC-0599144	111.67	76.42	1.97	43.70	15.50
IC-0599145	119.32	104.63	2.14	49.70	21.50
IC-0599146	97.50	83.92	1.90	46.67	25.32
IC-0599147	97.52	85.92	2.03	45.70	29.16
IC-0599148	101.67	75.45	1.86	44.95	17.83
IC-0599149	98.65	72.69	1.96	48.81	21.16
IC-0599150	93.30	83.58	2.01	47.38	32.67
Panniyur 1	97.64	71.98	2.05	42.80	32.65
CV (%)	15.54	19.24	10.57	17.28	12.68
SEm +/-	4.98	3.58	0.07	3.12	2.88
CD (0.05)	14.48	10.43	0.21	NS	8.38

the accessions varied from 66.91-104.63 m^2. Accession IC-0599145 also recorded the maximum leaf area (104.63 m^2) among the other accessions and were found to be much greater than the check variety 'Panniyur 1' Difference in growth characters, such as vine diameter and leaf area, among black pepper accessions might be attributed to various factors such as environment, soil moisture, availability of nutrients and genetic factor of the vine. The present study also showed the variation of petiole length from 1.67-2.14 cm. Three accessions (IC-0599145, IC-0599147 and IC-0599150) had petiole length of above 2.0 cm. Greatest petiole length was observed in accession IC-0599145 (2.14 cm) and was significantly greater than all the other accessions (Table 1). Similar finding was reported in Morogoro district in Tanzania by Shango *et al*. (2021) where they observed variation in petiole length of black pepper. Babu Kubwa types had significantly longer petiole (2.8 cm) than all other black pepper types grown in the district.

Spike characteristics

Number of spikes per lateral branch and fresh weight of 1,000 berries differ significantly among the accessions (Table 2). Accession IC-0599150 was found to produce higher number of spikes per lateral branch (17.83), much greater than 'Panniyur 1' (11.32). Bernawie *et al*. (2019) based on their study on black pepper variety Ciinten in three agro ecological conditions in Indonesia, reported differences in the number of spike/lateral branch, peduncle length as well as berry weight between locations, though the materials were clonally propagated from same mother vines. Accessions IC-0599145 and IC-0599150 produced the longest spike length and length of berry while the rest of the accessions were found to have shorter spikes, much smaller than 'Panniyur 1'. Among the black pepper landraces including 'Panniyur 1' evaluated in an experiment conducted in farmer's field of Uttar Kannada district in Karnataka, India, Naik *et al*. (2013) observed varying spike length, from 7.1-13.8 cm with 'Panniyur 1' (13.8 cm) producing longest spike length. Other yield associated traits, *viz*., number of berries per spike and number of spikes per kilogram had significant differences for the fresh and dry pepper yield (Table 2). Number of berries per spike was found to be significantly higher in accession IC-0599150 followed by IC-0599145 and the lesser number of spikes per kilogram can be attributed to more berries per spike. On the other hand, more spikes per kilogram was found in accession IC-0599148 followed by IC-0599144 which might be due to shorter spike length as compared to other accessions. Interestingly, the accession IC-0599147, with relatively more number of spikes per lateral branch, had the lowest number of berries per spike and recorded the least fresh as well as dry pepper yield. The accessions IC-0599150 and IC-0599145 had the highest fresh pepper yield with dry pepper yield increases of 43.62% and 34.89%, respectively over 'Panniyur 1'. Other accessions which

Table 2. Yield and yield associated traits of black pepper accessions under Assam condition

Accessions	No. of spikes/ lateral branch	Fresh weight of 1000 berries (g)	Spike length (cm)	Peduncle length (cm)	Length of berry portion (cm)	No. of berries /spike	No. of spike/kg	Fresh yield/ vine (kg)	Dry yield/ vine (kg)	% dry yield increase over check	% fresh yield increase over check	Dry recovery (%)
IC-0599138	12.00	100.00	10.91	1.51	9.49	56.07	226.06	5.20	1.76	18.12	5.26	33.84
IC-0599139	10.67	103.32	10.76	1.60	9.19	54.21	215.96	4.74	1.39	-6.71	-4.04	29.32
IC-0599140	9.33	93.33	11.26	1.55	9.72	59.45	233.18	5.19	1.70	14.09	5.06	32.75
IC-0599141	9.00	133.30	11.05	1.52	9.57	53.43	191.18	4.46	1.23	-17.44	-9.71	27.57
IC-0599142	9.83	96.67	10.85	1.44	9.32	57.37	223.28	3.94	0.99	-33.55	-20.24	25.12
IC-0599143	10.50	96.66	10.53	1.35	9.16	57.36	217.02	5.29	1.79	20.13	7.08	33.83
IC-0599144	9.34	96.65	9.57	1.26	8.31	50.63	242.67	4.35	1.17	-21.47	-11.94	26.89
IC-0599145	10.00	113.32	13.45	1.43	12.01	64.64	138.62	5.74	2.01	34.89	16.19	35.01
IC-0599146	12.16	96.64	10.08	1.25	8.74	55.80	230.37	3.95	1.25	-16.10	-20.04	31.64
IC-0599147	15.15	113.33	10.28	1.39	8.72	47.28	196.28	3.39	0.86	-42.28	-31.37	25.36
IC-0599148	9.50	103.30	9.58	1.27	8.35	52.22	340.20	3.50	1.08	-27.51	-29.14	30.85
IC-0599149	12.01	100.00	10.23	1.54	8.68	50.12	222.47	5.05	1.65	10.73	2.22	32.67
IC-0599150	17.83	110.00	12.97	1.37	11.59	80.69	160.33	6.02	2.14	43.62	21.86	35.54
Panniyur 1	11.32	133.33	12.85	1.50	11.33	52.58	149.69	4.94	1.49			
CV (%)	13.52	19.28	10.46	10.24	17.32	14.65	18.43	34.28	11.36			
SEm +/-	1.05	3.43	0.39	0.08	0.36	2.90	4.93	0.33	0.10			
CD (0.05)	3.07	9.97	1.14	0.18	1.06	8.46	14.39	0.98	0.32			

showed more modest increases in dry yield over check variety, are IC-0599138 (18.12%), IC-0599140 (14.09%), IC-0599143 (20.13%) and IC-0599149 (10.73%). Variation in fresh and dry yield among black pepper accessions were also reported by Kurian *et al*. (2002) and Kumar *et al*. (2003). In Indonesia, Bernawie *et al*. (2019) reported variation in yield of black pepper over the locations and seasons, which could be attributed to differences in agro-ecological condition.

Correlation studies

Correlation studies (Table 3) revealed that, fresh and dry yields were positively and significantly correlated with number of berries per spikes, number of spike per lateral branch, length of spikes and length of berry bearing portion, and will simplify selection methods to improve black pepper yield. The lack of significant correlations concerning vine diameter, length of lateral branch and number of nodes per lateral branch means selection methods to improve yield may ignore these traits to save the time and resources of the breeder. Sujatha and Namboothiri (1995) reported positive and significant influence of spike length on black pepper yield. Further, since fresh berry yield showed high positive significant correlation with dry yield, it can be considered for evaluation to save time and resources of

Table 3. Correlation among yield and yield associated traits of black pepper accessions/variety under Assam condition

	Vine diameter (cm)	Length of lateral branch (cm)	No. of spike/ lateral branch	No. of nodes/ lateral branch	Spike length (cm)	Length of berry bearing portion (cm)	No. of berries/ spike	Fresh yield/ vine (kg)	Dry yield/vine (kg)
Vine diameter (cm)		.409	-.180	-.070	.125	.160	.009	-.006	.017
Length of lateral branch (cm)			.170	.139	.032	.019	.201	.155	.190
No. of spike /lateral branch				.787**	.677**	.775**	.607**	.703**	.767**
No. of nodes /lateral branch					.506	.501	.358	.256	.298
Spike length (cm)						.996**	.669**	.708**	.663**
Length of berry bearing portion (cm)							.698**	.713**	.679**
No. of berries/spike								.690**	.723**
Fresh yield/vine (kg)									.968**

black pepper breeders. Ibrahim *et al*. (1985), Thanuja and Rajendran (2003), Preethy *et al*. (2018), Shivakumar and Saji (2019) and Shivakumar *et al*. (2020) also reported significant and positive correlation among fresh and dry berry weight. The results of this study indicated the presence of wide variations for important traits in the local black pepper populations in farmers' fields and importance of collection, conservation, characterization of local germplasm resources for utilization in the development of high yielding black pepper lines for cultivation in NER.

Review of black pepper as an excellent mix crop in arecanut farms has been done (Abraham, 1974; Thomas and Balasimha, 2011). Many authors have also emphasized the importance of black pepper as a mixed crop in coconut for increase in productivity and profitability of the mixed cropping system and also complementary effect of black pepper as a mixed crop towards the improvement of yield in the coconut (Maheswarappa *et al.,* 2012; Subramanian *et al*., 2016). Arecanut is grown in most of the states of NER and is an important crop due to its commercial value and food habit of Indians. Among the NER, arecanut is grown in an area of 81,000 ha in Assam alone (DASD, 2019). Other states of importance for cultivation of arecanut are Meghalaya, Mizoram and Tripura. Similarly, coconut is grown in an area of 19,917 ha in Assam followed by Tripura (5.201 ha) (CDB, 2018). So, there is ample scope to use arecanut and coconut plantations for mixed cropping with black pepper for increasing productivity and profitability per unit area. Hence, the implication of this study has far reaching consequences in improving livelihood security and enhancing profitability of the predominantly tribal communities in the agrarian scenario of NER, through cultivation of improved black pepper varieties suitable to the region.

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RESEARCH ARTICLE

Evaluation of French Bean Genotypes with Slow Rusting Components Against *Uromyces appendiculatus*

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In this study, 66 genotypes of French bean were screened against bean rust under glasshouse and field conditions, of which, 11 genotypes with different degree of resistance/susceptibility were selected for measuring the slow rusting components. Both, incubation period and latent period were significantly and negatively correlated with infection frequency, disease severity and area under disease progress curve (AUDPC). Infection frequency was significantly and positively correlated with disease severity and AUDPC. The AUDPC was also significantly and positively correlated with disease severity while negatively correlated with uredium size. Factor analysis calculated using principal components revealed that factor I and II explained 65.97 and 15.45 per cent of the total variance, respectively. On the basis of scoring, it could be inferred that the five germplasm accessions *viz*., EC400406, EC755318, EC405210, EC400442 and EC400390 having low and negative factor I scores demonstrated slow rusting characteristics *i.e*., longer incubation and latent periods, lower infection frequency, smaller uredia and less disease severity against bean rust. These germplasm lines might have slow rusting genes and therefore, can be effectively used in breeding programs for developing rust resistant cultivars.

Key Words: AUDPC, Infection frequency, Latent period, *Phaseolus vulgaris*, Rust, Slow rusting resistance

Introduction

Rust (*Uromyces appendiculatus*) is a serious disease of French bean (*Phaseolus vulgaris* L.) throughout the world that causes significant yield reduction due to premature defoliation of the infected plants (Gupta *et al*., 2008; Liebenberg and Pretorius, 2010). The disease usually appears in Himachal Pradesh during the last week of July to the second week of August when crop is in the flowering or pod formation stage. This period usually coincides with slightly warmer and humid weather, which favours growth, reproduction and spread of rust pathogen. Losses in green pods ranging from 4.7 to 69 per cent have been reported due to this pathogen (Devi *et al*., 2019). Though this disease can be kept in check with the application of fungicides, but these have some limitations like environmental pollution, residual effect, high cost of application and health hazards. Bean rust can be effectively controlled by deploying resistant varieties, however the resistance does not remain effective for too long especially if it is conferred by major genes. Major gene resistance breakdown is a frequent phenomenon because plant pathogens such as *Uromyces appendiculatus* evolve into new and more virulent isolates through mutation or sexual recombination (Stavely 1984).

Slow rusting is an effective way to achieve durable rust resistance and appears to be race non-specific and more durable than major gene resistance (Ohm and Shaner, 1976). Slow rusting retards disease development in the field even when the infection type appears susceptible. Most of the work in slow rusting has been carried out in wheat where it has been found associated with a longer latent period, reduced pustule (uredium) size, and fewer uredia per unit area.

Therefore, this study was carried out with an objective to determine the mechanism of slow rusting resistance, to ascertain the role of different components of slow rusting in French bean and to investigate the

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relationship of these components to each other and to the rust severity.

Materials and Methods

Selection of genotypes for slow rusting

Sixty six French bean genotypes were evaluated under glasshouse as well as natural epiphytotic conditions for two consecutive crop seasons, *i.e.,* 2015 and 2016. Out of these, ten genotypes *viz.,* EC400390, EC400406, EC400408, EC400411, EC400435, EC400442, EC405210, EC405224, EC755318 and EC283179, each having a different degree of resistance/ susceptibility were selected based on disease reaction under field conditions along with highly susceptible cv. 'Falguni' as check.

Pathogen selection and inoculum multiplication

Uromyces appendiculatus isolate collected from Nauni, Solan (Himachal Pradesh, India) was used as test pathogen and mass multiplied on susceptible variety Falguni. The plants of Falguni were raised in pots (12.5 cm diameter) filled with sterilized mixture of fine loam and farmyard manure (3:1). After 15 days of sowing, plants were inoculated with urediniospores of *U. appendiculatus* by leaf coating method. The inoculated plants were sprayed with fine mist of water and incubated in saturated humidity chambers for 24 h, then shifted to the glasshouse at 20±2°C. Fifteen days post inoculation (dpi) urediniospores were collected and stored at -20°C for further studies.

Raising of genotypes for inoculation

To evaluate the components of slow rusting resistance, plants of different French bean genotypes were raised in separate plastic pots as mentioned above. In each pot, 6-8 seeds of each genotype were sown and after seedling emergence three plants per pot were maintained. For inoculation, urediniospores of *U. appendiculatus* were suspended in Tween 20 (0.05%, v/v) at a concentration of 2.0×10^4 urediniospores/ml. Inoculation was done by spraying urediniospores suspension with an atomizer on both sides of the leaves. After inoculation, fine mist of water was sprayed on these plants with pneumatic hand sprayer (Aspee Agro Equipment Pvt. Ltd.) to provide leaf wetness. The inoculated plants were then kept in humid chamber to provide high humidity and leaf wetness for 24 h and then shifted to glasshouse benches at ambient temperature (20±2°C) and observed regularly for the appearance of the symptoms.

Measurement of slow rusting components and analysis

Incubation and Latent period: Incubation period (number of days from inoculation to the first appearance of symptoms) was recorded as described by Van der Plank (1963). Number of days from inoculation to the appearance of 50% of the uredia, were recorded as latent period (Habtu and Zadoks, 1995).

Infection frequency: The number of uredia/cm^2 that appears on the leaf area after uniform inoculation is called infection frequency (Ohm and Shaner, 1976). The number of uredia /cm^2 were counted at the end of latent period by making 1 cm^2 slit on luggage tag. The one cm^2 slit was then placed on basal, middle and end of the leaves and no. of uredia in the slit were counted (Singh, 1985).

Uredium size: The size of 10 randomly selected uredia was measured with the help of stage and ocular micrometer. The ocular scale (a glass disc engraved in 0.01 mm scale and fitted in the eye piece lens) was focused after superimposing it on the stage micrometer scale under stereo-zoom microscope. The number of ocular micrometer divisions coinciding with the fixed number of stage micrometer scale was recorded. The value of each division in the eye piece was calculated as per the formula given by Birchen (1963). The ocular scale was calibrated at 4.5 × 15X magnification and all the measurements were made by taking length and width (mm). The area of the uredium (mm^2) was calculated as the product of π (Pi) ×½ length ×½ width as adopted by Kochman and Brown (1975).

Disease severity (%): Rust severity was estimated 14 times with the aid of modified 0-5 scale of Stavely (1983) as 0 = no disease, 1 = 0.1–5%, 2 = 5.1–10.0%, 3 = 10.1–25.0%, 4 = 25.1–40% and 5 = > 40% leaf area covered with rust uredia and per cent disease severity was calculated according to formula proposed by McKinney (1923).

$$\text{Disease severity (\%)} = \frac{\text{Sum of all disease ratings}}{\text{Total number of ratings} \times \text{Maximum disease grade}} \times 100$$

Area under disease progress curve (AUDPC): The area under disease progress curve was calculated from disease severity as proposed by Shaner and Finney (1977).

$$AUDPC = {}^{n}\sum_{i=1} [(Y_{i+n1} + Y_i)/2] [X_{i+1} - X_i]$$

Where,

Yi = disease severity (per unit) at i^{th} observation,

Xi = time (days) at i^{th} observation, and

n = total number of observation

Apparent infection rate (r): The apparent infection rate (r) was calculated by the formula given by Van der Plank (1963).

$$r = \frac{2.3}{t_2 - t_1} \log \left\{ \frac{X_2}{1 - X_2} - \log \frac{X_1}{1 - X_1} \right\}$$

Where, r = apparent infection rate per unit per day, t_1 = first date of recording disease intensity, t_2 = second date of recording disease intensity, X_1 = disease intensity at time t_1, X_2 = disease intensity at time t_2, 2.3 = constant value

Data analysis. The data were analysed using the XLSTAT software to perform simple correlation, multiple regression and Factor analysis. Factor analysis using principal component utilizing correlation matrix was used to estimate the factor loadings. Eigenvalue >1 are considered for interpretation and indicate the proportion of total variance explained by each principal component (Sharma, 1996).

Results

Incubation period (IP) and Latent period (LP)

Incubation and latent periods varied significantly between in slow rusting and fast rusting French bean genotypes (Table 1). IP ranged from 168 h in Falguni to 196 h in EC400442. The genotypes *viz.,* EC400390, EC400406, EC755318 and EC405210 have shown longer incubation period of 175 to 185 h against fast rusting lines. LP ranged from 216 h (Falguni) to 288 h (EC400442) and the genotypes *viz.,* EC400390, EC400406, EC405210 and EC755318 showed longer latent period of 240, 252, 245 and 252 h, respectively.

Infection frequency

Of the eleven genotypes studied, number of uredia was minimum on the line EC755318 (4.0/cm^2) followed by EC400406 EC400442 and EC400390 with 6.33, 8.0 and 12.0 per cm^2, respectively, which differed significantly from other genotypes. The maximum infection frequency (63.67/cm^2) were recorded on Falguni (Table 1, Fig.1).

Uredium size

Size of uredia varied from 0.03 mm^2 in EC400442 to 1.53 mm^2 in EC755318. The genotypes *viz.,* EC400442, EC400411, EC400406, EC405224, EC400435 and EC400390 showed much smaller sized uredia ranging from 0.03 to 0.16 mm^2 (Table 1, Fig.1).

Area under disease progress curve (AUDPC) and apparent infection rate (r)

The data (Table 1) pertaining to area under disease progress curve and apparent infection rate (r) revealed that the values of both were minimum in the line EC400442 followed by EC400406, EC755318, EC405210 and EC400411 while the values were maximum in Falguni.

Disease severity (%)

Minimum disease severity was recorded in the genotype EC400442 (4.62%) followed by EC400406 and EC755318 each with 6.90 per cent, whereas, disease severity was maximum in Falguni (70.19%) followed by genotype EC400408 (68.82%) which were statistically at par with each other (Table 1).

Correlation and regression between components of slow rusting

The correlation coefficients between slow rusting components indicated that the incubation period (IP) was significantly and positively correlated with latent period (LP). Both IP and LP were significantly and negatively correlated with infection frequency, AUDPC and disease severity (Table 2). Infection frequency was significantly and positively correlated with AUDPC and disease severity. AUDPC was significantly and positively correlated with disease severity while negatively correlated with uredium size.

The multiple regression equations were also computed with disease severity as the dependent variable, and each of the components as independent variables.

$$Y = 313.31 - 1.81 X_1 + 0.13 X_2 + 0.63 X_3 - 5.64 X_4$$

Where, Y = Disease severity; X_1 = Incubation period; X_2 = Latent period; X_3 = Infection frequency; X_4 = Uredium size.

The coefficient of determination (R^2=0.905) between disease severity and group of independent variables indicated that 90.00 per cent bean rust severity was due to these variables, whereas rest of the variation was due to unexplained (error variation) factors and/or the factors not included in the investigation.

Factor analysis calculated using principal components

Table 1. Components of slow rusting resistance to *U. appendiculatus* in different genotypes of French bean

Genotypes	Incubation period (h)	Latent period (h)	Infection frequency (uredia/cm^2)	Uredium size (mm^2)	AUDPC	Apparent infection rate	Disease severity (%)
***Slow rusting*:**							
EC400406	184	252	6.33	0.08	176.50	0.069	6.90 (15.19)
EC755318	182	252	4.00	1.53	176.50	0.069	6.90 (15.20)
EC405210	185	245	16.33	0.27	434.88	0.089	22.42 (28.24)
EC400442	196	288	8.00	0.03	106.94	0.050	4.62 (12.34)
EC400390	175	240	12.00	0.16	817.74	0.101	38.86 (38.55)
***Fast rusting*:**							
EC400408	173	223	46.33	0.42	1477.71	0.130	68.82 (56.05)
EC400411	186	235	38.33	0.05	518.08	0.091	23.77 (29.15)
EC400435	175	225	42.67	0.12	1014.10	0.106	44.23 (41.67)
EC405224	175	225	48.67	0.10	1341.42	0.125	62.23 (52.06)
EC283179	185	235	22.00	0.29	624.92	0.098	29.48 (32.87)
Falguni	168	216	63.67	0.22	1655.36	0.134	70.19 (56.90)
LSD $_{0.05}$	(1.85)	(3.04)	(5.25)	(0.01)			(2.31)
±SE(m)	0.63	1.03	1.78	0.003			1.06

Figures in parentheses are arc sine transformed values

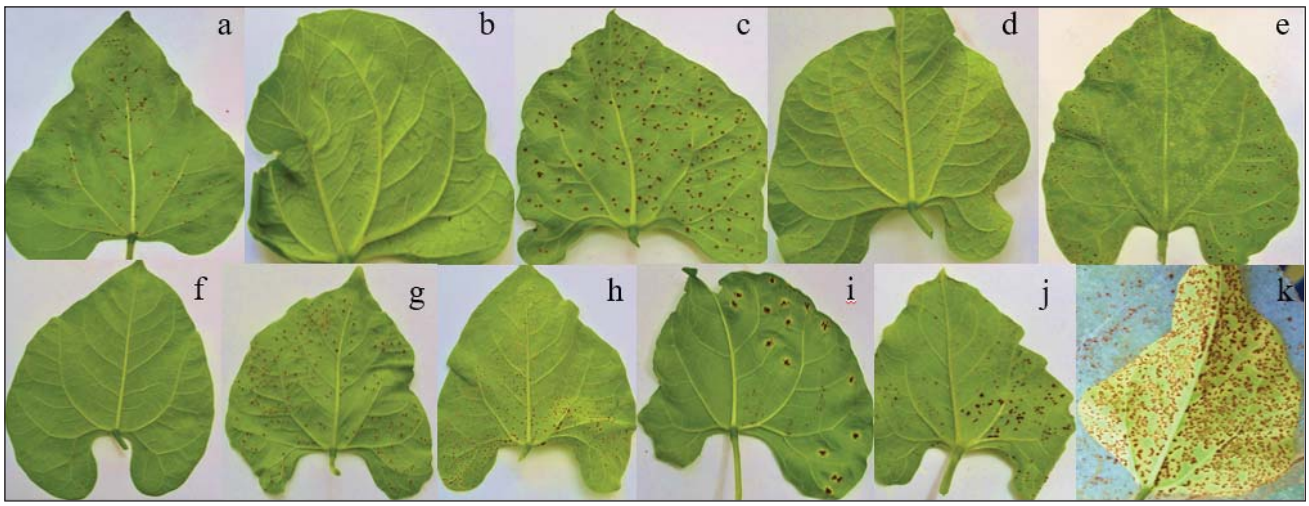

Fig. 1. leaves of genotypes, a) EC400390, b) EC400406, c) EC400408, d) EC400411, e) EC400435, f) EC400442, g) EC405210, h) EC405224, i) EC755318, j) EC283179 and k) Falguni, showing infection frequency

Table 2. Correlation coefficients among the components of slow rusting resistance in French bean genotypes

Variables	IP	LP	IF	US	AUDPC	r	DS
IP	1.00						
LP	0.86*	1.00					
IF	-0.69*	-0.80*	1.00				
US	-0.05	0.06	-0.30	1.00			
AUDPC	-0.87*	-0.83*	0.90*	-0.21	1.00		
r	-0.36	-0.09	0.19	-0.10	0.50	1.00	
DS	-0.85*	-0.83*	0.88*	-0.22	0.99*	0.52*	1.00

IP- Incubation period (h); LP- Latent period (h); IF- Infection frequency (No. of pustules/cm^2); US- Uredium size (mm^2); AUDPC- Area under disease progress curve; r-Apparent infection rate; DS- Disease severity (%)
*Significant at P≤ 0.05

revealed that factor I and II, each having eigenvalue greater than unity, explained 65.97 and 15.45 per cent of the total variance, respectively (Table 3). Factor I represented differences between average effects of infection frequency, AUDPC and disease severity which were interpreted as fast rusting characters. Incubation period and latent period which showed high and negative weights for factor I were interpreted as slow rusting characters to pathogen reproduction. Factor II represented differences for apparent infection rate and latent period which were interpreted as fast rusting characters. These two factors together accounted for 81.41 per cent of the total variance.

Table 3. Scores, eigen value and cumulative variance of the two major factors obtained from the PCA of slow rusting components

Variables	Factor I	Factor II
Incubation period	-0.900	0.068
Latent period	-0.879	0.362
Infection frequency	0.899	-0.206
Uredium size	-0.178	-0.076
Area under disease progress curve	0.995	0.087
Apparent infection rate	0.434	0.812
Disease Severity	0.991	0.114
Eigenvalue	4.617	1.081
Per cent variance	65.97	15.45
Cumulative (%)	65.97	81.41

Scoring of genotypes based on factor analysis

On the basis of scoring, it could be inferred that the germplasm lines *viz*., EC755318, EC400442, EC400406, EC400390 and EC405210 having negative values of Factor I scores possessed slow rusting characters while the genotypes *viz*. Falguni, EC400408, EC405224, EC400435, and EC283179 had positive and high factor I score values. The interpretation of factor II was not clear so it was not found very useful in differentiating between the genotypes (Table 4).

Thus only five French bean germplasm lines *viz*., EC755318, EC400442, EC400406, EC400390 and EC405210 demonstrated slow rusting characteristics *i.e.,* longer incubation and latent period, lower infection frequency, smaller uredia and less disease severity, therefore, these lines might have slow rusting genes.

Discussion

In the present study, slow rusting French bean germplasm lines showed longer incubation and latent periods than

Table 4. Scoring of genotypes based on factor analysis

Genotypes	Factor I	Factor II
Slow rusting:		
EC400406	-0.306	0.186
EC755318	-0.289	0.488
EC405210	-0.245	-0.593
EC400442	-0.238	-0.238
EC400390	-0.136	-0.178
Fast rusting:		
EC400408	1.115	-0.013
EC400411	0.066	-0.020
EC400435	0.320	0.264
EC405224	0.313	-0.011
EC283179	0.332	-0.213
Falguni	1.420	0.412

fast rusting lines. Similarly Habtu and Zadoks (1995) and Hegde (2001) reported extended latent period from 9.40 to 18.60 days and 9.17 to 16.22 days in bean and soybean genotypes, respectively and opined that the extended latent period played a major role in partial resistance.

Infection frequency, another important component of slow rusting, indicated that 11 genotypes differed in their ability to produce number of uredia/cm^2 leaf area. The germplasm lines with longer incubation and latent period exhibited uredial density of 4.00 to 16.33/cm^2 of leaf area, whereas the genotypes with shorter incubation and latent period had higher number of uredia (22.00 to 63.67/cm^2) which in turn reflected in high AUDPC and disease severity. Statler and McKey (1987) and Statler and Grafton (1988) recorded fewer number of uredia in resistant cultivars as compared to susceptible ones among French bean cultivars. Similarly lesser uredia/cm^2 in resistant bean cultivars against *U. appendiculatus* have been reported by Shaik (1985) and Habtu and Zadoks (1995), which is an important component of slow rusting mechanism.

Smaller sized uredia were noticed in slow rusting cultivars as compared to fast rusting cultivars except in germplasm line EC-755318 which was found to have largest sized uredia. Similar results were obtained with bean rust (Habtu and Zadoks, 1995) and leaf and stem rusts of wheat (Mabrouk *et al*., 2019).

In our studies, lowest AUDPC value (106.94) was recorded for the genotype EC-400442 and the maximum (1655.36) for Falguni. Differences in AUDPC have successfully been used to evaluate cultivars of wheat (Mabrouk *et al*., 2019) and soybean against rust (Hegde,

2001). AUDPC has been reported a more reliable criterion for quantifying slow rusting in wheat by Luthra *et al.* (1991). We observed that apparent infection rate was of little value in determining relative levels of slow rusting ability. Patil (1996) pointed out that "r" values were not as useful as AUDPC values, hence, 'r' should be coupled with AUDPC values to arrive at better conclusion in the mechanisms of slow rusting.

The genotypes *viz.*, EC400442, EC400406, EC755318, EC405210 and EC400390 showed lower disease severity, hence, these may be carrying slow rusting resistance. Van der Plank (1963) suggested the importance of disease severity in measuring the slow rusting mechanism in compound interest diseases like rusts. Rashid and Bernier (1991) recorded lower disease severity in the slow rusting cultivars of faba bean which had varied degree of tolerance.

Correlation between disease severity with the infection frequency, AUDPC and apparent infection rate was significant and positive whereas it was strong and negative with latent period. Similar strong correlations were also reported by Safavi *et al*. (2010) for wheat rust.

On the basis of factor analysis, two factors were extracted, where factor I represented the differences between average effects of disease severity, infection frequency, uredium size, infection rate and AUDPC. The factor I had high and negative weights for incubation period and latent period which were interpreted as slow rusting components. Scoring on the basis of this analysis revealed that germplasm lines namely, EC400442, EC400406, EC755318, EC405210 and EC400390 had low and negative scoring as compared to fast rusting genotypes. Similar scoring of genotypes on the basis of principal component analysis has been reported by Sandhu and Gupta (2001) in *Brassica* spp.

Conclusion

This study presents a preliminary report on the genotypic differences observed for the slow rusting components in 11 French bean genotypes. Based on the values of slow rusting components and their univariate analysis for different genotypes, it can be concluded that the French bean germplasm lines, *viz*., EC400442, EC400406, EC755318, EC405210 and EC400390 possessed slow rusting characters and can be effectively used in breeding programs for developing rust resistant cultivars. The genotypes exhibited variations in the level of slow rusting resistance which further suggests that there could be diversity in the number of genes involved and/or their effect in conferring the slow rust resistance against *Uromyces appendiculatus* in these genotypes.

Conflict of Interest: The authors declare that they have no conflict of interest.

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RESEARCH ARTICLE

Genetic Parameters, Diversity and Population Structure in Tomato based on Quantitative Traits and Microsatellite Markers

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Fifty accessions of tomato, including related species were evaluated to study genetics for yield and quality attributes and diversity based on quantitative traits and molecular markers. The results have shown high heritability and genetic advance for the number of fruits per plant (96.14 and 193.5), fruit weight (87.61 and 96.70), yield per plant (95.45 and 78.90) and lycopene content (98.31and 43.44) which indicated that these traits based selection is adequate. Among the accessions, extensive genetic diversity was observed for both quantitative traits and molecular analysis. From cluster means, accession Khasi Local was superior for fruit weight (80.30 g), LE-1-2 for lycopene (12.23 mg/100g) and *S. peruvianum* (1) for TSS (6.17) and vitamin-C (46.63mg/100g) content. In molecular analysis (35 microsatellite markers), a total of 118 alleles were observed with an average of 3.47 alleles per marker, and polymorphism information content ranged from 0.25 (SLM6-38) to 0.79 (LEat-015). From group based genetic diversity analyses, maximum diversity was observed in common cultivated tomato (100%) followed by cherry tomato (82.35%) and local landraces (Khasi Local and Tura-1). The molecular markers associated to QTLs for tolerance to bacterial wilt (SLM6-17, SLM6-14), fruit firmness (LEga7, LEaat7, and LEaat1), shelf life (LEat16) and yield (LEaat1 and LEaat18) have also shown wider diversity. Moreover, marker SLM6-17(150bp) tightly linked to major QTL associated with bacterial wilt was unique to accession LE-1-2, DMT-1 and BWT-3 and SLM-6-14 (240bp) for Sel-3, BWT-3, and RCT-3. These markers could be utilized in the selection of genotype having multiple desirable traits through MAS approaches.

Key Words: Genetic diversity, Quality, Molecular markers, Tomato, Yield

Introduction

Tomato (*Solanum lycopersicum* L.) is a worldwide grown Solanaceous vegetable crop. It is an important source of antioxidants such as lycopene, β-carotene, and ascorbic acid (Hanson *et al*., 2004) and used for cooking, table purpose, and in preparation of different processed products.

To develop varieties having a higher yield and superior quality, the information on the genetics of yield and quality attributes is essential. Deployment of a particular breeding strategy needs an insight into the components of genetic variability. The phenotypic expression of the plant is mainly controlled by the genetic makeup and the environment, in which it is growing. Therefore, it is necessary to partition the observed phenotypic variability into its heritable and non-heritable components with suitable parameters such as phenotypic and genotypic coefficient of variation, heritability, and genetic advance. Further, the genetic advance can be used to predict the efficiency of selection. It is a known fact that more the variability, higher the chance of getting desirable genotype. However, it is only the genetic variation which is heritable and hence significant. Solanaceae crops viz., potato, tomato, and pepper have greatly benefited from the use of wild relatives in breeding programs. Virtually all of the disease resistance in modern tomato varieties originated in related wild species (Rick and Chetelat, 1995). Among the crop species, genetics and genomics of tomato are well studied (Foolad, 2007). Genetic diversity can be evaluated using morphological traits or molecular markers. Morphological traits are the simplest way to investigate genetic diversity but they are often influenced by the environment. Molecular markers help to understand genetic variation at the DNA level without any influence of the environment. Further, breeding efficiency in tomato can be improved by using DNA markers to tag and transfer useful alleles from germplasm to elite cultivars (Foolad, 2007). There are several molecular marker systems which have been applied in tomato to the study of genetic diversity,

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fine mapping and marker-assisted selection of QTLs associated with yield and quality (Yogendra *et al*., 2013), drought (Foolad *et al*., 2003), heat (Xu *et al*., 2017), low temperature (Liu *et al*., 2016) bacterial wilt and late blight (Thoquet *et al*., 1996, Moreau *et al*., 1998).

The North-eastern region of India is known for diverse genetic resources in different crop species. The region falls under high rainfall zone, and due to mild warm temp (20-30 ºC) and humid (>75%) weather during crop season which is conducive for diseases, the incidence of bacterial wilt and late blight are severe (up to 100%). Besides yield loss, the quality of the product is also affected. These problems are more severe to the farmers adopting organic production package. The primary research work at country level in India has been focused on breeding of varieties against moisture stress and tomato leaf curl viruses, and limited work has been done for resistance to bacterial wilt and blight, but they are the primary production constraints of the region. For the development of tomato varieties well suited to the region, the present research work was initiated with following objectives: To study the genetics of yield and quality traits of tomato including wild relatives, and diversity among the accessions at the quantitative and molecular level.

Materials and Methods

Plant materials

This experiment was conducted using 50 accessions of tomato including commercial cultivar and relative species collected from different of institutes of Indian Council of Agricultural Research (ICAR) and state agricultural universities (Table 1). Among the accessions, the incidence of bacterial wilt ranged from 7.5-27.0 %. Advance breeding lines MCTR- 4B, RCMT-8, MT-11, MT-3, MT-2, MCTR-3, MCTR-4, Sel-2 and resistant check BT-317 were found resistant to bacterial wilt with < 10% incidence of bacterial wilt.

Genetic parameters and diversity based on quantitative traits

All the accessions were evaluated under open field condition for three consecutive years (January to April 2013-2016). The experimental site was Horticulture Farm, ICAR Research Complex for NEH Region, Umiam, Meghalaya (latitude 25º41'N and 92º55'E longitude) located at 960 m above mean sea level. Yearly, rainfall ranged from 2,200-2,551 mm and the average maximum and minimum temperatures during crop period were 28.3°C and 18.0°C, respectively. This location has inceptisol soils of sandy texture and acidic in reaction (pH: 5.4). The pre-treated seeds with Captan were sown in the nursery each year during the first week of January. One-month-old seedlings were transplanted on raised beds (3.5 m × 2.0 m size) at 45 cm × 30 cm spacing between the line to line and plant to plant, respectively. The accessions were evaluated in Augmented Block Design. Recommended doses of FYM (10 t/ha) and chemical fertilizers NPK applied at 120:80:60 kg ha^{-1} from urea, single super phosphate (SSP) and muriate of potash (MOP), respectively. Full dose of P and K with one-third of N was applied at the time of land preparation. The remaining dose of

Table 1. Accessions of tomatoes and their sources

S. No.	Name of the accessions	Source
1.	DVRT-2, H-86, BT-317, DMT-5, LE-1-2, BWT-3, LE-626, DMT-1, BT-1, Var-801, KSS-227, Cherry Tomato-1, Cherry Tomato-2, Cherry Tomato-3, Cherry Tomato-4 & Cherry Tomato-5	ICAR- AICRP on Vegetable Crops
2.	*S. peruvianum* (1), *S. peruvianum* (2) & *S. pimpinelifollium*	ICAR-IIVR, Varanasi
3.	TMC-1, TMC-2, RCT-3, RCMT-8, MT-5, MT-6, MT-11, MCTR-3, MCTR-4, MCTR-4A, MCTR-4B, MCTR-5, MCTR-7B, Sel-1, Sel-2, Sel-3, Sel-8, Sel-9A & Sel-11	ICAR Research Complex for NEH Region, Manipur Centre, Manipur
4.	MT-1-1, MT-2, MT-3 (Big), MT-10, Tura-1 & Khasi Local	ICAR Research Complex for NEH Region, Umiam, Meghalaya
5.	Pusa Rohini	ICAR-IARI, New Delhi
6.	Junagadh Tomato -1	Junagadh Agricultural University, Junagarh, Gujarat
7.	Arka Vikash	ICAR-IIHR, Bengaluru, Karnataka
8.	Pant T-10	GBPUAT, Pantnagar, Uttarakhand
9.	Solan Lalima	Dr Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan, Himanchal Pradesh
10.	V L Tamatar - 4	ICAR-Vivekananda Parvatiya Krishi Anushandhan Sansthan, Almora, Uttarakhand

N was applied in two split dosages at 30 and 60 days after planting. To control leaf miner and fruit borer insecticide, Imidacloprid (0.5 ml/L water) was sprayed prophylactically at 30 and 60 days after transplanting. Manual weeding and hoeing were done during 25-30 and 60-75 days after transplanting. Observations for growth and yield attributes were taken on six plants in each replication. Quality parameters such as TSS (^{o}B), acidity, total sugar (%), ascorbic acid (mg/100g) and lycopene content (mg/100g) were estimated by following the procedure as suggested by Ranganna (1985). The mean values of each replication for all the 13 quantitative traits were used to assess genetic parameters and dissimilarity between accessions.

Quantitative data analyses

The analysis of variance for the design of the experiment was carried out according to the procedure outlined by Panse and Sukhatme (1967). Phenotypic and genotypic coefficient of variability were calculated according to the method suggested by Burton and de Vane (1953). For estimation of genetic parameters such as heritability (broad sense), genetic advance and correlation were calculated according to Johnson *et al*. (1955). Genetic diversity was estimated following Mahalanobis's (1936) generalized distance (D^2) extended by Rao. Tocher's method (Rao, 1952) was followed for determining the group constellations.

Molecular characterization

Young actively growing leaves from one-month-old plants (pooled 5 plants in each accessions) were collected and used for DNA extraction. Total genomic DNA was extracted using a modified cetyltrimethylammonium bromide (CTAB) method (Saghai–Maroof *et al*., 1984). Nanodrop™ 1000 Spectrophotometer (Thermo Scientific, USA) used for DNA quantification. Total 40 SSR markers, of which 22 SSR (LE series associated with QTL for yield and quality attributes) developed by He *et al*. (2003) using Gene Bank Database and 18 SSR (SLM series associated with QTL for resistance to bacterial wilt) developed by Geethanjali *et al*., 2010 using genomic sequences of anchored BAC clones on chromosome 6 were surveyed to identify primers that were reproducible and generated the most polymorphic patterns. Veriti PCR machine (Applied Biosystem, USA) was used to carry out PCR reaction. The amplification products were electrophoresed on 3% agarose gel at 60 volts. Gels were stained with ethidium bromide and documented using a Chemidoc gel documentation unit (BioRad, California, USA).

Molecular data analyses

Molecular weights of bands were estimated by using 50 bp DNA ladder, and the homology of bands was based on the distance of migration in the gel. SSR amplicons obtained from each entry were resolved as a band on the gel system, and the data sets were used to calculate major allele frequency and the polymorphism information content (PIC) for each locus using Power Marker software. GenAlEx v.6.1 software was used to calculate pair-wise Nei's genetic distance and private alleles. Neighbor-joining dendrogram (cluster analysis) based on Nei's genetic distance for genic-SSR was generated using MEGA 7 software.

Population structure analysis was carried out using STRUCTURE 2.3.4 software (Pritchard *et al*., 2000). The optimum value of K was determined in Structure Harvester online software (Earl and vonHoldt 2012; http://taylor0.biology.ucla.edu/structureHarvester/) by loading STRUCTURE 2.3.4 software results. Principal coordinate analysis (PCoA) based on allele frequencies was done by using XLSTAT software.

Results and Discussion

Nowadays, the focus of crop improvement in tomato is not only limited to yield but also quality parameters with tolerance to biotic and abiotic stresses. To breed the varieties with multiple desirable traits, diverse genetic materials for different traits are essential. The genotypic coefficient of variation (GCV) is considered as the real indicator of the extent of genetic variability in the population. In present investigation, the coefficient of variability for quantitative traits, indicated the higher value for phenotypic coefficient of variation (PCV) than the GCV for all the traits (Table 2). However, the differences between PCV and GCV were small and indicated the influence of higher degree of the genotype over environment on phenotypic expression of these characters. It also suggests that selection based on all these traits would be useful for future crossing program. Among the traits number of fruits/plant (95.64% and 97.54%), fruit weight (50.06% and 53.48%) and yield per plant (48.0 and 56.0%) exhibited higher GCV and PCV values, respectively, indicating the higher variability for these traits in the accessions. These traits has also contributed maximum toward genetic diversity. Other traits like plant height, number of primary branches, fruit

Table 2. Genetic parameters for different quantitative traits in 50 tomato accessions

Traits	Mean	Range	Vg	Vp	GCV	PCV	h^2 (Broad Sense)	Genetic Advance	Genetic advance as percentage of mean	Contribution to genetic diversity (%)
Plant height (cm)	80.32	42.0-136.3	341.11	368.78	22.99	23.91	92.50	36.64	45.60	6.12
No of primary branches	7.47	4.0-14.0	2.45	2.70	20.95	22.00	90.74	3.08	41.20	0.08
No of flowers/cluster	6.10	4.1-9.0	0.91	1.18	15.63	17.78	77.23	1.73	28.31	0.00
Fruit length (cm)	3.93	0.8-5.9	0.78	0.81	22.47	22.90	96.30	1.79	45.50	0.33
Fruit diameter (cm)	4.15	0.8-6.6	1.28	1.30	27.26	27.47	98.46	2.32	55.80	0.90
Fruit weight (g)	38.08	1.5-88.0	363.34	414.72	50.06	53.48	87.61	36.81	96.70	21.55
No of fruits/plant	30.07	9.6-130.0	827.08	860.33	95.64	97.54	96.14	58.17	193.5	16.73
Yield/plant (kg)	1.17	0.2-2.3	0.21	0.22	48.0	56.0	95.45	0.92	78.90	23.59
TSS (^{0}B)	4.75	3.5-6.5	0.19	0.20	9.09	9.27	95.88	0.87	18.36	0.65
Acidity (%)	0.71	0.4-0.9	0.01	0.01	16.27	16.73	92.86	0.23	32.59	0.33
Total Sugar (%)	1.56	1.2-2.2	0.04	0.04	12.90	13.13	97.62	0.41	26.13	4.08
Vitamin-c (mg/100g)	23.72	14.0-48.0	23.09	33.37	24.24	24.34	69.21	8.25	34.78	13.80
Lycopene (mg/100g)	9.74	5.7-13.6	4.30	4.37	21.27	21.46	98.31	4.24	43.44	11.84

length and diameter, vitamin – C, and lycopene content exhibited moderate PCV, GCV values indicating that a moderate level of genetic variability (Table 2). Similar findings in tomato were also reported by Kouam *et al*. (2018) for these traits.

Further, perusal of data presented in Table 2 has also shown high heritability coupled with high genetic advance, as a percent of the mean for the observed characters like number of fruits per plant, fruit weight, yield per plant, fruit diameter, fruit length, plant height, and lycopene content. It indicates a strong influence of an additive gene on these traits are under action and hence, simple selection based on the phenotypic performance of these traits would be effective and efficient. Johnson *et al*. (1955) have suggested that traits with high heritability coupled with high genetic advance would respond to selection better than those with high heritability and low genetic advance. High heritability and moderate genetic advance as a percentage of mean (GAM) values were observed for total sugar and acidity, indicating expression of these traits are influenced by non-additive gene action and environment. These traits could be exploited through the manifestation of dominance and epistatic components. Similar findings was also observed by Dar and Sharma *et al*. (2011) while, Yogendra *et al*. (2013) observed low heritability and genetic advance for lycopene in the F_2 population.

For effective use of parental lines in crossing programs, it is considered imperative to have analyses of genetic divergence based on morphological along with molecular analysis. Selection of parents based on the extent of genetic divergence has been successfully utilized in different crop species. The concept of genetic distance for this has been of essential utility in differentiating distinct populations. Several measures of distance have been proposed to suit various objectives, of which Mahalanobis generalized distance (Mahalanobis, 1936 and Rao, 1952) has occupied a unique place in plant breeding. The results of cluster analysis based on quantitative data (D^2 analysis), 50 accessions were grouped into seven clusters. Cluster I and III comprised maximum 33 and 7 accessions, respectively. However, clusters IV, V, VI, and VII were mono-genotypic with accession LE-1-2, BT-1, Khasi Local, and *S. peruvianum*-1, respectively and found most diverse from other accessions. Between the clusters, maximum genetic distance (3064.0) was observed between cluster VI (Khasi Local) and cluster VII (*S. peruvianum*-1) followed by cluster II and cluster VII. Likewise, maximum intra-cluster distance (394.44) was observed in cluster III comprising accessions of *S. cerasiformae, S. pimpinellifolium* and *S. peruvianum*-2. The intra-cluster divergence indicated the existence of genetic diversity within the cluster III. Average inter and intra-cluster distances revealed that, in general, inter-cluster distances were much higher than those of intra-cluster distances, suggesting homogeneous and heterogeneous nature of the germplasm lines within and between the clusters, respectively. Similar findings were also observed in tomato by Thapa *et al*. (2014).

From cluster mean, accession Khasi Local of cluster VI was found superior for fruit weight (80.30g), and

fruit diameter (5.30 cm) hence could be utilized for higher yield. Moreover, wild species *S. peruvianum* (1) of cluster VII was found to be superior and could be utilized in a breeding program for traits like number of fruits (124.2) per plant, TSS (6.17) and vitamin-C (46.63 mg/100g) content. Similarly, accession LE-1-2 of cluster IV was superior for lycopene (12.23 mg/100g) content. The results of genetic analysis have also shown high heritability and genetic advance for yield and quality traits (number of fruits per plant, fruit weight, yield per plant, and lycopene content). Therefore, identified superior line with unique traits from the diverse group could be utilized for hybridization to get novel recombinants.

Among the traits, positive and significant association of fruit yield per plant was observed with plant height, number of primary branches, fruit length, diameter, and weight (Table 3). This may be explained by the higher photosynthetic products available for partitioning to fruit production. The direct selection of these traits will be very useful for identifying genotype having a higher yield. Similar findings were also reported by Kouam *et al*. (2018). Moreover, among the quality parameters, TSS and acidity were negatively correlated while lycopene content was positively correlated with total sugar, TSS. Thus, these traits can be selected together for identifying lines having superior quality. The results of the present study are also in agreement with the result reported Singh *et al*. (2018).

Principal component analysis (PCA) is a technique which identifies plant traits which contributed most to the observed variation within a group of genotypes and has a practical application in the selection of parental lines for the breeding purpose In present investigation, the cumulative variance of 72.17% by the first four principal components with eigenvalues of more than 1.0 indicated that the identified traits within this axes exhibited significant influence on the phenotype of the cultivars and could effectively be used for selection among them. Similar findings were also observed by Chernet *et al*. (2014). The first three PC was contributed by traits such as fruit weight, fruit length, fruit diameter, yield per plant, vitamin-C and lycopene content and are essential for improving yield and quality traits. Therefore, these variables might be taken into consideration for a valid selection of parents for hybridization programs to broaden the genetic base in the population as well as to develop elite lines or F_1 hybrids.

The power of molecular markers for analyzing genetic diversity has been well established in a range of vegetable crops, including tomato. In present investigation, polymorphism survey was carried out with a total of 40 SSR primers out of which 35 had shown amplification, and 34 (19 EST and 15 genomic SSR) markers were polymorphic (Table 4). Further, molecular analysis has also shown wide allelic diversity and frequency among the different accessions. The

Table 3. Phenotypic correlation coefficients between 13 quantitative traits in 50 tomato accessions

Traits	Plant height (cm)	Fruit length (cm)	Fruit diameter (cm)	Fruit weight (g)	No of fruits/ plant	No of primary branches	No of flowers/ cluster	TSS (0B)	Acidity	Total sugar (%)	Vitamin-C (mg/100g)	Lycopene (mg/100g)
Plant height (cm)												
Fruit length (cm)	0.050											
Fruit diameter (cm)	0.066	0.856**										
Fruit weight (g)	-0.019*	0.829**	0.930**									
No of fruits/plant	0.193	-0.716**	-0.723**	-0.77**								
No of primary branches	0.338**	0.061	0.217**	0.144	-0.076							
No of flowers/ cluster	-0.009	0.035	0.036	-0.043	0.167*	0.128						
TSS (0B)	0.118	-0.638**	-0.446**	-0.437**	0.714**	0.192*	0.162*					
Acidity	0.556**	0.140	0.237**	0.137	-0.033	0.432**	0.014	-0.057				
Total sugar (%)	0.210**	-0.341**	-0.279**	-0.309**	0.335**	-0.029	-0.054	0.284**	0.120			
Vitamin-C (mg/100g)	0.065	-0.203*	-0.326**	-0.345**	0.509**	-0.297**	0.004	0.327**	-0.168*	0.158		
Lycopene content (mg/100g)	0.028	-0.138	-0.134	-0.177*	0.221**	0.192*	0.118	0.241**	-0.028	0.246**	0.023	
Yield/plant(kg)	0.246**	0.594**	0.647**	0.680**	-0.216**	0.248**	0.130	-0.059	0.270**	-0.070	-0.069	0.006

*,** Significant at 1 and 5% level of significance, respectively

Table 4. Details of SSR primers used for genetic diversity analysis of tomato 50 accessions

Locus	Na	Ne	I	Ho	He	u He	Allele Frequency	PIC	Size of alleles (bp)
LEaat-006	4	1.94	0.91	0.10	0.48	0.49	0.66	0.50	160-190
LEat-016	4	2.07	0.94	0.10	0.52	0.52	0.64	0.45	160-195
LEcag-003	4	2.09	0.95	0.02	0.52	0.53	0.64	0.49	140-180
LEcaa-001	3	1.71	0.67	-	0.41	0.42	0.72	0.34	120-160
LEaat-001	5	2.65	1.17	0.10	0.62	0.63	0.50	0.63	160-240
LEaat-002	3	1.90	0.81	-	0.47	0.48	0.68	0.41	110-130
LEaat-007	4	3.56	1.32	0.02	0.72	0.73	0.34	0.68	95-125
LEat-015	4	3.83	1.37	0.91	0.74	0.75	0.26	0.79	380-410
LEat-017	3	2.40	0.95	1.00	0.58	0.59	0.78	0.32	160-210
LEat-018	4	3.55	1.33	0.88	0.72	0.73	0.34	0.75	400-450
LEct-003	5	3.68	1.40	0.07	0.73	0.74	0.32	0.75	220-245
LEctat-001	5	1.62	0.80	0.02	0.38	0.39	0.64	0.52	260-305
LEga-003	3	2.80	1.07	0.96	0.64	0.65	0.42	0.60	220-245
LEga-007	2	1.84	0.65	0.04	0.46	0.46	0.60	0.46	195-205
LEta-003	3	1.18	0.33	0.08	0.15	0.15	0.90	0.18	100-150
LEta-0019	2	1.17	0.27	0.02	0.14	0.15	0.82	0.29	240-245
LEta-0020	2	1.74	0.62	0.19	0.43	0.43	0.56	0.56	200-225
LEta-0014	2	2.00	0.69	-	0.50	0.50	0.42	0.61	150-170
LEta-0024	2	1.53	0.53	0.00	0.35	0.35	0.62	0.41	260-280
SLM-6-3	2	1.84	0.65	0.00	0.46	0.46	0.40	0.72	125-130
SLM-6-4	4	2.69	1.14	0.12	0.63	0.64	0.44	0.71	120-180
SLM-6-5	5	2.77	1.23	0.09	0.64	0.65	0.76	0.39	130-210
SLM-6-7	3	1.37	0.52	0.09	0.27	0.27	0.42	0.72	230-290
SLM-6-14	4	2.95	1.23	0.07	0.66	0.67	0.52	0.63	240-300
SLM-6-15	3	2.07	0.88	0.15	0.52	0.52	0.38	0.66	230-250
SLM-6-17	4	2.84	1.14	0.12	0.65	0.66	0.50	0.48	150-220
SLM-6-18	4	2.27	0.92	0.02	0.56	0.57	0.54	0.60	130-160
SLM-6-12	5	2.08	1.06	0.32	0.52	0.52	0.56	0.55	210-280
SLM-6-25	3	2.16	0.90	-	0.54	0.54	0.56	0.56	180-225
SLM-6-13	3	2.20	0.92	-	0.54	0.55	0.76	0.37	160-190
SLM-6-36	3	1.44	0.54	0.04	0.30	0.31	0.66	0.52	145-170
SLM-6-38	6	2.78	1.20	0.90	0.64	0.65	0.84	0.25	200-280
SLM-6-56	2	1.32	0.41	-	0.25	0.25	0.48	0.61	145-150
SLM-6-57	3	2.47	0.99	-	0.60	0.60	0.57	0.53	90-105
Mean	3.47	2.25	0.90	0.19	0.51	0.52	0.57	0.53	-

* Where: Na = No. of different alleles, Ne = No. of effective alleles, I = Shannon's Information Index, Ho = Observed heterozygosity, He = Expected heterozygosity, uHe = Unbiased expected heterozygosity, PIC = polymorphic information content (mean)

total numbers of observed alleles were 118, with an average of 3.47 numbers of alleles per marker. The number of alleles varied from 2 to 6 with allele size ranging from 90-105 bp (SLM-6-57) to 400-450 bp (LEat-018). The maximum number of alleles per marker was 6 in genomic marker SLM6-38. However, the most informative markers were EST marker LEat-015 with PIC (0.79) followed by LEat-018 and LEct-003 (0.75 each). The markers have also shown heterozygosity in the accessions, and the observed heterozygosity (Ho) was lower than the expected heterozygosity (He) and this may be due to an intensive selection of the accessions (Table 4). A large variation in polymorphism information content (PIC) of markers was observed for all the SSR loci and ranged from 0.25 (SLM6-38) to 0.79 (LEat-015) with the mean polymorphism of 0.53 per markers. The wide variation in PIC of SSR loci was also observed by Geethanjali *et al*. (2010), Zhou *et al*. (2015), and Gharsallah *et al*. (2016) in different accessions of tomato. According to high, medium and low locus polymorphism is defined as PIC > 0.5; PIC 0.25 - 0.5 and PIC < 0.25, respectively. Accordingly, PIC in our investigation indicated a wider genetic base by high locus polymorphism. This could be due to the involvement of cultivated as well as wild species of the tomato. Kwon *et al*. (2009), also reported higher PIC

value (0.628) ranging from 0.210 to 0.880 using 33 SSR markers while screening 63 tomato varieties.

The cluster analysis has shown broader diversity in the accessions of the tomato. Neighbor-joining dendrogram presented in Figure 1 shows the genetic relationships among the cultivated and relative species of tomato based on Nei's genetic distance using 34 cross transferable SSRs. All the accessions were grouped into six major clusters. Except *S. peruvianum* other species were distributed mixed with common cultivated tomato. *S. pimpinellifolium* was found closer to tomato cultivar BWT-3 while landrace Khasi Local was found closer to Cherry Tomato-5. Further, molecular analysis has also shown maximum variation within the population (86%) followed by between the populations (14%). From group based diversity analysis the maximum polymorphism was observed in common cultivated tomato followed by cherry tomato and local landraces (Khasi Local and Tura-1) while it was least in *S. pimpinellifolium* and indicated the broad genetic base of the cultivated species over the other species. Maximum genetic diversity was found in common and cherry tomato, and it could be due to the broad genetic base by hybridization and selection of the desired recombinants. Among the populations based on pairwise Nei genetic distance, the maximum genetic distance (1.34) was observed between *S. pimpinelifollium* and *S. peruvianum* followed by local landrace of cultivated tomato and accessions of *S. peruvianum.* This could be due to incompatibility between of *S. lycopersicum, S. pimpinelifollium* to *S. peruvianum* (Alvarez *et al*., 2001). Higher levels of genetic diversity in the self-incompatible species (*S. peruvianum*, *S. hirsutum*, *S. pennellii*, and *S. chilense*) than the self-compatible species (*S. esculentum*, *S. pimpinellifolium*, *S. cheesmanii*, *S. parviflorum*, and *S. chmielewskii*) have been reported (Miller and Tanksley, 1990). Further, principal coordinate analysis (PCoA)

Fig. 1. Neighbour-joining dendogram showing genetic relationships among the cultivated and wild species of tomato based on Nei's genetic distance using 34 cross transferable SSRs

proved the wider diversity in the common and cherry tomato. First three coordinates explained 38.0% of the total variation, with 18.61% defined by the first coordinate and 11.56% by the second coordinate. The accessions of *S. lycopersicum* and cherry tomato were distributed in both the coordinates. However, accessions of *S. peruvianum* and *S. pimpinelifllium* was separated by second coordinate.

Under structure analysis, the optimum number of the cluster was determined as per the procedure described by Evanno *et al*. (2005) using software STRUCTURE. The analysis detected the maximal ΔK at K=2, indicating that the entire population could be grouped into two sub-populations. The ΔK value decreases with an increase in K (Figure 2). Clusters differentiate ideally between and within the crop species. The genetic differentiation between clusters was low to moderate (F*st* = 0.10 – 0.59). Further, the structure result also agreed with the clustering and principal coordinate analysis. Among the accessions, the proportions of accessions with admixture were few, and it may be due to the different geographical origin. Moreover, the admixture was common in cultivated *S. lycopersicum* and cherry tomato (*S. lycopersicum* var. *cerasiforme*) which may be due to closeness, free natural gene flow between them, hybridization, and selection.

The markers used in the present studies were also associated with desirable QTLs and have differentiated the accessions. The more extensive allelic variation was observed for the markers associated with QTL for tolerance to bacterial wilt as well as fruit quality and yield. Among the tomato accessions, bacterial wilt tolerant lines BT-317 and MT-1-1 were identified by four

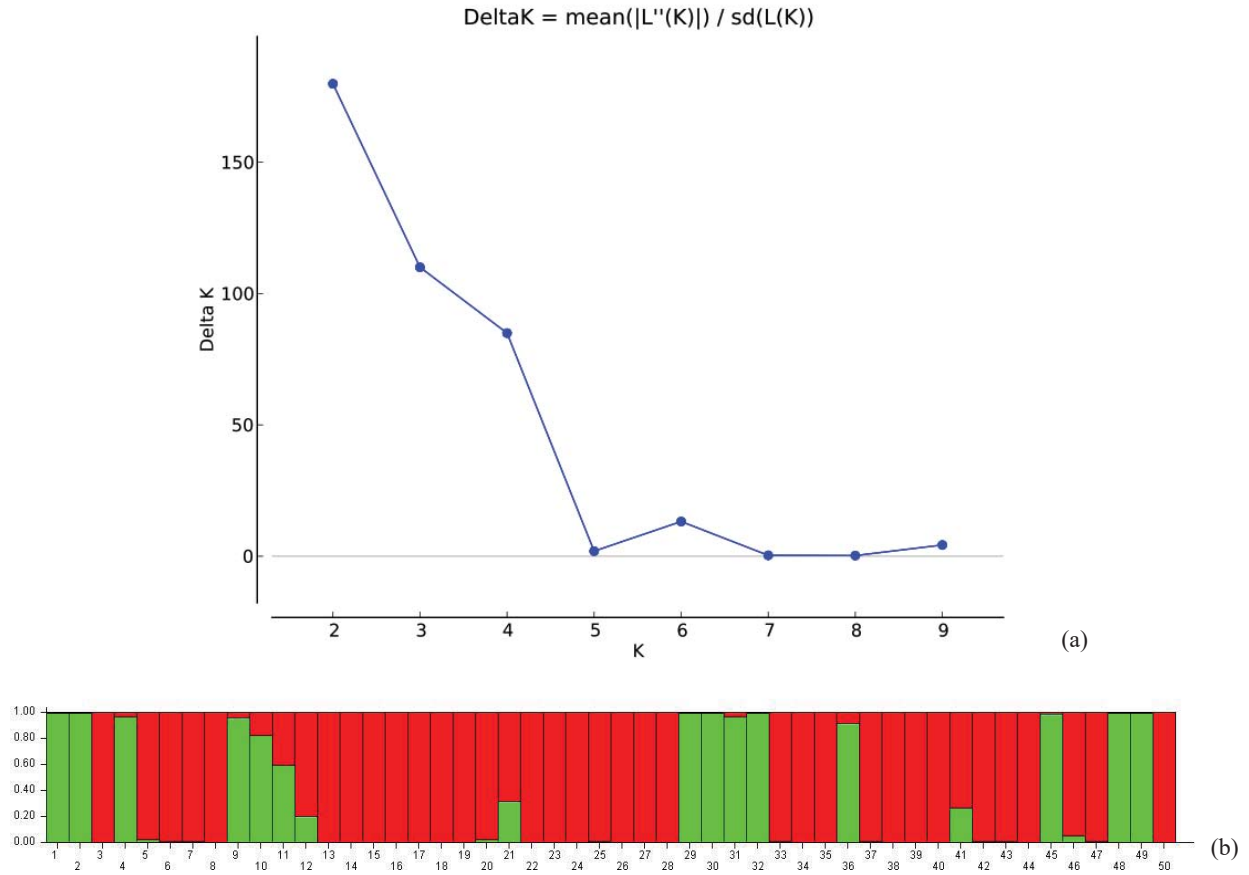

Fig. 2. Population structure of the accessions of cultivated and wild tomato accessions based on SSR markers. (a) ΔK graph, (b) population structure at ΔK =2.

Accessions Name: 1. DVRT-2; 2. VL Tamatar-4; 3. TMC-1; 4. TMC-2; 5. Pusa Rohini; 6. Junagadh Tomato-1; 7. Solan Lalima; 8. H-86; 9. RCT-3; 10. RCMT-8; 11. BT-317; 12. DMT-5; 13. LE-1-2; 14. BWT-3; 15. Arka Vikash; 16. LE-626; 17. Pant T-10; 18. DMT-1; 19. BT-1; 20. Var-801; 21. KSS-227; 22. MT-1-1; 23. MT-2; 24. MT-3 (Big); 25. MT-5; 26. MT-6; 27. MT-10; 28. MT-11; 29. MCTR-3; 30. MCTR-4; 31. MCTR-4A; 32. MCTR-4B; 33. MCTR-5; 34. MCTR-7B; 35. Sel-1; 36. Sel-2; 37. Sel-3; 38. Sel-8; 39. Sel-9A; 40. Sel-11; 41. Tura-1; 42. Khasi Local; 43. Cherry Tomato-1;44. Cherry Tomato-2; 45. Cherry Tomato-3; 46. Cherry Tomato-4; 47. Cherry Tomato-5; 48. *S. peruvianum* (1); 49. *S. peruvianum* (2); 50. *S. pimpinelifollium*

Which is correct: LEga-7 or **LEat-16 or LEaat-1 or LEaat-18?????**

markers SLM6-4 (120bp), SLM6-5 (130bp), SLM6-7 (230bp) and SLM6-12 (280bp) of chromosome-6. In earlier studies, Geethanjali *et al*. (2010) also identified these markers associated with QTL resistance to bacterial wilt strain Pss4. Further, marker SLM6-17 (150bp) tightly linked to major QTL associated with bacterial wilt was unique to LE-1-2, DMT-1, and BWT-3. Marker SLM6-14 (240bp) was also unique to bacterial wilt tolerant line Sel-3, BWT-3, and RCT-3. These identified lines and markers could be utilized in the mapping the gene linked to bacterial wilt and future improvement programs. Like quantitative traits, the molecular markers have also shown wider genetic diversity for fruit characteristics. Markers LEga-7, LEaat-7, and LEaat-1 from linkage group 3, 4 and eight associated with QTL for fruit firmness (Yogendra *et al*., 2013) have shown wider variability with PIC of 0.46, 0.68 and 0.63, respectively. Similarly, LEat-16 associated with QTL for shelf life was also found informative with PIC of 0.45 while marker LEaat-1 and LEaat-18 shown PIC value 0.63 and 0.75, respectively were associated with QTL for yield. These markers could be utilized in the selection of genotypes having multiple desirable traits through MAS approaches.

Conclusions

The overall analysis of the present investigation has shown the existence of wide genetic variability for different quantitative traits due to the significant contribution of the genotypic coefficient of variation. The traits for yield (number of fruits, size, and weight) and quality (lycopene) have shown high heritability, and genetic advance indicates for the higher role of additive gene action, and hence selection for these traits will be useful. Further, results of genetic diversity based on quantitative traits as well as molecular analysis revealed wider genetic diversity among the accessions for yield and quality attributes. The lines with unique traits from the diverse group could be utilized for developing new recombinants with many desirable traits. Moreover, molecular markers associated with unique traits (QTLs) could be used for MAS breeding. Further, there is also a need to introduce new lines having resistance to diseases, especially for early and late blight.

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RESEARCH ARTICLE

Moisture Induced Changes in Root Attributes and Selection Indices for Drought Tolerance in Desi, Kabuli and Wild Chickpea Accessions

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Root traits i.e. root length (RL), root weight (RW) and root area (RA) and root indices i.e. root length density (RLD) and root:shoot ratio (RSR) were evaluated in leaves of desi, kabuli and wild chickpea accessions at 70 DAS under receding moisture conditions (control, 50% and 75% reduction). The tolerance of desi genotypes was recorded to be significantly higher than kabuli and wild accessions. Check desi genotype PBG 7 was observed to be tolerant to moisture stress with 13.15% and 34.04% decline in root length density as well as root length under 50% moisture reduction conditions respectively. However, wild accessions sustained low stress injuries in terms of low membrane permeability index (MPI) (12.76 and 28.46%) and less decline in cellular respiration (26.06 and 43.23%) highlighting their resistance at 50 and 75% moisture reduction respectively. The tolerance capacity of the desi genotypes was attributed to high root area that contributed directly to root length density (P=0.89) and was instrumental in maintaining yield stability.

Key Words: *Cicer* species, Moisture stress, Path coefficient analysis (PCA), Root traits, Selection indices

Introduction

Chickpea (*Cicer* spp.) is an important *rabi* pulse crop of India as the country accounts for 75% of the total world production (Ahsan *et al.,* 2018). It is an important source for vegetarian protein. The major chickpea producing regions are the arid and semi-arid areas globally, including South Asia and sub-Saharan Africa, where residual soil moisture is the only source to fulfil plant water requirement.

Increasing incidence of abiotic stress due to change in global precipitation levels, increasing temperature due to global warming and decline in the water strata has immensely added to the adversity. All has led to a water deficit condition in soil and prevailing consistency of such conditions over a longer period of time renders the plant to suffer at every stage i.e. prior to germination and till maturity. The low water availability at the terminal stage affect the pod formation, causing up to 40–50% yield losses, and resulting in stagnancy in chickpea productivity (Fang *et al.*, 2010; Kashiwagi *et al.*, 2015). The yield losses reported in crops facing terminal drought stress at the reproductive phase are greater than the drought stress at vegetative stage (Krishnamurthy *et al.*, 2010).

Water deficiency alters the shoot and root systems of the plant by inducing complex morphological, physiological and biochemical mechanisms. Amongst morphological modifications an increase in root depth, biomass and density facilitates efficient water uptake under terminal drought conditions (Turner *et al*., 2001). Consistent water availability is necessary for reproductive success and ultimately for crop grain yield (Kato *et al*., 2008). Thus, the chickpea germplasm that descend the rhizosphere via the root length elongation and increase in root hairs surface under moisture stress are less susceptible and have low or negligible effect of the same on the yield (Turner *et al.*, 2001). Root traits have been shown to influence not only transpiration via soil moisture utilisation but also yield attribute *viz*., harvest index under terminal drought (Zaman-Allah *et al.*, 2011).

Thus, the current study focusses on comparative responses in the behavioural changes in root traits of desi, kabuli and wild chickpea accessions under receding moisture conditions at 70 DAS (days after sowing).

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Materials and Methods

Plant material

Ten *Cicer* accessions (Desi: GL 12020, PBG 7, PBG 5, PDG 4, GNG 1581, Kabuli: HK-10-103, GNG 2285 and three wild chickpea accessions *C. judaicum 185B, C. judaicum 185* and *C. judaicum 182*) were procured from Pulses Section, Department of Plant Breeding and Genetics, PAU Ludhiana. PBG 7, PBG 5 and PDG 4 are three released varieties by Punjab Agricultural University (PAU, 2021).

Chickpea accession	Check
PBG 7	Irrigated conditions
PBG 5	Irrigated conditions in humid areas
PDG 4	Rainfed conditions

Desi (GL 12020, GNG 1581), Kabuli (HK-10-103, GNG 2285) and wild accessions (*C. judaicum 185B, C. judaicum 185* and *C. judaicum 182*) were selected to draw the comparisons under receding moisture conditions.

Cylinder culture

The lysimetric culture (as described by Kashiwagi *et al.,* 2005 with slight modifications) were grown in 18 cm diameter and 1.6 m tall PVC pipes in a randomised block design (3 treatments i.e. control, 50% and 75% reduction with respect to irrigation) with 3 replications in the first and second *Rabi* trials (Fig. 1). The PVC pipes were placed in 1.2 m deep soil pits with a spacing of 30 cm between adjacent pipes and 3 feet spacing within replications. A 2 m pit was dug and a polythene sheet was placed in order to avoid water leaching within successive treatments. The PVC pipes were filled with an equi-mixture of sand and soil. The soil-sand mixture was air dried 15 days before the sowing. Soil moisture content was calculated as below:

$$\frac{(\text{Wet weight of soil-Dry weight of soil})}{\text{Dry weight of soil}} \times 100$$

Each PVC pipe was filled with 10 kg of mixture of sand and soil with same soil level in all the pipes. Initially, in each pipe 1 litre of water was added into each treatment. After the irrigated water had penetrated the soil profile, 2 kg of dry soil was added over the surface of each pipe. At the time of sowing, five seeds were planted in each pipe. Irrigation was done at regular interval of five days with respect to irrigation treatment i.e., 1 litre of water was added in control during each irrigation followed by 500 ml and 250 ml of water in the treatment i.e. 50% and 75% reduction, respectively. The plants were grown in rainout shelter to avoid moisture from precipitation. Plants were harvested at 70 days after sowing (DAS) by placing the pipes in running water, with the soil-sand mixture which was removed gently. After three-fourth of the mixture was removed, the PVC were erected and plants are gently slipped out and the data was recorded on root traits and stress indices.

Root attributes

Root area and root length density (RLD) was measured using root scanner (Delta T-root Scan software). The mean of three roots were expressed in mm^2 and g cm^{-3} /plant, respectively.

Three random plants were harvested and roots of the plant were dipped into the measuring cylinder. After removing soil particles, the roots were stretched to measure the root length (cm).

Fig. 1. Field view of lysimetric screening of chickpea accessions

Morphophysiological parameters

The plants were air dried for 2h to remove excess water and then fresh root and shoot weights (g) were measured. The root and shoot fresh weight were compared and root:shoot ratio was calculated.

For estimation of allocation of resources towards the shoot, shoot weight: root length density (SW: RLD) (g $g^{-1}cm^3$) was calculated.

Three plants from each replication were selected and their plant length was measured and their average values are expressed in cm/plant. The number of leaflets was counted in three plants at 70 DAS.

Selection indices

The damage in leaves due to moisture stress was measured in terms of membrane permeability index (MPI) by the method of Fletcher and Drexlure (1980). Leaflets (0.1 g) were washed and kept in distilled water for 24 h. The electrical conductivity (EC) was measured using a conductivity meter (Mettler Toledo FEP 30). After recording, the same tissue was kept for boiling in water bath for 30minutes and after cooling the EC was measured again. MPI was calculated as below:

$$\text{MPI (\%)} = \frac{\text{EC after boiling- EC before boiling}}{\text{EC after boiling}} \times 100$$

The effect of moisture stress on cellular respiration (viability) of leaves was determined by the method of Steponkus and Lamphear (1967). Leaf tissue (0.1 g) was placed in water and was subjected to two temperature treatments *viz*., at 25^0C and 49^0C for 1.5 h. Thereafter, 10 ml of 2,3,5-triphenyl-tetrazoliumchloride (TTC) solution was added per tube for 24 h at 25 ºC in dark. Rinsing of the incubated tissue was done with distilled water, then 4 ml of 95% ethanol was added for 24 h and optical density (O.D.) was recorded in a spectrophotometric cuvette at 530 nm in relation to formazon.

$$\text{Cellular respiration in terms of \% TTC reduction} = (OD_h/OD_c) \times 100 \quad (\text{h=heat treated, c=control})$$

Statistical analysis

Tukey's HSD through SPSS 16.0 was used to validate the differences within treatments and accessions for lysimetric screening. Correlation within various attributes was determined using Pearson's correlation coefficients. Path coefficient analysis using polynomial regression coefficients between root length density and morphophysiological parameters were analysed using Microsoft office Excel version 2010. Percent increase or decrease data was calculated at 50% and 75% receding moisture conditions with respect to control conditions to assess the tolerance capacity of chickpea accessions.

Results and Discussion

In general, there was a reduction of 29.29 and 50.45% in root weight at 50% and 75% moisture content respectively (Table 1). The fine tuning of partitioning of photosynthates between below and above ground organs is disrupted whenever plant comes across stressful situations. Genotypes did not depict a static trend in root:shoot biomass reduction after exposure to water reduction. Desi check genotype PBG 5 expressed a minimum decline of 10.80% at 50% moisture reduction with respect to control. PDG 4 depicted an interesting behaviour though showing a decline of 11.52% in root weight at 75% reduction, but an increase of 21.63% in root weight when moisture content reduced to 75% from 50%.

Table 1. Root traits and root indices of chickpea accessions under receding moisture conditions

	Root weight (g)			Root length (cm)			Root:Shoot ratio		
Chickpea Accessions	Control	50%Reduction	75%Reduction	Control	50%Reduction	75%Reduction	Control	50% Reduction	75%Reduction
GL 12020	7.70±0.22^{c}	6.06±0.12^{b}	4.57±0.05^{d}	52.00±0.06^{e}	49.46±0.11^{d}	46.31±0.11^{d}	0.90±0.02^{d}	0.94±0.02^{d}	0.79±0.01^{d}
PBG 7	6.94±0.04^{e}	4.96±0.01^{d}	3.68±0.03^{f}	37.74±0.29^{i}	50.59±0.16^{c}	53.80±0.09^{c}	0.92±0.01^{d}	0.73±0.01^{e}	0.71±0.01^{e}
PBG 5	8.62±0.02^{a}	7.69±0.07^{a}	6.66±0.03^{a}	51.99±0.08^{e}	47.56±0.13^{e}	37.78±0.20^{f}	0.96±0.01^{d}	1.18±0.03^{c}	1.07±0.01^{c}
PDG 4	7.37±0.05^{d}	5.36±0.04^{c}	6.52±0.05^{b}	43.55±0.07^{g}	39.79±0.09^{g}	37.14±0.40^{f}	0.96±0.01^{d}	0.96±0.01^{d}	1.51±0.03^{b}
GNG 1581	8.09±0.01^{b}	6.28±0.22^{b}	5.49±0.03^{c}	56.38±0.10^{c}	50.99±0.13^{c}	44.54±0.14^{e}	1.71±0.05^{b}	1.69±0.07^{a}	2.17±0.06^{a}
GNG 2285	6.64±0.14^{f}	5.49±0.05^{c}	4.06±0.01^{e}	82.24±0.18^{a}	63.50±0.39^{b}	57.67±0.26^{b}	0.46±0.02^{f}	0.71±0.01^{e}	0.68±0.01^{e}
HK-10-103	8.59±0.04^{a}	7.54±0.01^{a}	3.51±0.01^{g}	75.01±0.12^{b}	73.22±0.30^{a}	60.64±0.14^{a}	0.92±0.01^{d}	0.91±0.01^{d}	0.63±0.04^{e}
C judaicum 185B	1.74±0.04^{i}	0.63±0.04^{g}	0.38±0.01^{h}	54.68±0.29^{d}	49.55±0.39^{d}	43.76±0.16^{e}	0.66±0.01^{e}	0.44±0.03^{f}	0.32±0.01^{f}
C judaicum 185	2.43±0.07^{h}	1.21±0.01^{f}	0.32±0.02^{h}	46.75±1.17^{f}	41.05±0.18^{f}	37.32±0.90^{f}	1.48±0.01^{c}	1.27±0.10^{bc}	1.08±0.03^{c}
C judaicum 182	2.86±0.01^{g}	1.74±0.01^{e}	0.36±0.01^{h}	39.54±0.54^{h}	38.21±0.49^{h}	33.12±0.20^{g}	1.99±0.02^{a}	1.31±0.01^{b}	0.37±0.01^{f}

Data was subjected to tukey's test. Each value represents mean value ± S.D. Mean values marked with same alphabets are significantly not different. Data pooled for both years (2015-16 and 2016-17) for control, 50% reduction and 75% reduction.

Table 2. Morpho-physiological parameters of chickpea accessions under receding moisture conditions

	Shoot weight (g)			Plant height (cm)			Number of leaves		
Chickpea Accessions	Control	50% Reduction	75%Reduction	Control	50% Reduction	75%Reduction	Control	50% Reduction	75%Reduction
GL 12020	8.52±0.01^{e}	6.49±0.04^{d}	5.79±0.04^{b}	83.47±0.24^{bc}	78.49±0.14^{c}	73.12±0.11^{c}	64.19±0.32^{c}	50.24±0.29^{c}	23.59±0.22^{g}
PBG 7	7.57±0.07^{d}	6.76±0.01^{c}	5.20±0.01^{d}	70.62±0.33^{bc}	81.13±0.35^{b}	83.52±0.08^{b}	70.53±0.62^{a}	44.44±0.38^{d}	39.82±0.07^{b}
PBG 5	9.02±0.02^{f}	6.53±0.06^{d}	6.21±0.03^{a}	81.10±0.16^{bc}	71.66±0.05^{e}	60.33±0.03^{g}	65.87±0.08^{b}	51.37±0.59^{bc}	29.32±0.14^{e}
PDG 4	7.70±0.02^{d}	5.59±0.05^{e}	4.31±0.04^{e}	74.80±1.14^{bc}	65.48±0.11^{f}	61.64±0.26^{f}	58.85±0.24^{e}	52.97±0.06^{b}	31.89±0.10^{d}
GNG 1581	4.84±0.13^{c}	3.74±0.02^{f}	2.57±0.05^{f}	80.78±0.08^{bc}	74.40±0.98^{d}	66.32±0.36^{d}	53.26±0.28^{f}	44.67±0.64^{d}	40.95±0.11^{a}
GNG 2285	14.80±0.33^{g}	7.73±0.01^{b}	6.08±0.13^{a}	114.37±0.53^{a}	76.98±2.33^{c}	82.61±0.31^{b}	61.43±0.92^{d}	56.63±0.44^{a}	27.17±0.45^{f}
HK-10-103	9.35±0.04^{g}	8.30±0.11^{a}	5.59±0.10^{c}	108.35±0.21^{a}	104.40±0.44^{a}	87.32±0.29^{a}	54.57±0.47^{f}	44.09±0.57^{d}	34.03±0.34^{c}
C. judaicum 185B	2.62±0.03^{b}	1.43±0.01^{g}	1.18±0.01^{g}	75.29±0.23^{ab}	71.81±0.13^{e}	63.45±0.24^{e}	43.58±0.29^{g}	32.65±0.46^{f}	27.30±0.54^{f}
C. judaicum 185	1.64±0.01^{b}	0.96±0.08^{h}	0.30±0.03^{i}	61.79±1.59^{c}	58.18±0.16^{g}	60.48±0.94^{g}	43.08±0.06^{g}	33.61±0.98^{e}	26.61±0.30^{f}
C. judaicum 182	1.44±0.01^{a}	1.33±0.02^{g}	0.97±0.03^{h}	59.14±0.96^{c}	54.17±0.52^{h}	45.68±0.20^{h}	44.06±0.67^{g}	28.49±1.07^{g}	18.34±0.22^{h}

Data was subjected to tukey's test. Each value represents mean value ± S.D. Mean values marked with same alphabets are significantly not different. Data pooled for both years (2015-16 and 2016-17) for control, 50% reduction and 75% reduction.

Table 3. Root traits and root indices of chickpea accessions under receding moisture conditions

	RLD (gcm^{-3})			Root area (mm^{-2})			SW:RLD (g/gcm^{-3})		
Chickpea Accessions	Control	50% Reduction	75% Reduction	Control	50% Reduction	75% Reduction	Control	50% Reduction	75% Reduction
GL 12020	1.32±0.21^{b}	0.87±0.11^{abc}	0.80±0.06^{de}	1062.60±13.68^{d}	1447.90±16.29^{d}	1559.90±13.89^{d}	4.61±1.10^{c}	3.36±0.96^{a}	3.83±1.02^{a}
PBG 7	1.41±0.07^{b}	1.33±0.09^{ab}	1.23±0.02^{a}	1145.00±9.87^{c}	1512.50±8.88^{d}	1657.00±14.77^{d}	5.36±1.02^{c}	5.11±1.15^{d}	4.24±1.62^{d}
PBG 5	1.09±0.20^{c}	1.01±0.15^{abc}	0.94±0.02^{bcd}	1108.00±18.09^{cd}	1594.00±17.74^{c}	2691.40±15.90^{a}	8.47±0.98^{b}	6.60±1.77^{c}	6.63±1.67^{b}
PDG 4	0.93±0.06^{d}	0.78±0.01^{bc}	0.63±0.01^{c}	1485.00±12.06^{a}	2223.20±9.16^{a}	2406.80±21.14^{b}	8.28±2.14^{b}	7.20±2.78^{b}	6.87±1.16^{b}
GNG 1581	1.61±0.04^{a}	1.35±0.02^{a}	1.21±0.11^{b}	1333.70±10.09^{b}	1945.00±11.44^{b}	2435.20±17.21^{b}	3.01±0.16^{f}	2.78±0.78^{e}	2.14±0.90^{f}
GNG 2285	1.58±0.07^{a}	1.14±0.02^{ab}	1.04±0.21^{abcd}	874.26±9.00^{f}	1275.00±20.08^{e}	1590.10±14.12^{d}	9.39±3.01^{a}	6.79±1.11^{c}	6.15±1.65^{c}
HK -10- 103	1.58±0.08^{a}	1.10±0.11^{ab}	1.08±0.14^{abc}	2181.70±23.11^{c}	3968.60±24.45^{b}	7926.00±13.37^{c}	5.93±1.19^{d}	7.62±1.89^{a}	5.30±1.76^{d}
C. judaicum 185B	1.08±0.21^{c}	0.68±0.04^{c}	0.88±0.34^{cd}	721.22±11.11^{g}	847.90±9.44^{g}	997.80±8.15^{f}	2.47±0.45^{g}	2.10±0.22^{f}	1.49±0.05^{g}
C. judaicum 185	1.37±0.37^{b}	1.13±0.37^{ab}	0.98±0.15^{abcd}	825.68±16.44^{f}	727.90±7.11^{h}	997.29±6.37^{f}	1.26±0.23^{h}	0.93±0.02^{g}	0.30±0.01^{i}
C. judaicum 182	1.00±0.09^{d}	1.01±0.17^{abc}	1.20±0.08^{ab}	972.50±10.01^{e}	1071.70±10.39^{f}	1264.30±14.16^{e}	1.44±0.15^{h}	1.33±0.16^{g}	0.80±0.01^{h}

Data was subjected to Tukey's test. Each value represents mean value ± S.D. Mean values marked with same alphabets are significantly not different. Data pooled for both years (2015-16 and 2016-17) for control, 50% reduction and 75% reduction.

The mechanisms of differential response can be explained via the root-shoot allometric relationships. Genotype PBG 7 and wild accessions exhibited decrease (20.02% and 27.36%) at 50% reduction in root:shoot resulting in the accumulation of photosynthates towards shoot growth, thus being less affected by drought stress (Table 1). In contrast some of the genotypes depicted increased root:shoot ratio thus partitioning its photosynthates towards below ground system for the expansion of root area. Among kabuli genotypes, GNG 2285 exhibited maximum increase of 53.46% and 46.36% at 50 and 75% moisture reduction in comparison to control. Higher root:shoot in turf grass explained the drought tolerance to channelize and expand its roots against water scarcity that ultimately improved the hydraulic status of the former (Karcher *et al.,* 2008).

The root length density (RLD) declined in desi and kabuli chickpea genotypes at 50 and 75% reduction levels. The tolerance level of wild accessions was exhibited in terms of considerable increase of 1.29 and 1.18 folds in *C. judaicum* 185B and *C. judaicum* 182 from 50% to 75% reduction to combat reduction in moisture conditions (Table 3). PBG 7, a desi chickpea check variety, showed lowest decline of 1.06 and 1.15 folds at 50% and 75% moisture reduction, respectively. The tolerance mechanism of this variety against water stress coincided with resistance against Ascochyta blight, wilt and dry root rot (PAU, 2021). Poorter *et al.,* (2012) observed that plants exposed to severe drought stress portray an array of responses *viz*., increase in root mass fraction (root biomass relative to total biomass), root biomass being compensatory, as a drought avoidance strategy.

There was an overall significant decrease in the root length of 4.77 % at 50% reduction and 13.69 % at 75% reduction respectively (Table 1). The drought stress impeded serious effects of 22.79% and 29.88% reduction in root length in GNG 2285 at 50% and 75% water reductions, respectively. On the contrary there were some genotypes that expanded its length to acquire water

from the deeper strata. Desi genotype PBG 7 increased root length by 34.04% and 42.54% at 50 and 75% water reduction. Root area expanded on exposure to water deficit conditions by depicting an overall upheaval of 36.21% and 65.73% at 50 and 75% water reduction, respectively (Table 3). Desi genotypes were more efficient in expansion of root horizons in comparison to wild and kabuli accessions. Amongst them, check varieties PDG 4 and PBG 5 indicated maximum root area increase of 49.71 and 43.86% at 50% water reduction and 62.07 and 142.91% at 75% reduction in water in comparison to control.

Kabuli variety HK-10-103 exhibited increase in root area of 66.59% and 71.45% at 50% and 75% water reduction, respectively, in comparison to control. However, the major proportion in increase of root area (52.75%, 78.64%) in the kabuli genotypes was recorded when the moisture content was reduced to 75% from 50% in comparison to control. *C. judaicum* 185B wild accession expanded its root horizons by 17.56 and 38.35% when water was reduced to 50% and then to 75% in comparison to control, respectively. The results are in complete agreement with the response of maize and sunflower cultivars that maintains high carbon assimilation and water use efficiency during the course of drought tolerance by depicting deeper root system (Ghannoum, 2009). Increase in root surface area via root hairs promote contact with soil particles thus compensating for reductions in root elongation in extremely dry soils (Wasson *et al.,* 2012). In *Silene vulgaris* Franco *et al.* (2008) reported that branching of the roots, root surface area and total root length increased under moderate drought stress.

Moisture stress negated the growth of plant by reducing the plant length on exposure to receding moisture conditions. There was an overall decline of 7.83% and 14.30% in plant length thus deciphering the stress conditions (50 and 75% reduction, respectively) in comparison to control (Table 2). Amongst all genotypes, GNG 2285, a kabuli genotype, exhibited 32.70 and 27.77% decline at 50 and 75% moisture reduction amongst all genotypes. However, PBG 7 recorded an upheaval of 14.88 % decline at 50% reduction and further 2.95 % on exposure to 75% reduction in plant length. Ammar *et al.* (2014) reported reduction in plant height in Faba bean on being exposed to drought stress created by different concentrations of PEG in managed and open field conditions. Drought stress in Faba bean suppressed cell elongation due to low turgor pressure (Shao *et al.,* 2008) that caused interruption of water flow from the xylem to the surrounding elongating cells. Drought-induced alteration in the homoeostasis of phytohormones was another reason for growth reduction under water deficit conditions (Farooq *et al.,* 2009).

The number of leaflets is directly proportional to the photosynthetic efficiency of plant. Drought stress alters the capacity by negatively affecting the number of leaves under drought stress. Analysed data shows significant differences at both 50% and 75% moisture reduction levels (Table 2). The decline was evident in genotypes GL 12020 (63.24%), *C. judaicum* 182 (58.36%) and GNG 2285 (55.77%) at 75% water reduction in comparison to control. At 50% water reduction, PBG7 showed significant reduction of 36.99% in comparison to control.

In concordant to our studies, there had been a decline in number of leaves in variants of *Arabidopsis* when exposed to mild stress conditions (Clauw *et al.,* 2015). Emergence of leaves is a genetic phenomenon rather than morphological one. During the onset of stress, several other defence and signalling (ABA) genes get upregulated. The upregulation in the levels of ABA altered the emergence of leaf in *Arabidopsis* (Baerenfaller *et al.,* 2012).

The effect was more pronounced in shoot biomass when levels were reduced from 50 to 75% moisture reduction by declining mean shoot weight by 26.10%. Among kabuli types, GNG 2285 showed maximum reduction of 47.75% at 50% moisture level (Table 2). Stress disturbs the delicate balance of partitioning of photosynthates to below ground system thus reducing the probability of biomass storage and utilizing it in extension to withdraw more water from lower soil strata (Jaleel *et al.,* 2009). Webber *et al.* (2006) reported that common bean and green gram exhibited decrease in root and shoot weight on being exposed to drought stress. Similar responses have also been reported in field and laboratory experiments where decline in biomass compensates for drought avoidance strategies (Herzog *et al.,* 2014, Zang *et al.,* 2014). Drought stress tends to disturb the carbon partitioning and source sink relations that ultimately limit the storage pools in the above and below ground systems. The dynamics are further interfered when the assimilates like sucrose are deviated to form osmolytes for osmolyte adjustment rather than the formation of storage pools thus leading

to breakdown of sucrose and overall reduced biomass (Hasibeder *et al.,* 2015).

Shoot dry weight to root length density ratio (SW:RLD) is the relevant trait portraying effectiveness of roots in shoot production. This parameter explores the behavioural changes of partitioning the photosynthates that occur on account of facing stress. There was an overall decrease of 8.29 % and 28.82 % in the ratio at 50 and 75% water reduction over control (Table 3). The maximal decline in ratio was observed in *C. judaicum* 185 (74.82%) and GNG 2285 (37.67%) at 75% reduction in comparison to control. The tolerance of HK-10-103 showed striking results by an increase in ratio by 27.39 % at receding moisture levels. Drought stress immensely affects the shoot weight. So exploring the equation, if the decline in SW kept constant, the ratio mainly depends on the value of RLD. The variation in ratio thus is compensated by alteration in RLD values. The ratio was severely compromised in mini-core chickpea accessions by showing low values of heritability pointing towards the negative influence of soil drying and ultimately water deficit (Kashiwagi *et al.,* 2005).

The tolerance of accessions has an inversely proportional relationship with the MPI that is an indicator of leakiness of membranes. There was significant variation amongst all accessions depicting an overall damage of 56.66 and 84.57% at 50 and 75% reduction respectively in comparison to control (Fig. 2). Certain genotypes were affected very severely thus reporting a damage of 176.4% (PBG 7), 163.78% (PDG 4) and 125.73% (HK-10-103) at 75% moisture reduction in comparison to control. On the contrary, there were several genotypes that were able to maintain homeostasis by being reluctant to any change on exposure to drought conditions. Wild accessions *viz*., *C. judaicum* 185 and *C. judaicum* 182 effectively managed to show reluctance to damage by having only 11.04 and 18.41% increase in MPI when stress levels were elevated from 50 to 75%. Stress alleviates the damage to membranes, thus disturbing the ionic homeostasis. The maize tolerant cultivar Giza 2 exhibited high concentration of osmolytes *viz*., proline, glycine betaine, trehalose to nullify the effects of drought stress condition (Moussa and Abdel-Aziz, 2008).

Stress affects viability in terms of respiratory enzymes (dehydrogenases) exhibiting significant differences amongst accessions at receding moisture levels. There had been overall decline of 27.45 and 44.51% in cellular respiration at 50 and 75% moisture reduction (Figure 3). Amongst desi genotypes, PBG 5 showed fragile behaviour by recording 44.02 and 59.42 % decline in dehydrogenase activity at 50 and 75% reduction, respectively. The enzymes remained efficient in GNG 1581 (9.65%) at 50% reduction and at 75% reduction in PDG 4 (20.30%) highlighting their tolerant behaviour of resisting denaturing of dehydrogenases at respective moisture stress levels. Kabuli genotypes in proportion showed lower efficiency of dehydrogenases than desi and wild accessions reporting maximal damage of 56.60% in HK-10-103 at 75% water reduction. Tolerant lines effectively shield dehydrogenases via osmolytes from getting denatured under stress conditions. The cells with active respiratory enzymes will more efficiently produce ATP and eventually explore water by expanding its horizons in deep soil strata. Bent grass cultivar 'Independence' maintained optimal root viability from 0-20 cm of soil depth thus providing better drought tolerance capacity (McCann and Huang, 2008).

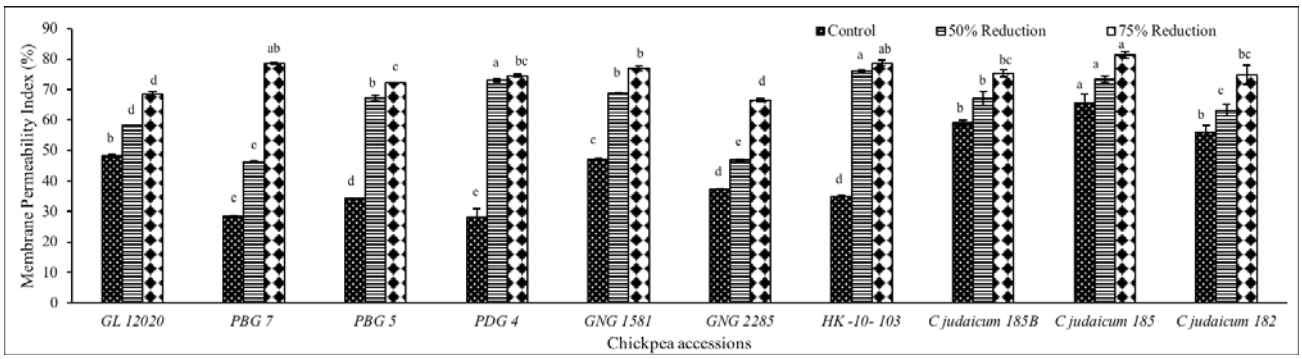

Fig. 2. Membrane permeability index (%) of chickpea accessions under receding moisture conditions. Each bar represents mean value ± S.E. Mean values marked with same alphabets are significantly not different. Data pooled for both years (2015-16 and 2016-17) and for control, 50% reduction and 75% reduction.

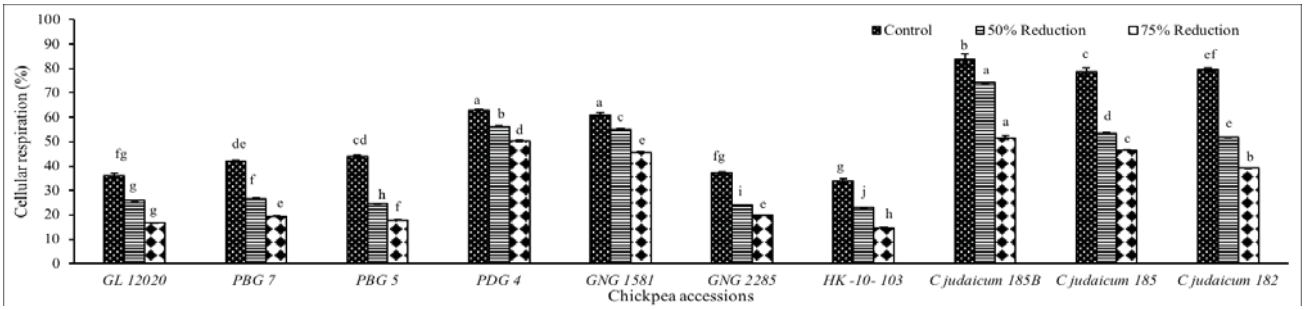

Fig. 3. Cellular respiration (%) of chickpea accessions under receding moisture condition. Each bar represents mean value ± S.E. Mean values marked with same alphabets are significantly not different. Data pooled for both years (2015-16 and 2016-17) and for control, 50% reduction and 75% reduction.

Table 4. Correlation between root indices, morphological parameters and stress indices under receding moisture conditions

	RLD	MPI	CR	RA	SRLD	RW	RL	RSR	SW	PH	NOL
RLD	1.00										
MPI	-0.15	1.00									
CR	-0.38	0.69	1.00								
RA	0.05	-0.37	-0.34	1.00							
SRLD	-0.16	**-0.75***	**-0.74***	**0.61***	1.00						
RW	0.18	-0.59	**-0.69***	**0.88***	**0.81***	1.00					
RL	0.47	-0.34	-0.59	0.11	0.43	0.36	1.00				
RSR	0.28	0.38	0.29	0.41	-0.33	0.18	-0.40	1.00			
SW	0.18	**-0.82***	**-0.88***	0.52	**0.93***	**0.80***	**0.66***	-0.36	1.00		
PH	0.41	-0.49	**-0.72***	0.30	**0.61***	0.56	**0.94***	-0.42	**0.80***	1.00	
NOL	0.24	**-0.79***	**-0.69***	**0.71***	**0.81***	**0.88***	0.37	-0.05	**0.84***	0.59	1.00

*Significant at 0.05 level.

RLD- root length density, MPI- membrane permeability index, CR- cellular respiration, RA-root area, SRLD- shoot weight: root length density, RW-root weight, RL-root length, RSR- root:shoot ratio, SW-shoot weight, PH- plant height, NOL-number of leaves.

Path coefficient and correlation analysis

The tolerance capacity of the desi genotypes is attributed to high root area that contributes directly to root length density (P=0.89) (Table 5). Root area of genotypes positively and strongly correlated with the partitioning of photosynthates i.e. SW:RLD (r=0.61) and root weight (r=0.88) (Table 4). Under receding moisture conditions morphophysiological parameters are tightly linked *viz*., shoot weight that further strongly correlated with plant height (r= 0.80) and number of leaves (r=0.84) and shoot biomass forms an important component to the root length density (P=0.62). The epicenter of moisture deficit tolerance relies strongly on the relative values of root length density, that further is instrumental in maintaining yield stability under terminal drought stress as was evidenced in sorghum (Kashiwagi *et al*, 2005).

Table 5. Path coefficient analysis depicting the direct effects of membrane permeability index (MPI), CR (cellular respiration), RL (root length), SW (shoot weight), RW (root weight), RSR (root shoot ratio), PH (plant height), NOL (number of leaves) and RA (root area) on root length density (RLD) under receding moisture conditions.

Physiological parameters	RLD
MPI	P=0.68
CR	P=0.27
RL	P=0.17
SW	P=0.62
RW	P=0.62
RSR	P=0.43
PH	P=0.23
NOL	P=0.50
RA	P=0.89

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RESEARCH ARTICLE

Screening of Black Gram (*Vigna mungo*) and its Crop Wild Relatives against *Callosobruchus maculatus* (Fab.) and Correlation of Resistance with Seed Physical Parameters

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Black gram [*Vigna mungo* (L.) Hepper] a potential grain legume is greatly affected by bruchid infestation in production and post-harvest storage. Moreover, there is dearth of natural and reliable sources of bruchid resistance in cultivated black gram. Therefore, a set of 69 germplasm accessions, representing landraces of black gram and its crop wild relatives (CWR) were assessed for resistance against *Callosobruchus maculatus* (Fab.). Accession IC259504 (*V. vexillata*) showed complete resistance whereas IC424616 (*V. mungo*) was highly resistant to bruchid infestation based on the three key growth traits viz., adult emergence (AE), per cent seed weight loss (PSWL) and growth index (GI). Correlation heat matrix indicated AE was positively correlated with GI (r= 0.80) and PSWL (r= 0.72). Seed hardness showed a significant negative correlation with AE (r= -0.38). The resistant accessions could be utilized in various breeding programs for the development of bruchid resistant cultivars in black gram and its other related *Vigna* species.

Key Words: Black gram, Bruchid, Crop wild relatives, Landraces, Resistance, Wild *Vigna*

Introduction

Pulses occupy a significant position in Indian agriculture as they form the cheapest source of plant-based proteins (25-40%). They have been rightly called the "poor man's meat" providing an ideal blend of minerals and essential amino acids (Jayasena and Abbas, 2016; Vishwajith *et al*., 2020). Black gram [*Vigna mungo* (L.) Hepper], an Indian origin potential grain legume, is grown and consumed primarily in South East Asian region (Indhu *et al*., 2018). India tops in black gram production being cultivated over an area of 3.81 million ha, thus accounting for more than 70% of global production. In 2018-19, the national production of black gram accounted for 3.36 million tonnes, with Madhya Pradesh being the leading producer state (Anonymous, 2019). But production, storage and nutritional quality of black gram is significantly constrained by post-harvest damage from bruchids especially *Callosobruchus maculatus* (Fab.). Bruchid infestation during storage has been observed to cause around 50% decrease in seed weight and protein in black gram (Gujar and Yadav, 1978). Indiscriminate use of insecticides and other chemical control measures for its management present high risk of persistent toxic residues, resistant strains and environmental pollution (Soumia *et al*., 2017). Identifying natural and sustainable sources of bruchid resistance therefore appears to be a cost-effective and environmentally beneficial approach for reducing storage damage (Tripathi *et al*., 2020). However, bruchid resistance sources are scarce in cultivated black gram (Duraimurugan *et al*., 2014; Tripathy, 2016). Hence, the present experiment was undertaken to evaluate black gram germplasm and its crop wild relatives (CWRs) for resistance against *C. maculatus* under artificial infestation set up following no-choice protocol.

Materials and Methods

Experiments were carried out in the laboratory of Division of Plant Quarantine, ICAR-National Bureau of Plant Genetic Resources, New Delhi. Insect cultures were reared on a local black gram variety and maintained in B.O.D incubator at 28º and 65% RH. A total of 55 landrace accessions of black gram and 10 wild *Vigna* accessions representing its CWRs (Table 1) were screened during 2020-21 against the test insect, *C. maculatus* following "no-choice" artificial infestation

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Table 1. Collection sites of germplasm accessions

S.No.	Accession	State
1	IC426766	Andhra Pradesh
2	IC436702	Andhra Pradesh
3	IC436770	Andhra Pradesh
4	IC541882	Andhra Pradesh
5	IC598464	Andhra Pradesh
6	IC628759	Andhra Pradesh
7	IC628781	Andhra Pradesh
8	IC394168	Assam
9	IC394232	Assam
10	IC394276	Assam
11	IC394301	Assam
12	IC394436	Assam
13	IC394448	Assam
14	IC394479	Assam
15	IC394551	Assam
16	IC394940	Assam
17	IC395030	Assam
18	IC545207	Assam
19	IC398744	Bihar
20	IC417875	Bihar
21	IC628811	Jammu & Kashmir
22	IC392275	Jharkhand
23	IC424616	Jharkhand
24	IC447791	Jharkhand
25	IC393540	Karnataka
26	IC471981	Kerala
27	IC472021	Kerala
28	IC472034	Kerala
29	IC472054	Kerala
30	IC396791	Madhya Pradesh
31	IC568908	Odisha
32	IC568947	Odisha
33	IC569057	Odisha
34	IC569080	Odisha
35	IC557279	Sikkim
36	IC557292	Sikkim
37	IC557300	Sikkim
38	IC401376	Tamil Nadu
39	IC436519	Telangana
40	IC436644	Telangana
41	IC626440	Tripura
42	IC626441	Tripura
43	IC626446	Tripura
44	IC371765	Uttar Pradesh
45	IC393526	Uttar Pradesh
46	IC393543	Uttar Pradesh
47	IC393545	Uttar Pradesh
48	IC393550	Uttar Pradesh
49	IC393551	Uttar Pradesh
50	IC541046	Uttar Pradesh
51	IC140825	Uttarakhand
52	IC436951	Uttarakhand
53	IC556551	Uttarakhand
54	IC449258	West Bengal
55	IC449265	West Bengal
56	IC524639	Andhra Pradesh
57	IC553505	Andhra Pradesh
58	IC553510	Andhra Pradesh
59	IC553520	Andhra Pradesh
60	IC553532	Andhra Pradesh
61	IC553516	Andhra Pradesh
62	IC331454	Chattisgarh
63	IC331457	Odisha
64	IC553517	Andhra Pradesh
65	IC259504	Goa

*Germplasm were obtained from the National Genebank, ICAR-National Bureau of Plant Genetic Resources, New Delhi, India

Table 2. Responses of various accessions to *C. maculatus* infestation in terms of growth parameters

S.No.	Trait	Minimum	Maximum
1	OP	9.67 (IC331454)	56.0 (IC436519)
2	AE (%)	0.00 (IC259504)	89.44 (IC553517)
3	Exit holes (No.)	0.00 (IC259504)	19.33 (IC371765.IC394479)
4	PSWL (%)	0.84 (IC259504)	63.48 (IC541046)
5	GI	0 (IC259504)	22.46 (IC524639)
6	MDP (days)	0 (IC259504)	13.0 (IC140825)

*OP- oviposition (per 20 seeds), AE- adult emergence, PSWL- % seed weight loss, GI- growth index, MDP- mean development period

protocol as described by Giga (1995). Experimental set up also contained four checks viz., KU-6, MASH-114, PU-11-14 and IPU-2-43. Identified resistant and susceptible accessions were validated to confirm their responses to bruchid.

Insect growth parameters: Newly emerged adults were released for oviposition (72 hrs) @ 2 pairs per 20 seeds each accession replicating 5 times. Observations on adult emergence (24 hr interval) were continued till no fresh adults appeared. Various insect growth parameters were calculated as follows:

Adult emergence (AE) % = $\frac{\text{(no. of adults emerged/}}{\text{No. of eggs laid)}} \times 100$ (Howe, 1971).

Mean development period (MDP) = $\frac{D_1A_1 + D_2A_2...+D_nA_n}{}$

Table 3. Reaction of studied accessions to *C. maculatus* based on GI

Groups	Range	Number of accessions	List of accessions
I	0	1	IC259504
R	0.1-2.0	4	IC424616, IC436519, IC598464, IC626440
MR	2.0-4.0	9	IC140825, IC436644, IC436702, IC541882, IC557300, IC569057, IC569080, KU-6, MASH-114
MS	4.0-6.0	16	IC371765, IC393540, IC394168, IC393545, IC394232, IC394448, IC394940, IC396791, IC449265, IC472054, IC556511, IC557279, IC568908, IC568947, IC626441, IC394551
S	6.0-8.0	12	IC392275, IC393526, IC394276, IC394301, IC395030, IC398744, IC401376, IC426766, IC471981, IC472034, IC557292, IC628781
HS	>8.0	27	IC393543, IC393550, IC393551, IC394436, IC394479, IC417875, IC436770, IC436951, IC447791 IC449258, IC472021, IC541046, IC545207, IC626446, IC628759, IC628811, IC331454, IC524639, IC553505, IC553510, IC553516, IC553517, IC553520, IC553532, PU-11-14, IPU-2-43, IC331457

*I- immune; R- resistant; MR-moderately resistant; MS- moderately susceptible; S- susceptible; HS- highly susceptible

Total no. of adults emerged

where D_1 is the day on which adult emergence commenced (first day), A_1 is the number of adults emerging on D_1th day (Howe, 1971)

$$\text{Growth index (GI)} = \frac{\text{AE}}{\text{MDP}} \text{ (Jackai and Singh, 1988)}$$

$$\text{Percent seed weight loss (PSWL)} = \frac{(N_1 - N_2)}{N_1} \times 100$$

where N_1 is weight of fresh seeds (g) and N_2 is weight of damaged seeds (g) (Eker *et al*., 2018)

Seed parameters: Quantitative and qualitative seed traits were also studied to comprehend the physical basis of resistance in the accessions. Observations on qualitative parameters of seed viz., seed coat colour, texture, lustre, seed shape and hilum concavity were recorded as per descriptor list of IBPGR (1985). The quantitative physical traits included 100-seed weight, seed length and width, length-width ratio and seed hardness. Hundred seed weight (in grams) was determined by weighing 100 seeds of uniform size in an analytical balance. Seed length and width were measured in millimetre using Vernier calliper. Texture analyzer was employed to measure the seed hardness and expressed in Newton.

Statistical Analysis: The strength of the relationship and extent of correlation between physical seed parameters, and specific growth parameters of bruchid was analysed by simple linear correlation estimation with the help of PAST-3 (Paleontological Statistics) software (Hammer *et al*., 2001).

Results and Discussion

Insect growth parameters: Studies showed that accessions had significant differences in their response to *C. maculatus* in terms of growth traits viz., total oviposition, number of exit holes, AE%, GI, PSWL and MDP (Table 2). Accession IC259504 (*V. vexillata*) exhibited complete resistance and IC424616 (landrace from Jharkhand) showed high resistance to bruchid infestation in terms of three vital life parameters viz., AE, PSWL and GI. Based on GI, the accessions were categorized as immune, resistant, moderately resistant, moderately susceptible, susceptible and highly susceptible (Table 3). Two checks (KU-6 and MASH-114) were observed to be moderately resistant whereas, other two checks (PU-11-14 and IPU-2-43) showed high degree of susceptibility to *C. maculatus*.

Physical seed parameters: Considerable variability was observed for quantitative seed traits (Table 4). The studied accessions exhibited wide variation in their qualitative seed traits (Figure 1). Majority of the accessions were being smooth textured (88.23%), black coloured (55.88%) coupled with dull appearance (86.76%), ovoid shaped (52.94%) with non-concave hilum (52.94%).

Table 4. Variable response of studied accessions to *C. maculatus* infestation in terms of quantitative seed parameters

S.No.	Trait	Minimum	Maximum
1	SH (N)	11.64 (IC553510)	77.37 (IC472021)
2	HSW (g)	0.76 (IC331454)	5.91 (IC628781)
3	SL (mm)	2.33 (IC331454)	5.98 (IC436770)
4	SW (mm)	1.63 (IC553532)	4.47 (IC628759)
5	LWR	1.13 (IC436519)	1.74 (IC436770)

* SH- seed hardness, HSW-100-seed weight, SL- seed length, SW- seed width, LWR- length-width ratio

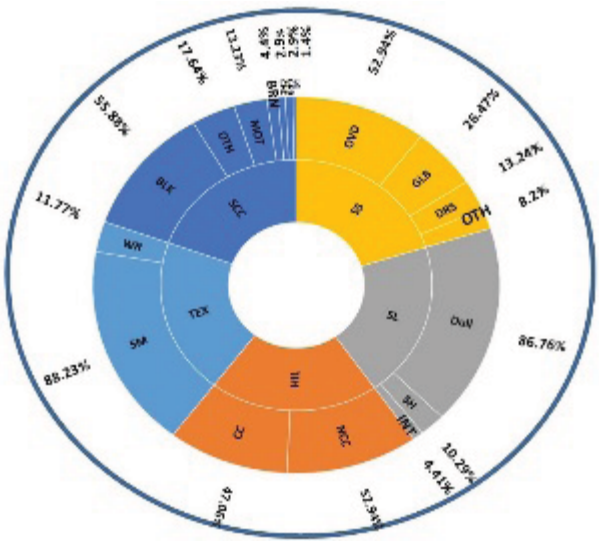

Fig. 1. Frequency distribution of qualitative seed traits
*SS seed shape (OVD ovoid, GLB globose, DRS drum shaped, OTH others); SL seed lustre (Dull dull, SH smooth, INT intermediate); HIL hilum (NC non concave, CC concave); TEX seed texture (SM smooth, WR wrinkled); SCC seed coat colour (BLK black, MOT mottled, BRN brown, CHC chocolate, GRB greenish brown, LG light green, OTH others)

Correlation studies: Correlation heat matrix (Figure 2) of growth and quantitative seed traits of various accessions revealed a significant positive correlation of AE with GI (r= 0.80) and significant negative correlation with MDP (r= -0.54) and seed hardness (r = -0.38). This was in conformity with earlier reports (Soumia *et al*., 2017; Amusa *et al*., 2018) where chemical deterrent inside the seed and physical barriers on seed coat obstructed adult emergence and prolonged the developmental period. Moreover, PSWL was significantly positively correlated with AE (r=0.83) and number of exit holes (r=0.71) which was congruent with earlier findings of Dasbak *et al*. (2009) and Tripathi *et al*. (2020). This could be attributed to the fact that larva of the bruchid is the sole damaging stage on account of its feeding on starchy seed contents. Significant positive correlation of total oviposition with 100-seed weight (r=0.51) indicated bruchid's preference to lay eggs on seeds with more storage reserves. Further, in the present study number of eggs laid by bruchid did not exhibit strong and uniform correlation with any of the studied qualitative seed parameters of the accessions. Similar findings were also reported by Pankaj *et al*. (2011).

Conclusion

In the present study, it was found that the black gram and its CWR accessions exhibited considerable variability in their physical seed attributes. Besides, the growth parameters of the bruchid, *C. maculatus* varied significantly among the accessions. Resistant accessions identified in the study namely, IC259504 and IC424616 displayed immunity and high degree of resistance respectively in terms of three vital growth parameters (AE, PSWL and GI). Hence, these validated accessions could be further utilized in breeding programmers to introgress bruchid-resistant genes into agronomically improved black gram cultivars which could increase the production manifold and prolong shelf life.

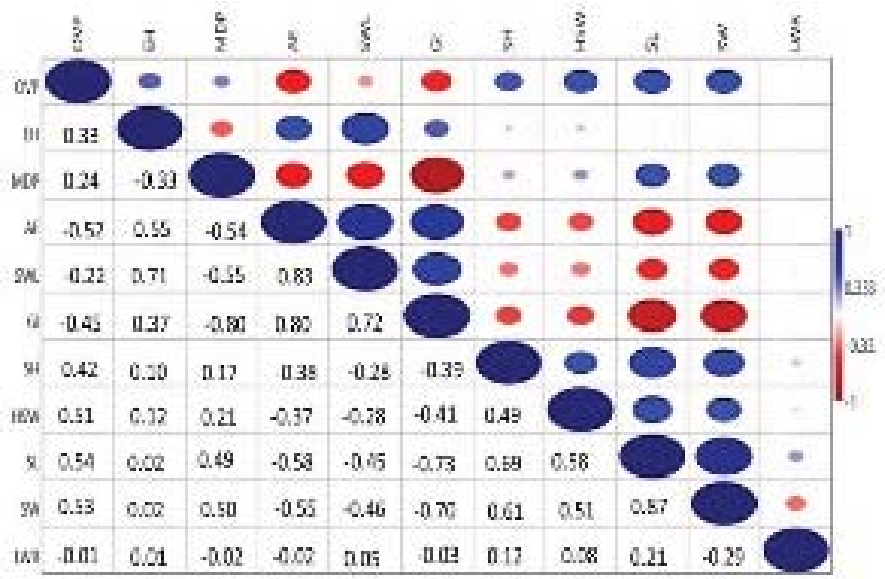

Fig. 2. Correlation heat map of insect parameters and physical traits of seeds
∗Wtl % seed weight loss; Ade % adult emergence; GI growth index; EMH emergence holes; MDP mean development period; SH seed hardness; HS 100-seed weight; LWR length-width ratio. Significant correlations are coloured either in blue (positive) or red (negative).

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Conflict of Interest

The authors declared no conflict of interest in the content of the manuscript and study undertaken.

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RESEARCH ARTICLE

Comparison of Three Cryotechniques for Conservation of Banana (*Musa* AAA, Cavendish Subgroup) Genotypes

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The aim of this study was to compare three cryotechniques, namely, droplet vitrification (DV), vitrification-cryo-plate (VCP) and dehydration-cryo-plate (DCP) on proliferating meristems of two *Musa* (AAA, Cavendish Subgroup) genotypes. The experiment was set up as factorial completely randomized design for the two *Musa* accessions (IC 250462 cv 'Borjahaji' and EC 493718 cv 'Williams') in five different dehydration duration (30 -150 min). Regrowth after cryopreservation in all treatments was predominantly of two types - shoot regeneration and callusing. In both the cultivars, shoot regrowth of non-frozen and LN treated meristems were not impacted significantly by duration of dehydration exposure, however, callus growth was significantly different with increasing time of exposure. The mean shoot regrowth for all dehydration treatments in Borjahaji was not significantly different. However, the VCP method in Williams, with mean shoot regrowth of 75.5 ± 2.8% was found to be significantly ($P \leq 0.05$) better than that obtained from DV (63.1 ± 2.5%) and DCP (64.8 ± 1.9%). Quantitatively, highest shoot regrowth was obtained after 120 min PVS2 exposure in both cultivars, but using VCP method in Williams (86.7 ± 7.7%) and DV method in Borjahaji (73.1 ± 2.7%). Taking into consideration practical aspects for each technique, any of the three cryopreservation approaches can be used for cryobanking of proliferation meristems of *Musa*, as DV offers cost-effectiveness, while VCP and DCP have ease of explant handling, and DCP avoids chemical toxicity of PVS2.

Key Words: D-cryo-plate (DCP), Dehydration duration, Droplet vitrification (DV), *Musa* germplasm, Plant vitrification solution 2 (PVS2), Proliferating meristems, V-cryo-plate (VCP)

Introduction

The genus *Musa* (Family Musaceae, Order Zingiberales) native to South-East Asia (Simmonds, 1962), is globally known for the commercially important crops of bananas and plantains. These provide starchy staple fruit/food across some of the poorest parts of the world, including Africa and Asia. Both bananas and plantains serve as an important source of carbohydrates, dietary fibre, protein, fat and essential vitamins such as A, B_6 and C and also contain moderate amount of potassium, magnesium, phosphorus, calcium and iron (Pareek, 2016). They are cultivated in more than 135 countries and the annual world production accounts for about 158 million tons i.e. 116.8 million tons for banana and 41.6 million tons for plantain (FAOSTAT, 2019). Out of recognized 50 subgroups of *Musa*, about 51% of the global production come from Cavendish subgroup of banana (ProMusa, 2016).

India is the leading country in banana production, with current annual production of nearly 30 million t. A large diversity of genus *Musa* comprising seeded wild species to seedless cultivars with a variety of ploidy (2x, 3x, 4x) and different genomic composition (AA, AAA, AAB, AB, ABB, BB, ABBB) are widely distributed especially in the North-eastern India (Uma *et al*., 2019, 2020). Banana production is threatened by several significant pests and diseases such as black Sigatoka, *Fusarium* wilt, bacteria wilt, banana bunchy top virus, banana streak virus, nematodes (Jones, 2018; Ploetz, 2021). Climate change is likely to increase frequency, intensity, and duration of biotic stresses as well as abiotic stresses whether from water, salinity, wind or temperature and can lead to the instability and fluctuation in yield, production and price. The alternative solution is breeding of new cultivars combining disease resistance, abiotic stress tolerance and value-added nutritional and agronomic qualities of cultivated clones.

Plant genetic resources are valuable genepools for many desirable traits including yields, nutrition quality, resistance to biological and environmental effects for

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crop improvement. Safeguarding genetic resources for ensuring food security and for the development of varieties that are more productive and resistant to biotic and abiotic stresses is an urgent need. Complementary conservation of *Musa* genetic resources will enhance the availability and maintenance of plant genetic resources as a safeguard for the changing world (Van den Houwe *et al*., 2020) Conservation of *Musa* genetic resources, including cultivated varieties and wild species, has been carried out in the past in field genebanks and in *in vitro* genebanks and more recently in cryogenebanks (Agrawal *et al*., 2004, 2008, 2019; Panis *et al*., 2020). Numerous germplasm accessions of *Musa* have been collected and conserved around the world, including those in Indonesia, Malaysia, Thailand, the Philippines, India, Honduras, Jamaica, Brazil, Cameroon, and Nigeria, with the largest collections in *in vitro* being at International Transit Centre (ITC) in Leuven, Belgium with a total of 1,617 accessions (Van den houwe *et al*., 2020) and 444 accessions in ICAR-NBPGR, India.

For the long-term and biosecure storage of key germplasm collections, cryo-storage or cryopreservation is the best option as a backup of *in vitro* collection. Cryopreservation in which plant materials are stored at ultra-low temperatures usually in liquid nitrogen (-196˚C) and/or in the vapour phase of LN (-135°C to -180 ºC) is the most practical method for long-term conservation since all physical and chemical reactions and time-related changes are almost arrested at these ultra-low temperatures allowing biological samples to be preserved for unlimited periods (Agrawal and Tyagi, 2014; Panis *et al*., 2020). Currently, it is feasible to use this technique for conservation of different variety of plant germplasm and also different explants including pollen, seeds, shoot tips, dormant buds, cell suspensions, embryonic cultures, somatic and zygotic embryos and callus tissue (Agrawal *et al*., 2019, Bettoni *et al*., 2021).

Cryopreservation protocols in *Musa* have been reported in embryogenic cell suspension cultures using slow-freezing method (Panis *et al*., 1990), simple freezing of sucrose precultured proliferating meristematic clumps (Panis *et al*., 1996), vitrification of banana apical meristems (Thinh 1999), droplet vitrification (DV) of *Musa* proliferating meristems (Agrawal *et al*., 2004), droplet vitrification of *Musa* shoot tips (Panis *et al*., 2005) and air-desiccation method for seeds/zygotic embryos of diploid *Musa* spp.(Singh *et al*., 2021). In recent years, to facilitate easier handling of samples during cryopreservation stages, new-age protocols called vitrification cryo-plate (VCP), dehydration cryo-plate (DCP) and cryo-mesh have been successfully developed for several species (Yamamoto *et al*., 2011; Niino *et al*., 2013; Funnekotter *et al*., 2017). The cryo-plates techniques are adaptation to the encapsulation-vitrification and encapsulation-dehydration protocols, aimed at providing greater stability, safety and tolerance of the explants to sudden changes in temperature, using aluminum microplates containing 10-12 oval wells. The VCP is based on PVS2 dehydration, while the DCP is based on air dehydration in the laminar flow chamber for a controlled time. However, there is constraint that the use of specific protocol is genotype specific i.e. some genotypes are recalcitrant to some protocols. Although about 100 accessions of *Musa* have been cryobanked in our laboratory at ICAR-NBPGR, results show high genotypic variability, with some accessions remaining recalcitrant to the DV protocol using proliferating meristems. Thus, the aim of this study was to compare three cryotechniques (DV, VCP and DCP) on proliferating meristems of two *Musa* (AAA, Cavendish Subgroup) genotypes, as a pilot study to further optimize cryobanking of diverse *Musa* genetic resources.

Material and Methods

Plant Material

In vitro shoot cultures of *Musa* belonging to genomic group AAA and subgroup Cavendish were sourced from the *in vitro* Gene Bank (IVGB) of ICAR-National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India. Two genotypes namely 'Borjahaji' (IC 250462) and 'Williams (Bell South Johnstone)' (EC 493718, henceforth referred as 'Williams') were used for this study. Shoot cultures were multiplied on a standard P5 multiplication medium comprising MS salts supplemented with 10 µM 6-benzylaminopurine (BAP), 1 µM Indole-3-acetic acid (IAA), 1 µM ascorbic acid, 3% sucrose (0.09 M) and gelled with 0.25% Phytagel™ (Panis, 2009). All chemicals used were of Sigma Aldrich, USA, except sucrose which was from HiMedia Lab, Mumbai, India. Cultures were maintained at 25 ± 2°C with 40 µE $m^{-2}s^{-1}$ photon flux density at 16 h light/8 h dark photoperiod.

Generation of Proliferating Meristems

The basic steps for cryopreservation were followed as per previous protocol of Agrawal et al. (2014a). Shoot cultures of the two accessions were subcultured by splitting the shoots vertically into two halves, to trigger proliferation of axillary meristems at leaf bases. These were raised on P5 medium for two culture cycles, after which they were subcultured on P4 medium (P5 medium with 10x higher BAP concentration i.e 100 µM), and incubated in the dark (Panis, 2009). Subsequently, the smallest shoots with clusters of meristems at their leaf bases were selected and subcultured on p4 medium, until groups of proliferating 'cauliflower-like meristems' (about 0.5 cm in diameter) are produced by subculturing about one-month intervals for 6-12 subculture cycles, depending on the cultivar. White proliferating meristematic clumps with 6-10 mm in diameter were excised and precultured on C0 medium containing all P5 medium constituents but with 0.4 M sucrose for pre-growth desiccation of 2 weeks and incubated at 25 ± 2°C in the dark.

Cryopreservation by Three Techniques

Meristematic explants (2-3 mm dia) containing at least 3-5 apical domes were excised from pregrown clusters and placed in a plastic container containing filter-sterilized loading solution (LS). The LS comprised 2 M glycerol + 0.4 M sucrose in MS medium, at pH 5.8 (Sakai *et al*., 1990). Explants were treated with LS at room temperature (RT, 25 ± 1°C) for 20 min, after the last meristematic clump was excised. After this step, explants were subjected to three different techniques for the subsequent steps of desiccation and freezing.

Droplet Vitrification Technique (DV)

After LS treatment, the meristematic clumps were transferred into a Petri dish (5 cm dia) containing ice-cold plant vitrification solution 2 (PVS2) (Sakai *et al*., 1990). The filter-sterilized PVS2 solution consisted of 30% glycerol (3.26 M) + 15% ethylene glycol (2.42 M) + 15% dimethyl sulfoxide (DMSO) (1.9 M) + 0.4 M sucrose in MS medium, at pH 5.8. The explants were subjected to PVS2 treatment at 0°C for 30, 60, 90, 120, 150 min. Five minutes before the completion of each incubation period, 10 meristematic clumps were transferred to a drop of fresh, chilled PVS2 solution placed on a strip of pre-autoclaved aluminum foil (20×5 mm). Then, the aluminum foil strips with the meristems were plunged directly into liquid nitrogen (LN) and carefully placed into sterile cryogenic tubes (2 ml). The cryotubes were held in a polycarbonate cryobox, kept immersed in LN in a storage dewar. Explants were held in the LN for at least 30 min.

Vitrification Cryo-plate Technique (VCP)

This technique was followed according to Yamamoto *et al.* (2011) and Niino *et al*. (2014), using aluminum cryo-plates No. 3 (37 mm length, 7 mm breadth, 0.5 mm thickness), with 10 oval-shaped wells of 1.5 mm dia, 2.5 mm long and 0.75 mm depth. Sterilized cryo-plates were placed in a Petri dish (9 cm) and 2% (w/v) sodium alginate solution (~2.0–2.5 µl) in a calcium-free MS basal medium with 0.4 M sucrose were poured in each well of the cryo-plate. The osmoprotected explants (in LS) were individually placed in the depth of cryo-plate and slightly pressed to allow the explants to position firmly in the well. Thereafter, calcium solution (0.1 M calcium chloride in basal MS medium and 0.4 M sucrose) was poured drop-wise on the section of the aluminum plates where the explants were located until it covered all the samples and left for 15 min to achieve complete polymerization of alginate gel. Excess calcium solution was removed by tapping the cryo-plates on autoclaved filter papers. The cryo-plate with encapsulated explants was transferred into Petri dish containing 20 ml ice-cold PVS2 solution. Exposure time to PVS2 were 30, 60, 90, 120, 150 min at 0°C. After appropriate time, the cryo-plate with the explants was transferred into a 2 ml sterile cryogenic tube immersed in LN and was kept for at least 30 minutes.

Dehydration Cryo-plate Technique (DCP)

The D-plate technique of Niino *et al*. (2013) was adapted, in which the explant embedding in alginate was same as in V-plate. Thereafter, the cryo-plates were desiccated by air-drying in a laminar flow cabinet for 30, 60, 90, 120 and 150 min to reduce the moisture content of the explants. Subsequently, cryo-plates with explants were transferred to a 2 ml cryotube immersed in LN with exposure time of at least 30 min.

Recovery, Regeneration and Data Analysis

After exposure to LN, the aluminum foil strips in DV technique and cryo-plates in V-plate and D-plate techniques were taken out and rapidly rinsed in 10 ml of recovery/reloading solution (RS) held in Petri dish (5 cm dia) at RT for 20 min. The RS comprised MS medium supplemented with 1.2 M sucrose, at pH 5.8.

Control in each technique consisted of explants along with aluminum foils/cryo-plates subjected to desiccation but not freezing; the explants were directly washed with RS solution after PVS2/air dehydration treatment without plunging in LN.

For regrowth, explants were placed on two sterile filter papers (Whatman No. 1) on top of semi-solid hormone-free MS medium (recovery medium) containing 0.3 M sucrose in Petri dish, and incubated in the dark for 48 h. Then meristems were transferred to the semi-solid regeneration (C3) medium (MS medium + 2.22 µM BA + 0.09 M sucrose + 0.25% phytagel) without filter papers and continuously keeping in the dark for 14 d. Thereafter meristems were transferred to p6 regeneration medium (MS medium + 0.09 M sucrose +1 µM BAP + 0.25% phytagel) and kept in 16 h light/8 h dark (40 μMm^{-2} s^{-1}) at 25±2°C.

Post-thaw recovery data was recorded after 4-6 weeks. Shoot regeneration and/or non-morphogenic callus formation of surviving explants were observed under a binocular microscope at weekly intervals up to 6 weeks; meristematic clumps which remained pale or white and did not show any further change and which turned partially or completely black were considered non-responsive. For each experiment, 5–20 explants were used for control treatment (non-LN-exposed) and 8–44 explants were used for LN treatment. As the experiments were performed three times for each treatment, a total of 75-92 control explants and 172-209 LN-treated explants were used. The experiment was set up as factorial completely randomized design (CRD). The percentage responses of the cryopreserved meristems that produced shoot and/or callus in 6-week-old cultures were calculated. Results are presented as means (%) ± standard error (SE). Data were subjected to analysis of variance (ANOVA) to test significant differences using SPSS ver. 22.0 package.

Results

Proliferating 'cauliflower like' meristems were generated after 8-12 subculture cycles in P4 medium in *Musa* cv Borjahaji while in Williams it took only 6-7 subcultures. The different steps of three cryopreservation techniques (DV, VCP and DCP) and post-regeneration growth are represented in Fig. 1. Regrowth after cryopreservation in all treatments was predominantly of two types – shoot regeneration and callusing. Some 5-10% infection

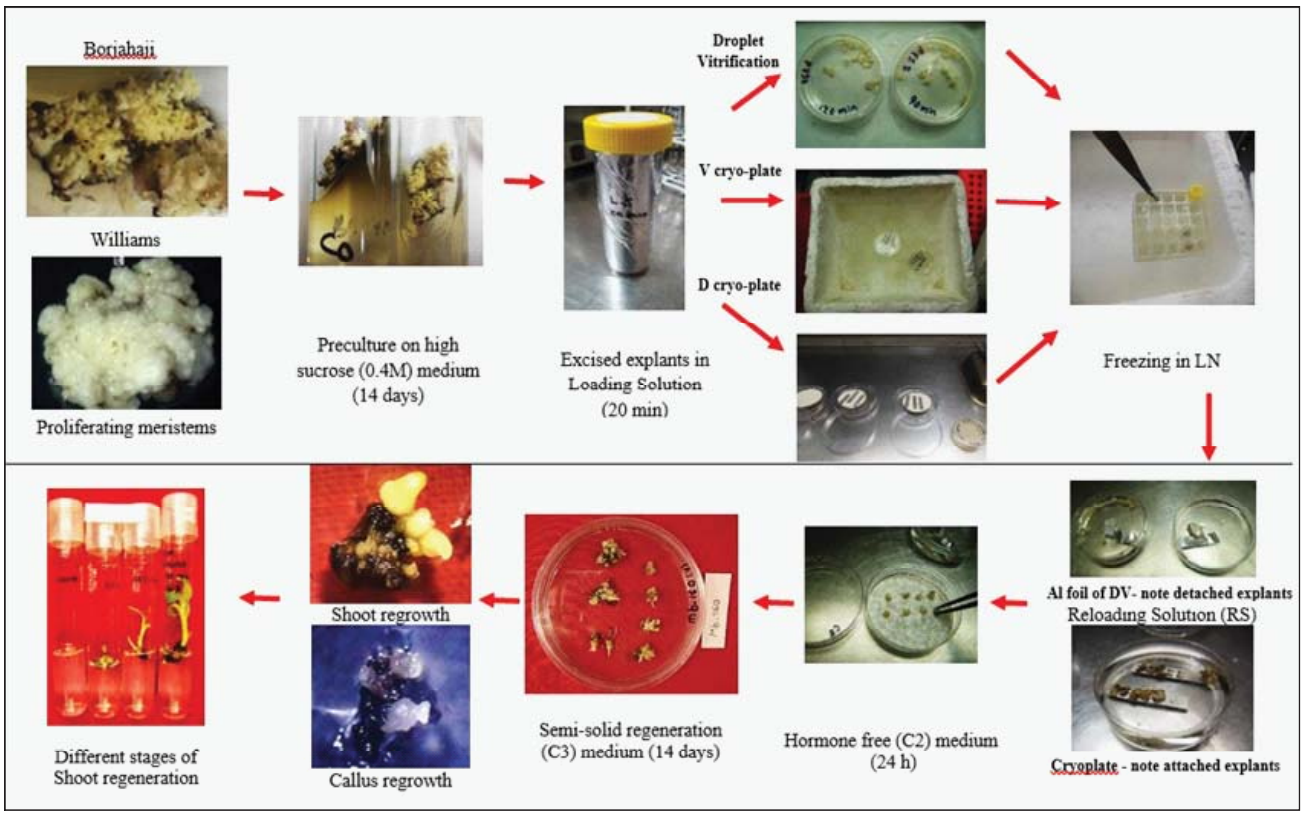

Fig. 1. Different stages of the three cryopreservation techniques – Droplet Vitrification (DV), V Cryo-plate (VCP) and D Cryo-plate (DCP), in *Musa* cv Borjahaji

(bacterial) was obtained in a few samples initially, and these were discarded and not used for computation of results.

Effect of dehydration duration

To determine the optimal duration of dehydration treatment, meristem explants in both the cultivars Borjahaji and Williams were treated with 30, 60, 90, 120 and 150 min. Post-thaw regeneration of shoot and callus in different duration of dehydration treatments for three cryopreservation techniques is shown in Table 1. In both the cultivars, shoot regrowth of non-frozen meristems was >82-85%, and not impacted significantly by duration of dehydration exposure (Table 2). In contrast, callus formation increased significantly ($P \leq 0.01$) in the non-frozen explants with increase in dehydration duration (120 and 150 min) in both the genotypes (Table 1, 2). Similar trend was recorded overall with the LN treated samples, where shoot regrowth was not meaningfully impacted with increase in dehydration duration but callus growth increased very significantly ($P \leq 0.01$) with increasing time of exposure of PVS2. The callus developed in all explants failed to convert into shoots at any stage, and eventually turned black and necrotic. In general, longer dehydration duration increases not only shoot regeneration, but also callus growth (Table 1).

Effect of technique and genotype

The mean regrowth of control and LN treated meristematic explants in the three techniques (DV, VCP, DCP) for Borjahaji and Williams is represented in Fig. 2. In Borjahaji, irrespective of the technique applied, it was found that 60-150 min duration of dehydration exposure gave statistically similar shoot regrowth after LN, within a technique (Table 1, 2). The mean shoot regrowth for all dehydration treatments in Borjahaji were 67.5 ± 2.8% in DV, 66.5 ± 2.4% in VCP and 58.4 ± 3.7% in DCP (Fig. 2A). In case of Williams, dehydration duration had no significant effect within a technique with respect to shoot regrowth after LN treatment (Table 1, 2). However, unlike Borjahaji, the VCP method in Williams, with mean shoot regrowth of 75.5 ± 2.8% was found to be significantly ($P \leq 0.01$) better than that obtained from DV (63.1 ± 2.5%) and DCP (64.8 ± 1.9%) (Fig 2B). In absolute terms, highest shoot regrowth was obtained after 120 min dehydration

Table 1. Shoot and callus regeneration (%) after different PVS2 treatment/dehydration durations, in *Musa* cvs Borjahaji and Williams, in control and LN treated meristematic explants.

Technique	Dehydration duration (min)	Borjahaji				Williams			
		Regrowth response (-LN)		Regrowth response (+LN)		Regrowth response (-LN)		Regrowth response (+LN)	
		Shoot (%)	Callus (%)	Shoot (%)	Callus (%)	Shoot (%)	Callus (%)	Shoot (%)	Callus (%)
DV	30	95.8 ± 1.9 ^{ab}	7.5 ± 0.0 ^{cde}	67.5 ± 4.3 ^{a}	27.5 ± 4.3 ^{cd}	90.0 ± 4.5 ^{a}	8.8 ± 0.1 ^{de}	56.7 ± 6.6 ^{cd}	18.4 ± 2.2 ^{e}
	60	95.0 ± 2.2 ^{ab}	6.3 ± 0.3 ^{de}	66.6 ± 2.0 ^{a}	25.7 ± 0.4 ^{cde}	93.3 ± 3.0 ^{a}	12.4 ± 0.1 ^{cd}	60.9 ± 9.5 ^{bcd}	19.1 ± 2.1 ^{e}
	90	82.2 ± 4.3 ^{b}	12.0 ± 0.8 ^{abc}	72.4 ± 4.4 ^{a}	27.9 ± 7.0 ^{cd}	93.3 ± 3.0 ^{a}	19.5 ± 1.2 ^{ab}	64.9 ± 5.8 ^{bcd}	28.1 ± 1.6 ^{cd}
	120	95.0 ± 2.2 ^{ab}	15.5 ± 0.2 ^{ab}	73.1 ± 2.7 ^{a}	35.7 ± 5.1 ^{bc}	85.0 ± 2.6 ^{a}	23.3 ± 0.2 ^{a}	64.3 ± 5.2 ^{bcd}	41.9 ± 5.8 ^{a}
	150	87.5 ± 3.2 ^{ab}	15.0 ± 0.0 ^{ab}	58.1 ± 9.8 ^{ab}	45.6 ± 2.5 ^{ab}	89.7 ± 3.4 ^{a}	18.0 ± 0.8 ^{b}	68.9 ± 8.7 ^{bcd}	40.0 ± 2.9 ^{a}
VCP	30	100.0 ± 0.0 ^{a}	11.0 ± 1.2 ^{bcd}	62.1 ± 2.9 ^{ab}	11.3 ± 0.8 ^{f}	100.0 ± 0.0 ^{a}	9.8 ± 0.6 ^{cde}	70.8 ± 5.3 ^{abcd}	24.0 ± 0.6 ^{de}
	60	100.0 ± 0.0 ^{a}	11.0 ± 1.2 ^{bcd}	71.1 ± 3.1 ^{a}	16.1 ± 2.0 ^{def}	100.0 ± 0.0 ^{a}	17.5 ± 0.3 ^{b}	72.5 ± 1.4 ^{abc}	32.5 ± 4.3 ^{bc}
	90	95.0 ± 2.2 ^{ab}	11.4 ± 1.8 ^{abcd}	67.4 ± 9.0 ^{a}	24.3 ± 2.3 ^{cde}	88.9 ± 5.0 ^{a}	14.6 ± 1.4 ^{bc}	75.2 ± 2.2 ^{ab}	42.5 ± 0.2 ^{a}
	120	95.0 ± 2.2 ^{ab}	11.5 ± 0.8 ^{abcd}	61.0 ± 4.0 ^{ab}	24.5 ± 2.9 ^{cde}	95.0 ± 2.2 ^{a}	17.8 ± 1.6 ^{b}	86.7 ± 7.7 ^{a}	36.7 ± 1.9 ^{ab}
	150	91.7 ± 3.7 ^{ab}	16.5 ± 1.6 ^{a}	70.9 ± 6.9 ^{a}	49.7 ± 4.7 ^{a}	92.9 ± 3.2 ^{a}	19.0 ± 0.6 ^{ab}	72.5 ± 7.2 ^{abc}	32.5 ± 1.4 ^{bc}
DCP	30	94.4 ± 2.5 ^{ab}	5.5 ± 0.1 ^{e}	45.5 ± 7.9 ^{b}	5.1 ± 0.1 ^{f}	100.0 ± 0.0 ^{a}	5.0 ± 0.0 ^{e}	63.9 ± 5.2 ^{bcd}	18.8 ± 2.0 ^{e}
	60	94.4 ± 2.5 ^{ab}	11.0 ± 0.3 ^{bcd}	61.1 ± 7.3 ^{ab}	7.9 ± 1.2 ^{f}	100.0 ± 0.0 ^{a}	5.5 ± 0.1 ^{e}	69.4 ± 1.6 ^{bcd}	27.8 ± 3.2 ^{cd}
	90	94.4 ± 2.5 ^{ab}	12.5 ± 0.6 ^{abc}	62.9 ± 7.4 ^{ab}	6.3 ± 0.2 ^{f}	100.0 ± 0.0 ^{a}	6.8 ± 0.5 ^{e}	68.1 ± 1.2 ^{bcd}	25.3 ± 2.4 ^{cde}
	120	100.0 ± 0.0 ^{a}	12.5 ± 0.1 ^{abc}	62.6 ± 9.6 ^{ab}	14.2 ± 1.4 ^{ef}	100.0 ± 0.0 ^{a}	9.4 ± 0.9 ^{de}	67.5 ± 4.3 ^{bcd}	23.0 ± 1.5 ^{de}
	150	100.0 ± 0.0 ^{a}	12.0 ± 0.0 ^{abc}	59.7 ± 10.1 ^{ab}	14.2 ± 1.4 ^{ef}	96.7 ± 1.5 ^{a}	12.5 ± 0.6 ^{cd}	55.0 ± 2.9 ^{d}	20.2 ± 0.2 ^{e}

Data represents mean ±SE of three replicates, recorded 8 weeks after culture; Values followed by the same letter are not significantly different ($P < 0.05$).

Table 2. Analysis of variance (ANOVA) for shoot and callus regeneration in the two *Musa* cvs Borjahaji and Williams

Regrowth	Source		Borjahaji				Williams			
		DF	SS	MS	F	P	SS	**MS**	**F**	**P**
Shoot (Control)	Techniques	2	291.2	145.6	1.7	0.198	618.4	309.2	3.2	0.057
	Dehydration duration	4	280.5	70.1	0.8	0.520	160.5	40.1	0.4	0.800
	Techniques*Dehydration duration	8	421.1	52.6	0.6	0.755	281	35.1	0.4	0.934
	Error	30	2551.9	85.1			2938.6	98.0		
Callus (Control)	Techniques	2	19.5	9.7	0.9	0.413	678.8	339.4	38.7	0.000**
	Dehydration duration	4	258.2	64.5	6.1	0.001**	493.9	123.5	14.1	0.000**
	Techniques*Dehydration duration	8	132.7	16.6	1.6	0.181	186.9	23.4	2.7	0.024*
	Error	30	320.2	10.7			263.0	8.8		
Shoot (LN)	Techniques	2	753.9	377.0	2.8	0.077	1356.6	678.3	7.2	0.003**
	Dehydration duration	4	479.1	119.8	0.9	0.483	443.3	110.8	1.2	0.343
	Techniques*Dehydration duration	8	869.0	108.6	0.8	0.603	716.9	89.6	1.0	0.494
	Error	30	4044.9	134.8			2838.5	94.6		
Callus (LN)	Techniques	2	4110.8	2055.4	40.8	0.000**	860.1	430.1	21.3	0.000**
	Dehydration duration	4	2765.8	691.4	13.7	0.000**	1041.1	260.3	12.9	0.000**
	Techniques*Dehydration duration	8	919.2	114.9	2.3	0.049*	1157.7	144.7	7.2	0.000**
	Error	30	1511.9	50.4			604.8	20.2		

* Significant ** Highly significant

exposure in both cultivars, but using VCP method in Williams (86.7 ± 7.7%) and DV method in Borjahaji (73.1 ± 2.7%).

Combined effect of genotype, technique and dehydration duration

Table 3 presents the overall ANOVA of combined effect of the two genotypes, three techniques and five dehydration duration tested in the present work, with respect to shoot and callus regrowth in non-frozen controls and LN treated meristematic tissues of banana. Shoot regeneration rate in dehydration treatment (control) was slightly influenced ($P \leq 0.014$) by cryotechnique applied, but interaction of genotypes and dehydration duration was not significant. Callus formation was highly influenced by genotypes, techniques and dehydration duration ($P \leq 0.01$), in both non-frozen and frozen meristems. In terms of shoot regrowth after LN treatment, which is the most important parameter, it is seen that genotype *per se* has no significant effect, but technique influences shoot recovery at highly significant level ($P \leq 0.005$) and genotype × technique has significant (P

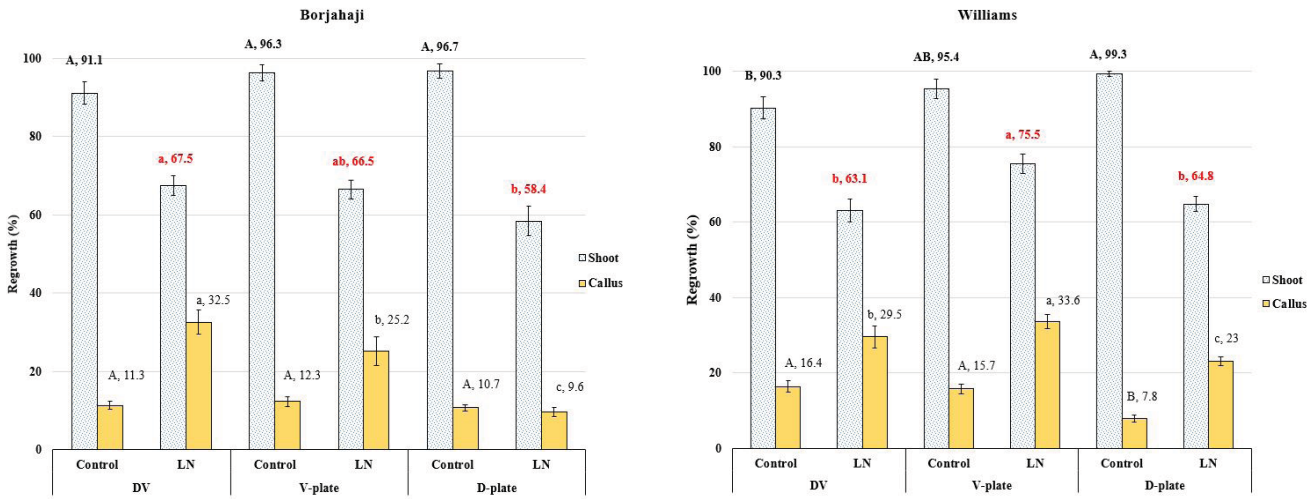

Fig. 2. Shoot and callus regeneration in control and LN treated meristem explants of the two cultivars of *Musa* (A-Borjahaji, B-Williams) using the three cryopreservation techniques. Same alphabets amongst C and LN treated samples for shoot and callus denote non-significant differences (P≤0.05)

Table 3. Analysis of variance (ANOVA) for shoot and callus regeneration (control and LN) for both genotypes, different techniques and various dehydration duration in cryopreserved *Musa* meristems

	Source	DF	SS	MS	F	P
Shoot (Control)	Genotypes	1	1.8	1.8	0.0	0.889
	Techniques	2	845.6	422.8	4.6	0.014*
	Dehydration duration	4	329.4	82.3	0.9	0.470
	Genotypes*Techniques	2	64.1	32.0	0.4	0.706
	Genotypes* Dehydration duration	4	111.5	27.9	0.3	0.874
	Techniques* Dehydration duration	8	268.1	33.5	0.4	0.934
	Genotypes*Techniques*Dehydration duration	8	434.0	54.3	0.6	0.780
	Error	60	5490.4	91.5		
Callus (Control)	Genotypes	1	81.8	81.8	8.4	0.005**
	Techniques	2	432.1	216.1	22.2	0.000**
	Dehydration duration	4	718.1	179.5	18.5	0.000**
	Genotypes*Techniques	2	266.1	133.0	13.7	0.000**
	Genotypes* Dehydration duration	4	34.0	8.5	0.9	0.485
	Techniques* Dehydration duration	8	196.3	24.5	2.5	0.019*
	Genotypes*Techniques*Dehydration duration	8	123.3	15.4	1.6	0.149
	Error	60	583.2	9.7		
Shoot (LN)	Genotypes	1	306.7	306.7	2.7	0.107
	Techniques	2	1351.0	675.5	5.9	0.005**
	Dehydration duration	4	806.6	201.7	1.8	0.149
	Genotypes*Techniques	2	759.5	379.7	3.3	0.043*
	Genotypes* Dehydration duration	4	115.8	29.0	0.3	0.907
	Techniques* Dehydration duration	8	242.5	30.3	0.3	0.975
	Genotypes*Techniques*Dehydration duration	8	1343.4	167.9	1.5	0.190
	Error	60	6883.3	114.7		
Callus (LN)	Genotypes	1	900.3	900.3	25.5	0.000**
	Techniques	2	3908.0	1954.0	55.4	0.000**
	Dehydration duration	4	2906.7	726.7	20.6	0.000**
	Genotypes*Techniques	2	1062.9	531.5	15.1	0.000**
	Genotypes* Dehydration duration	4	900.1	225.0	6.4	0.000**
	Techniques* Dehydration duration	8	1253.8	156.7	4.4	0.000**
	Genotypes*Techniques*Dehydration duration	8	823.1	102.9	2.9	0.008**
	Error	60	2116.6	35.3		

* Significant ** Highly significant

$\leq$ 0.043) impact. In our study, duration of dehydration alone, or in combination with genotype and technique was not important factor to change the shoot recovery results (Table 3).

Discussion

The droplet vitrifcation (DV) and cryo-plate methods (VCP and DCP) are contemporary cryopreservation techniques that are being increasingly used to successfully cryopreserve shoot tips of many tropical and temperate plant species (Wang *et al*., 2021). In case of *Musa,* vitrification and DV of isolated shoot tips or proliferating meristems have been practically applied for cryobanking of germplasm at the *Musa* International Transit Centre (ITC), Leuven, Belgium (Panis *et al*., 2020) as well as at

ICAR-NBPGR, India (Agrawal et al., 2019) with varying rates of post-thaw recovery (20-90%). However, the VCP and DCP protocols have not been applied so far. Hence, the present study was undertaken to test the comparative efficacy DV, VCP and DCP in cryopreservation of *in vitro* raised proliferating meristems in two Cavendish (AAA) cultivars of *Musa*. We focused on effect of dehydration periods (30, 60, 90, 120 and 150 min) before immersion in LN in all the three techniques and two genotypes (Borjahaji and Williams).

Finding the best duration of treatment with a cryoprotection solution is important to determine the correct balance between toxicity and acceptable dehydration of explants, by reducing the chance of lethal ice crystal formation in the regenerating tissue while avoiding overproduction of reactive oxygen species which cause serious oxidative damage to proteins, nucleic acids and polyunsaturated lipids (Turner *et al*., 2000; Panis *et al*., 2005). The optimal dehydration treatment of different plant species (with variable water content and membrane permeability) varies considerably (Faltus *et al*., 2021). In the present work, osmotic dehydration using varied duration (30 to 150 min) of PVS2 in both DV and VCP methods did not prove harmful for the tissues in non-frozen controls and LN treated samples. Shoot conversion rates were high in Borjahaji (58.1 – 73.1% in DV; 61.0-71.1% in VCP) as well as Williams (56.7 – 68.9% in DV; 70.8-86.7% in VCP) explants subjected to LN treatment. The high regrowth rate is attributed to the optimal cryoprotection by PVS2. The conversion of cytoplasmic and extracellular solutions to a glass by vitrification following cooling avoids the damaging stresses associated with the crystalline ice formation and cryodehydration, which increases viability following the recovery from cryopreservation (Agrawal *et al*., 2004). Our results are in agreement with the findings of Faltus *et al*. (2021) who have demonstrated that PVS2 in full concentration give optimal cooling and warming rates and its toxicity is significantly reduced when used at 0°C, as done in the present study.

Formation of non-morphogenic callus in the two genotypes was positively correlated with increase in PVS2 duration. This is in contrast to the results obtained in the Monthan subgroup (ABB) by Agrawal *et al*. (2008), where callus formation (0-30%) was genotype-dependent and not impacted greatly with PVS2 duration (30-120 min). Similarly, Agrawal *et al*. (2014b) investigating on five wild *Musa* species for different duration of PVS2 treatment reported that four species (*M. acuminata, M. basjoo, M. jackeyi* and *M. textilis*) could tolerate PVS2 dehydration up to 120 min, whereas in *M. balbisiana* a 90 min PVS2 incubation time was optimal in DV of proliferating meristems. The optimal length of PVS2 treatment (30-50 min) at 0°C was observed by Panis *et al.* (2005) in isolated shoot tips (not proliferating meristems) where DV cryopreservation on eight different groups of *Musa* spp. including AAA genomic group, gave 68-74% regeneration for Williams (AAA) and 74-83% for Grand Naine (AAA) genotypes.

Panis *et al*. (2005) reported that in banana cryopreservation, callus formation occurs in meristematic samples by mechanical injury of tissue due to excision and processing. Hence, it was expected that in VCP and DCP techniques, due to use of alginate gels to secure samples to aluminum cryo-plates, may reduce some mechanical injuries during handling and thus the callus formation. As seen in Fig. 2 and Table 3, all three factors, namely technique, genotype and desiccation duration and their interaction were highly significant variables that determined degree of callus development. Hence, not only mechanical injury but the genotype and method applied are critical in case of callus formation in *Musa* cryopreservation.

Cryopreservation protocols for more than 25 species (e.g. *Allium*, *Mentha*, *Saccharum*, *Solanum* etc.) by the VCP method and at least 15 species (e.g. *Solanum*, *Dendranthema*, *Juncus*, *Saccharum* etc.) by DCP are successfully reported (Niino *et al*., 2019). Yamamoto et al. (2011) reported the shoot regrowth rate of 65–90% (with an average of 77%) for seven genotypes of Dalmatian chrysanthemum (*Tanacetum cinerariifolium*) by VCP technique. Comparable regeneration rates have been reported using both VCP and DCP techniques in *Prunus* spp. (Vujović *et al*., 2015) and *Solanum* spp. (Yamamoto *et al*., 2015), while higher regeneration rates using DCP than VCP in *Ullucus tuberosus* (Arizaga *et al*., 2017). Higher regeneration rate for VCP than DCP is published in *Clinopodium odorum* (Sylvestre and Engelmann, 2015). Niino and coworkers, who successfully developed the DCP technique, reported cryo-storage of mat rush germplasm obtaining regeneration rates of 73–97% with a mean shoot regrowth of 86.3% across the 20 tested genotypes (Niino *et al*., 2013). The DCP technique is recommended for species which are sensitive to cryoprotective chemicals like PVS2 (Niino *et al*., 2019).

Table 4. Comparative advantages and drawbacks for droplet vitrification versus cryo-plate techniques of cryopreservation

Process/ steps during cryopreservation	DV technique	Cryoplate (VCP and DCP) technique
Support for mounting explants during freezing	aluminum foils are cost-effective and universally available.	The cryoplates are so far only available in Japan and costlier than aluminum foils.
Steps for handling during preparation for freezing	Steps are fewer and overall time taken is less. However, treating with LS and PVS2 often results in damage or loss of meristematic clumps by repeated pipetting or handling with forceps.	There are more handling steps (mounting explants on cryoplate, alginate drop on cryoplate, explant mounting, polymerization with calcium solution followed by desiccation) which needs extra time and well-trained technician.
Freezing tissue in LN	Placing the aluminum foil into cryovial requires skill and practice, as sometimes the foil folds during placing in cryovials (especially in larger explants like *Musa* proliferating meristems)	Overall handling of material and placing the samples in cryovials for freezing is easier, as explants are securely adhered to cryoplates with alginate matrix.
Thawing of frozen tissue	When frozen samples are thawed and placed in recovery solution, the tissue easily detach from the aluminum foil and readily float in solution, undergoing rapid thawing.	Frozen samples often remain attached to the cryo-plates in recovery solution (in DCP technique) due to the alginate matrix. Sometimes, it requires physical detachment with help of forcep/needle, and this can cause mechanical injuries to the explants.

So far, there is no publication related to cryopreservation of proliferation meristems of *Musa* using VCP and DCP techniques. The present work has shown that in *Musa* (AAA) cv Williams, VCP can be the preferred technique as compared to DV and DCP while for cv Borjahaji, all the three methods are equally effective (Table 1, Fig. 2). It is suggested that in general, VCP and DCP lead to higher and uniform survival after cryopreservation and have a wider spectrum for desiccation duration, which is especially useful for samples which are sensitive to exposure to toxic chemicals (Niino *et al*., 2013, 2014; Tanaka *et al*., 2018). There are findings in *Prunus* spp. (Vujović *et al*., 2015), *Solanum* spp. (Yamamoto *et al*., 2015) and mat rush (Niino *et al*., 2013) in which longer durations of dehydration treatment is required for DCP compared to VCP techniques. We hypothesize that longer air desiccation periods in DCP may increase higher regeneration rate in *Musa* proliferating meristems after cryopreservation. Also, due to absence of use cryoprotecting agents like PVS2 in DCP, it may be considered as an alternative choice for *Musa* germplasm cryobanking, as it would eliminate the risks of chemical stress and possible genetic alternations (Benson, 2008; Harding, 2004; Wang *et al*., 2014; 2018).

In terms of practical cryobanking of germplasm of *Musa*, use of proliferating meristems has the advantage in ease of explants isolation, although some genotypes are recalcitrant to proliferate (Agrawal *et al*., 2014a; Panis *et al*., 1990). Based on the findings of the present work and published literature (Yamamoto *et al*., 2011; Matsumoto 2017; Niino *et al*., 2019), Table 4 provides a comparative summary of advantages and drawbacks for DV, VCP and DCP techniques.

In India, a large diversity of genus *Musa* with a variety of ploidy and different genomic composition (AA, AAA, AAB, AB, ABB, BB, ABBB, etc.) are widely distributed. So far, some 100 accessions of *Musa* have been cryobanked at ICAR-NBPGR, New Delhi, out of which nearly 65 accessions are conserved in the form of proliferating meristems, using either simple vitrification or DV technique (Agrawal *et al*., 2014 a,b, 2019). The present study has demonstrated that besides DV, the VCP and DCP technique can also be successfully applied for cryobanking of *Musa* germplasm, provided cryo-plates are easily available to the genebank curators and managers. The DCP technique has the additional advantage of absence of use of PVS2, a toxic reagent. Both DCP and VCP may prove more useful in isolated meristems, which are more delicate to handle. However, more studies across all genotypes of *Musa* are required before practical cryobanking using DCP or VCP techniques. Taking into consideration practical aspects for each technique, any of the three cryopreservation approaches can be used for cryobanking of proliferation meristems of *Musa*, as DV offers cost-effectiveness, while VCP and DCP have ease of explant handling, and DCP avoids chemical toxicity of PVS2.

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RESEARCH ARTICLE

Studies on Diversity of Acid Lime (*Citrus aurantifolia* Swing.) based on Important Fruit Characters under West Bengal Conditions

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The study on diversity of acid lime was undertaken using one hundred genotypes collected from twelve districts of West Bengal in the duration of 2015-17. Genotypes were selected for studies on the basis of *in situ* observation as well as information provided by the farmers. Based on consumer's preference, six important quantitative fruit characters (fruit weight, rind thickness, juice percentage, number of seed, TSS: acid, ascorbic acid) were recorded. Descriptive analysis revealed a wide range of variation particularly in fruit weight, rind thickness, juice percentage and number of seed. The genotypes were grouped into eighteen clusters by Hierarchical cluster analysis. The major characters responsible for such grouping by canonical discriminant function were fruit weight, number of seed and ascorbic acid. PCA of six fruit characters of acid lime showed three components with cumulative variance of 67.6% According to biplot analysis, genotypes HRA 3, PMA 2, NAA 21 may be exploited for higher fruit weight; genotypes NAA 2, HGA 2, MUA 3, PMA 4 for higher juice content and genotypes NAA 24, PMA 6, NAA 5, NAA 1, BRA 5 for excellent biochemical quality.

Key Words: Biplot, Cluster analysis, Descriptive analysis, Discriminant analysis, PCA

Introduction

Acid lime, an important commercial species of citrus, is considered to be indigenous to India and is extensively cultivated in many states under tropical and subtropical climatic conditions. India is the largest producer of acid lime in the world (IIFPT, 2021). Lime commonly called lebu or nimbu are very popular for its refreshing juice. Demand of fresh fruit is always high all the year round, particularly during the summer months when the price of a fruit goes up. The fruits having bigger in size with more juice and less seed content are always in market demand. In spite of tremendous potentiality for commercial exploitation, acid lime is yet to be given due importance in India. It is mostly grown in homestead and kitchen gardens in India. Few varieties have been developed for acid lime but they are not well accepted throughout India. The diverse eco-geographical distribution in India and the occurrence of spontaneous mutation and natural hybridization have given rise to a wide range of variability in citrus (Dubey *et al*., 2016).

In West Bengal, no named varieties are available. Varieties are classified only 'Pati' (round) and 'Kagzi' (oval) based on fruit shape although this state is endowed with extremely diverse population of lime in diverse agro-ecological zones (Kundu *et al*., 2010). It emphasizes the needs for varietal improvement. Acid lime should have got the importance which definitely demands the survey, identification of elite germplasm and its subsequent utilization through proper fruit characterization and comprehensive variability study. The quality of acid lime fruits primarily depends on fruit weight, rind thickness, juice percentage, number of seeds, TSS/Acid ratio and ascorbic acid which may vary according to the climate, temperature, soil fertility and genotypes. However study of fruit characters at different agro ecological regions of West Bengal is meager in the past and very limited literatures are available in this aspect. Therefore, the present study was undertaken to determine the variation of fruit characters of acid limes grown in different districts of West Bengal and select elite genotypes for commercial cultivation.

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Materials and Methods

One hundred genotypes of acid lime covering twelve districts of West Bengal were selected by thorough survey and first hand information from growers during 2015-17. The different collections were named based on code of different districts and the first letter 'A' of acid lime. Thus different genotypes were named as BNA (collected from Bankura), BRA (Bardhaman), BIA (Birbhum), HGA (Hooghly), HRA (Howrah), MUA (Murshidabad), NAA (Nadia), PNA (North 24 Parganas), PMA (Paschim Medinipur), PRA (Purba Medinipur), PUA (Purulia) and PSA (South 24 Parganas). Based on consumer's preference, six important quantitative characters (fruit weight, rind thickness, juice percentage, number of seed, TSS: acid, ascorbic acid) were chosen from 'Citrus Descriptor' of Bioversity International (IPGRI, 1999) and studied for characterization of selected lime genotypes. Twenty fully matured and healthy fruits from each genotype were collected randomly from different directions of the canopy and brought to laboratory of Fruit Science of Bidhan Chandra Krishi Viswavidyalaya for recording six core quantitative observations including analytical works. All the genotypes were seedling in origin and naturally each genotype consists of single plant. However survey was conducted during 2015-16 for recording observations of each genotype during two successive years 2016 and 2017 to minimize the effect of environment. Electronic (digital) balance was used for recording fruit weight. Fruit rind thickness was measured by slide calipers. Total soluble solids content of fruits was determined with the help of a digital refractometer and calibrated in ºbrix at 20 ºC. Titratable acidity is estimated by treating against standard alkali (N/10 NaOH) solution using phenolphthalein as an indicator and ascorbic acid was estimated by the method as described by Ranganna (2000).

The data obtained was statistically processed for descriptive analysis, hierarchical cluster analysis, discriminant analysis, principal component and biplot analysis. Descriptive statistics used the data to provide descriptions of the population. Hierarchical cluster analysis following single linkage (nearest neighborhood) method, where distance matrix was Euclidian, was attempted to identify relatively homogeneous groups of varieties. Cluster members were further subjected to canonical discriminant analysis for multiple group problems to find out characters responsible for such clustering. Principal component Analysis and Biplot analysis were done to clarify the relation between genotypes and variables. Data was analyzed by using SAS 9.3 software.

Results and Discussion

Descriptive analysis revealed a wide range in fruit weight (20.75 – 100.00 g), rind thickness (0.91 – 2.86 mm), juice percentage (22.56– 78.79), seed number (0.00 – 40.00), TSS/acid ratio (0.92–1.90) and ascorbic acid (28.30 – 54.60 mg/100 ml juice) among one hundred lime collections (Table 1). Prominent variation of physical characters of lime fruits was obtained earlier by Kundu *et al*. (2010) in West Bengal, Shambhulingappa *et al*. (2015) and Abhilash *et al*. (2017) in Karnataka. Among 6 quantitative characters, coefficient of variation in the present study was much higher (≥25) in fruit weight (37.29), rind thickness (25.81), juice percentage (30.78) and seed number (57.69). Higher coefficient of variation obtained in these four characters could be interpreted with a high degree of heterozygosity and therefore genetic variation with regard to these traits might be high. Hence these traits can be utilised for selection in plant breeding. Lesser coefficient of variation (<15) in fruit characters was found earlier by Shambhulingappa *et al*. (2015) and Yadlod *et al*. (2018) whereas Dubey *et al*. (2016) obtained much wider coefficient of variation (9.52-227.12) in lime.

Hierarchical cluster analysis following single linkage divided 100 acid lime collections into 18 clusters with allowed distance 1.701 considering six core quantitative characters. All the clusters were distant each other and cluster 1 was the largest one consisting of 78 lime

Table 1. Variability study of core quantitative characters of acid lime

Characters	Minimum	Maximum	Mean	Std. Deviation	CV (%)
Fruit weight (g)	20.75	100.00	50.85	18.96	37.29
Rind thickness (mm)	0.91	2.86	1.84	0.48	25.81
Juice percentage	22.56	78.79	46.02	14.16	30.78
Number of seeds	0.00	40.00	15.22	8.78	57.69
TSS: Acid	0.92	1.90	1.39	0.22	16.11
Ascorbic acid (mg/100 ml juice)	28.30	54.60	37.56	5.48	14.59

genotypes followed by cluster 10 with three genotypes (Table 2). Canonical discriminant function revealed the major characters responsible for such clustering were fruit weight, seed number and ascorbic acid (Table 3). Cluster analysis is able to identify physico-chemical variability among different clusters. The variation among clusters might be due to heterozygosity, seedling population and nucellar embryony. From earlier studies it was noted five main clusters from 19 genotypes of lime and lemon by Zandkarimi *et al*. (2011), 5 clusters by Shrestha *et al*. (2012) and 4 clusters by Kumar *et al*. (2013) from lime diversity. The more number of clusters in the present study might be due to more collection of lime genotypes from different agro-climatic zones.

Principal component analysis of six core quantitative characters of acid lime resulted three components with cumulative variance of 67.583 per cent with reference to eigen value more than 1 (Table 4). The eigen value was high in F1 (1.758) and low in F3 (1.047). The component matrix F1 alone contributed 29.315 per cent of total variance with highly positive loading of juice percentage (0.917) and negatively loading of fruit weight (-0.933). The component F2 explained 20.813 per cent of total variance having positively loaded higher scored variables of TSS: Acid (0.721) and ascorbic acid (0.679). The component F3 explained 17.454 per cent of total variance. The positively loaded characters in this component were rind thickness (0.793) whereas number of seed was negetatively loaded (-0.442). So, the cumulative variance for quantitative traits revealed great variability with high genetic diversity and the traits had significant contribution in lime diversity. The present results are more or less similar with the earlier findings of Shrestha *et al.* (2012) who obtained 71.3 % accumulative variance using seven quantitative characters. In contrast, Zandkarimi *et al*. (2011) obtained higher accumulated variance of 85.99% using 31 characters and Dubey *et al*. (2016) obtained cumalative variation of 99% using 11 physico-chemical characters.

Biplot analysis of 100 lime genotypes indicated that six core quantitative fruit characters distributed in loading plot contributed a considerable role to the

Table 2. Clusters of acid lime genotypes based on six core quantitative characters using single linkage clustering method on squared Euclidean distance matrix

Cluster number	Cluster member
1	BNA 1, BNA 2, BRA 1, BRA 2, BRA 3, BRA 4, BRA 5, BRA 6, BRA 7, BRA 9, BRA 10, BRA 11, BRA 12, BIA 1, BIA 3, BIA 4, HGA 1, HGA 2, HGA 3, HGA 4, HGA 7, HGA 8, HGA 9, HGA 10, HGA 12, HRA 1, HRA 2, HRA 3, HRA 4, MUA 1, MUA 2, MUA 3, MUA 4, MUA 5, MUA 7, NAA 1, NAA 2, NAA 3, NAA 4, NAA 5, NAA 6, NAA 7, NAA 8, NAA 9, NAA 10, NAA 11, NAA 12, NAA 13, NAA 14, NAA 15, NAA 16, NAA 17, NAA 18, NAA 19, PNA 1, PNA 6, PNA 7, PNA 9, PNA 10, PNA 11, PMA 1, PMA 2, PMA 3, PMA 4, PMA 5, PMA 6, PRA 2, PRA 3, PUA 1, PUA 2, PUA 4, PSA 1, PSA 2, PSA 3, PSA 4, PSA 5, PSA 6, PSA 7
2	BNA 3
3	BNA 4
4	BIA 2
5	HGA 5
6	HGA 6, NAA 23
7	HGA 11
8	MUA 6
9	MUA 8
10	NAA 20, NAA 21, NAA 25
11	NAA 22
12	NAA 24, PNA 3
13	PNA 2
14	PNA 4
15	PNA 5
16	PNA 8
17	PRA 1
18	PUA 3

Table 3. Canonical Discriminant function Coefficient based on six core quantitative characters ofacid lime

Variable coefficients	Function		
	1	2	3
Fruit weight	0.072	0.009	0.031
Number of Seed	-0.137	0.017	0.081
Ascorbic acid	0.016	0.244	-0.034
Eigen value	2.542a	1.225a	.187a
% of Variance	64.3	31.0	4.7
Cumulative %	64.3	95.3	100.0
Canonical Correlation	0.847	0.742	0.397
(Constant)	-2.216	-9.837	-1.537
Unstandardized coefficients			

Table 4. Component matrix resulted by PCA for core quantitative characters of acid lime

Variables	Component matrix		
	F1	F2	F3
Fruit weight	**-0.933**	0.021	0.028
Rind thickness	-0.064	-0.266	**0.793**
Juice percentage	**0.917**	-0.063	-0.016
Number of Seed	-0.138	0.437	**-0.442**
TSS: Acid	0.144	**0.721**	0.136
Ascorbic acid	0.025	**0.679**	0.448
Eigen value	1.758	1.248	1.047
Variability %	29.315	20.813	17.454
Cumulative %	29.315	50.129	67.583

differentiation of acid lime genotypes. Genotypes from the 1st quadrant (NAA 24, PMA 6, NAA 5, NAA 1, BRA 5, PUA 1, BIA 1, BIA 2, HRA 1, BNA 1 etc.) had higher mean values of ascorbic acid and TSS: acid ratio Genotypes distributed in the 2nd quadrant of scoring plot had higher number of seed (HGA 7, BIA 3, PNA 5, HGA 8, PSA 1, PNA 4 etc.) and fruit weight (HRA 3, PMA 2, NAA 21 etc.) Similarly, in the 3rd quadrant of biplot, genotypes (HGA 9, HRA 2, PNA 9, NAA 25, BRA 6, HGA 1, PRA 2, NAA 18 etc.) had higher rind thickness and higher in juice percentage (NAA 2, HGA 2, MUA 3, PMA 4, NAA 7, MUA 2, PNA 8 etc.) .

Conclusion

From the present study, it could be concluded that there is a profound phenotype diversity among acid lime collections. Genotypes HRA 3, PMA 2, NAA 21 may be exploited for higher fruit weight, genotypes NAA 2, HGA 2, MUA 3, PMA 4 for higher juice content and genotypes NAA 24, PMA 6, NAA 5, NAA 1, BRA 5 for excellent biochemical quality. Few may be utilized as important breeding material for development of improved varieties.

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RESEARCH ARTICLE

Characterization of Peach [*Prunus persica* (L.) Batsch] Germplasm Using UPOV Test Gudielines

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Fifteen peach accessions namely July Elberta, Early Redhaven, Suncrest, Tropic Sweet, Paradelux, Saharanpur Prabhat, Earligrande, Flordaprince, Tropic Snow, Flordaglo, Vallegrande, Tropic Beauty, Pratap, Shan-i-Punjab and Glohaven were evaluated for their tree, foliage, floral and fruit characters using UPOV test guidelines. Varied serration was observed from crenate (4 accessions) to shallow serrate (6 accessions) to deep serrate (5 accessions). Nectaries (glands) were observed as globose in five accessions whereas in other accessions these were reniform. Mucron tip was present in seven accessions whereas absent in eight accessions. Shape of fruit varied from circular (8 accessions) to broad elliptic (5 accessions) to medium oblate (2 accessions). Flesh colour was observed as white in Saharanpur Prabhat, Flordaglo and Tropic Snow whereas yellow in all other accesssions under study. Adherence of stone to flesh was absent in nine accessions whereas, present in rest of the accessions. Ovary was pubescent in all the accessions studied. The present work has revealed considerable variation in majority of the characters studied and the descriptive database so developed will help in DUS testing and in multiplication of true-to-type planting material.

Key Words: Accessions, Biopiracy, DUS testing, Peach cultivars, *Prunus persica*

Introduction

Peach [*Prunus persica* (L.) Batsch] a member of family Rosaceae is an important fleshy stone fruit. This delicious fruit, a native of China got domesticated 4,000-5,000 years ago and has spread to various parts of the world used both fresh as well as processed products (Faust and Timon, 1995). Its cultivation extends from 10º N and 49º S latitude where strong light, clear skies, long seasons and warm temperature prevail mainly in low and mid hills within altitudinal range of the 1000-2000 m above mean sea level. Apart from its cultivation in temperate regions, peaches are also grown in warmer climates owing to availability of low chill requiring cultivars. In India, peach got introduced in early 20th century and is cultivated mostly in Himalayan region starting from Jammu & Kashmir and extending up to North - eastern hills.

Most of the peach cultivars require 500-1,000 or more chilling hours at or less than 7.2 °C to foliate and bloom normally in the spring (Hancock *et al*., 2008). The improvement work on peach taken up in different parts of India has resulted in the development of some varieties which are suitable only to local agro-climatic conditions (Devi *et al.,* 2012). Peach growing got a further boost with introduction of low chill cultivars requiring 100-300 hours at or less than 7.2 °C especially from Florida, USA which laid a foundation for peach cultivation in sub-tropical plains of North India (Singh *et al.,* 2014). In fact, all the commercial peach cultivars available in India are exotic introductions made from time to time and the process is continuing.

The existing peach gene pool comprising different cultivars and selections carry synonyms and local names over and above their original nomenclature and perhaps this happened due to lack of characterization and documentation which makes the identification and propagation of true-to-type material difficult. Characterization and evaluation of germplasm plays an important role in identifying the cultivars for their utilization either as a cultivar or to be used as a propagating material. Moreover, proper identification and specific characterization would also facilitate taxonomic description and identification (Wolfe and Strang, 2010). and in addressing the issues related to germplasm exchange and registration. Recent developments of peach DUS test guidelines by PPV&FRA (The Protection of

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Plant Variety and Farmers Right Authority), New Delhi offer a new tool to identify and characterize peach genotypes on the basis of their morphological and fruiting characters and provide a checklist for defined characterization and evaluation. It will also serve as a quick reference guide when developing a new descriptor list for peaches. Keeping in view the above facts, the present study was undertaken with the objective to characterize 15 accessions of peach [*Prunus persica (*L.) Batsch] existing as a part of germplasm collection in the Experimental Block of Department of Fruit Science, Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan with the objective to develop descriptive database for identification and characterization of the peach germplasm as well as to help in multiplication of true to type planting material.

Materials and Methods

The present investigation was carried out in Peach Experimental Blocks of Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh. A total of 15 peach varieties, namely July Elberta, Early Redhaven, Suncrest, Tropic Sweet, Paradelux, Saharanpur Prabhat, Earligrande, Flordaprince, Tropic Snow, Flordaglo, Vallegrande, Tropic Beauty, Pratap, Shan-i-Punjab and Glohaven were evaluated for their tree, foliage, floral and fruit characters in a Randomized Block Design with three replications. UPOV (The International Union for the Protection of New Varieties of Plants, 2014) Test guidelines were followed to assess and characterize accessions for morphological characters. In each accession, three bearing plants (6-7 years old) grafted on wild peach were taken to record the observations. The observations for the assessment of distinctiveness, uniformity and stability (DUS) were made on three plants from each variety. For foliage characters randomly picked fifteen mature leaves (five in each replication) were taken. Similarly, for fruit and stone characters, 15 representative fruit samples (five in each replication) were taken at optimum maturity. All colour characteristics were assessed using the Royal Horticultural Society (RHS) colour chart. Characters were categorized in different groups and notes were assigned as shown below:

- GH (Growth habit): Upright (3), upright-spreading (5), Spreading (7)
- TV (Tree Vigour): Strong (3), Medium (5)
- SC (Shape in cross section of leaf blade): Concave (3), Flat (5)
- LM (Leaf margin): Shallow Serrate (3), Deep Serrate (5), Crenate (7)
- AA (Angle at apex of leaf blade): Right Angled (3), Acute (5), Obtuse (7)
- AB (Angle at base of leaf blade): Acute (3), Obtuse (5), Right angled (7)
- LBC (Leaf blade colour): Green Group 137 A (3), Green Group 137 C (5), Green Group 137 B (7), Yellow Green Group 146 A (9)
- RM (Red mid vein on lower side): Present (3), Absent (5)
- PN (Shape of petiole nectaries): Reniform (3), Globose (5)
- PAC (Presence of anthocyanin colouration): Present (3), Absent (5)
- FSAC (Flowering shoot: Intensity of anthocyanin colouration): Medium (3), Weak (5)
- FB (Density of flower buds): Dense (3), Medium (5), Sparse (7)
- FT (Flower type): Rosette (3), Campanulate (5)
- CC (Corolla main colour): Medium Pink (3), Light Pink (5)
- PS (Petal shape): Medium Ovate (3), Circular (5)
- SPP (Stamen position compared to petal): Below (3), Above (5)
- SPA (Stamen position compared to anther): Below (3), Above (5)
- PO (Pollen): Present (3), Absent (5)
- OP (Ovary pubescence): Present (3), Absent (5)
- FS (Fruit shape): Circular (3), Broad elliptic (5), Medium Oblate (7)
- MT (Mucron tip at pistil end): Present (3), Absent (5)
- PMT (Shape of pistil end excluding mucron tip): Flat (3), Prominently Pointed (5), Weakly Depressed (7), Weakly Pointed (9)
- FSM (Fruit symmetry): Symmetric (3), Asymmetric (5)
- PSU (Prominence of suture): Weak (3), Strong (5), Medium (7)
- GC (Ground colour of skin): Greenish Yellow (3), Greenish White (5), Green (7)

- RAO (Relative area of over colour): Medium (3), Large (5), Absent (7)
- HC (Hue of over colour of skin): Dark Red (3), Orange Red (5), Pink Red (7), Pink (9), Medium Red (11)
- PC (Pattern of over colour of skin): Mottled (3), Solid Flush (5)
- PU (Pubescence of skin): Present (3), Absent (5)
- DPU (Density of pubescence): Medium (3), Dense (5), Sparse (7), Very Sparse (9)
- TS (Thickness of skin): Medium (3), Thin (5)
- AF (Adherence of skin to flesh): Strong (3), Medium (5), Weak (7)
- CC (Carotenoid colouration of flesh): Light Yellow (3), Orange Yellow (5), Greenish White (7), Cream White (9)
- ACFS (Anthocyanin colouration of flesh next to skin): Present (3), Absent (5)
- IAFS (Intensity of anthocyanin colouration of flesh next to skin): Weak (3), Strong (5)
- ACCF (Anthocyanin colouration of flesh in central part of flesh): Present (3), Absent (5)
- IACF (Intensity of anthocyanin colouration of flesh in central part of flesh: Weak (3), Strong (5)
- AFS (Anthocyanin colouration of flesh around stone): Present (3), Absent (5)
- IAFS (Intensity of anthocyanin colouration of flesh around stone): Weak (3), Strong (5)
- FF (Flesh fiber): Weak (3), Medium (5), Strong (7)
- SSI (Stone size in relation to fruit): Large (3), Medium (5), Small (7)
- SS (Stone shape): Circular (3), Obovate (5), Elliptic (7), Oblate (9)
- ACS (Stone anthocyanin colouration): Absent/Very weak (3), Medium (5), Strong (7)
- IBC (Stone intensity of brown colour): Medium (3), Dark (5), Light (7)
- ROS (Stone relief of surface): Equally pits and grooves (3), Prominently pits (5), Only grooves (7), Only pits (9)
- AF (Adherence to flesh): Present (3), Absent (5)
- DAF (Degree of adherence to flesh): Weak (3), Strong (5)

Results and Discussion

Considerable variations were recorded among 15 peach accessions for various morphological characters (Supplementary Table 1). Cultivars are known to exhibit substantial variation in growth and form of tree. In the present study most of these characters revealed significant differences except vigour which differed moderately. Such variation in growth characters has also been reported by previous workers (Bisla and Chitkara, 1980; Gautam *et al.,* 1986; Singh *et al.,* 2005; Saran *et al.,* 2010). Growth habit of the peach accessions was observed to be upright, spreading or upright-spreading (Supplementary Table 1, Fig.1). Similarly, PPV&FRA guidelines for DUS test, indicate growth habit to vary from upright to semi-spreading among different peach accessions (PPV&FRA, 2015). Upright nature of Suncrest, Earligrande, Flordaglo and Glohaven as observed in the present study suggests their suitability for high density planting, also contended by Gradziel and Beres (1993).Variation in growth parameters characterizing tree form has been observed earlier also (Bassi *et al.,* 1994; Tworkoski and Scorza, 2001; Frecon *et al.,* 2002; Scorza *et al.,* 2002; Scorza *et al.,* 2006; Jana, 2015).

Leaf characters are commonly used to distinguish and identify various fruit crop species and varieties. However, in the present study no marked variation was observed in respect of angle at base of leaf blade, angle at apex, red mid vein on lower side of the leaf, shape in cross section of leaf blade except for nectaries (glands) and leaf margins (Supplementary Table 1, Fig. 1). Leaf margin in the present study was observed to vary from crenate to shallow serrate to deep serrate which is in agreement with previous studies of Chalak *et al.* (2006) and UPOV (2014). Presence or absence and shape of petiole glands are an important feature for identification and characterization of peaches (Wolfe and Strang, 2010). Glands were observed in all the accessions under study whereas the shape of glands was globose in Vallegrande, Earligrande, Paradelux, Shan-i-Punjab, Pratap and reniform in remaining ten accessions (Supplementary Table 1) These findings are largely in line with reference varieties as per PPV&FRA guidelines for DUS test as they have reported round glands in Earligrande whereas reniform in Early Red June (PPV&FRA, 2015). Similar variation in leaf glands has been reported by Rouse and Byrne (1990) as globose in Vallegrande and Earligrande whereas reniform in Tropic

Beauty, Tropic Sweet, Flordagrande and Flordaprince. Variation in type of petiole glands has been reported by other workers also (Andersen *et al.,* 2001; Chalak *et al.,* 2006). Leaf blade colour in all the accessions fell in Green Group (137) and Yellow Green Group (146) however slight variation was observed in the shade of colour. Variation in leaf colour as an aid in identifying peach cultivars was also used by Wolfe and Strang (2010). Variation in leaf blade colour as greenish yellow in Redhaven, light yellow in Silver Fire, medium green in Robin and dark green in Fiesta Red peach is reported by UPOV (2014).

Similar to foliage characters, some floral characters also varied considerably among peach accessions, however flower type, number of petals, presence of anthocyanin colouration on flowering shoot, density of flower buds, stigma position compared to anther, stamen position compared to petals and pubescence of ovary did not show any significant variation (Supplementary Table 1, Fig. 2). The flower type was found to be rosette in all the peaches which is in support of PPV&FRA guidelines for DUS test except for 'July Elberta' where campanulate type of flowers are present (PPV&FRA, 2015). Significant differences among various fruit characters such as shape, colour of skin and flesh were observed in peach accessions under study (Supplementary Table 1, Fig. 3). These fruit characters are crucial in making any variety acceptable to the end user *i.e.* the consumer. Several researchers have worked on the physical aspects of peach fruits (Sherman *et al.,* 1984; Matta *et al.*, 1986) in the past and have reported considerable variation in fruits of different peach cultivars. The colour of fruit (flesh and skin) are important indices to differentiate between various peach cultivars and to some extent are considered as indices of fruit maturity. However, in the present study the peach accessions exhibited no significant variation in fruit skin colour except that slight variation was observed in the shade of the colour but the flesh colour varied from white to yellow and several studies conducted elsewhere has also reported similarly (Sherman and Lyrene, 1988; Andersen *et al.*, 2001; Chalak *et al.,* 2006). The shape of fruit varied from medium oblate to broad elliptic to circular (Supplementary Table 1, Fig. 3). Andersen *et al*. (2001) reported round to oblong fruit shape of peach. However Devi *et al.* (2012) found round, ovate, oblong and elongated fruit shapes in different varieties. Chalak *et al*. (2006) also reported similar fruit shape in Elberta as flat whereas round in Redhaven. Fruit shape has been defined as medium oblate in Earligrande, circular in Red Globe, broad elliptic in Nimla and medium elliptic in Peshawari and Southland as per PPVFRA guidelines (PPV&FRA, 2015). Shape of the pistil end excluding mucron tip was observed to be flat in July Elberta, Earligrande, Flordaglo, Saharanpur Prabhat; prominently pointed in two accessions; weakly depressed in 5 accesssions and weakly pointed in 4 accessions (Supplementary Table 1, Fig. 3).

Adherence of stone to flesh was observed as free, semi-free or clinged stone type (Supplementary Table 1, Figure 5). Such a variation in stone type was in accordance with UPOV (2014) guidelines. All the cultivars with melting flesh are genetically clingy because they ripen before pit and flesh has time to separate (Andersen *et al.,* 2001). Stone were observed as free in Early Redhaven, Glohaven, July Elberta, Pratap, Suncrest, Saharanpur Prabhat, Shan-i-Punjab, Tropic Snow and Tropic Sweet whereas semi free in remaining accessions. Bowen (1980**)** reported cv. Earligrande to be as semi-free stone type whereas stone adherence to flesh as semi-cling in Flordaprince was reported by Sherman *et al*. (1984). Variation in stone type has been reported by several earlier workers (Rouse and Bryne, 1990; Andersen *et al.,* 2001; Singh *et al.,* 2014; PPV&FRA, 2015). The relief of surface of stone was observed to vary from prominently pits to only grooves to only pit to equally pits and grooves (Supplementary Table 1, Fig. 5). Jana (2015) also categorized presence of grooves on stone as present or absent in six low chill peach cultivars. UPOV (2014) indicated similar categorization in peach accessions. This kind of work generates the reference database which could be utilized for comparison with new candidate varieties applied for protection through PPV&FRA as well as developing a new descriptor list for peaches.

Conclusion

Fifteen peach varieties namely July Elberta, Early Redhaven, Suncrest, Tropic Sweet, Paradelux, Saharanpur Prabhat, Earligrande, Flordaprince, Tropic Snow, Flordaglo, Vallegrande, Tropic Beauty, Pratap, Shan-i-Punjab and Glohaven studied here recorded variation for characters like, tree vigor, growth habit, serration of the leaf, nectaries, petal shape, fruit shape, flesh colour, mucron tip at pistil end of fruit, redness towards pit, stone adherence to flesh. The descriptive database so developed will help in DUS testing and

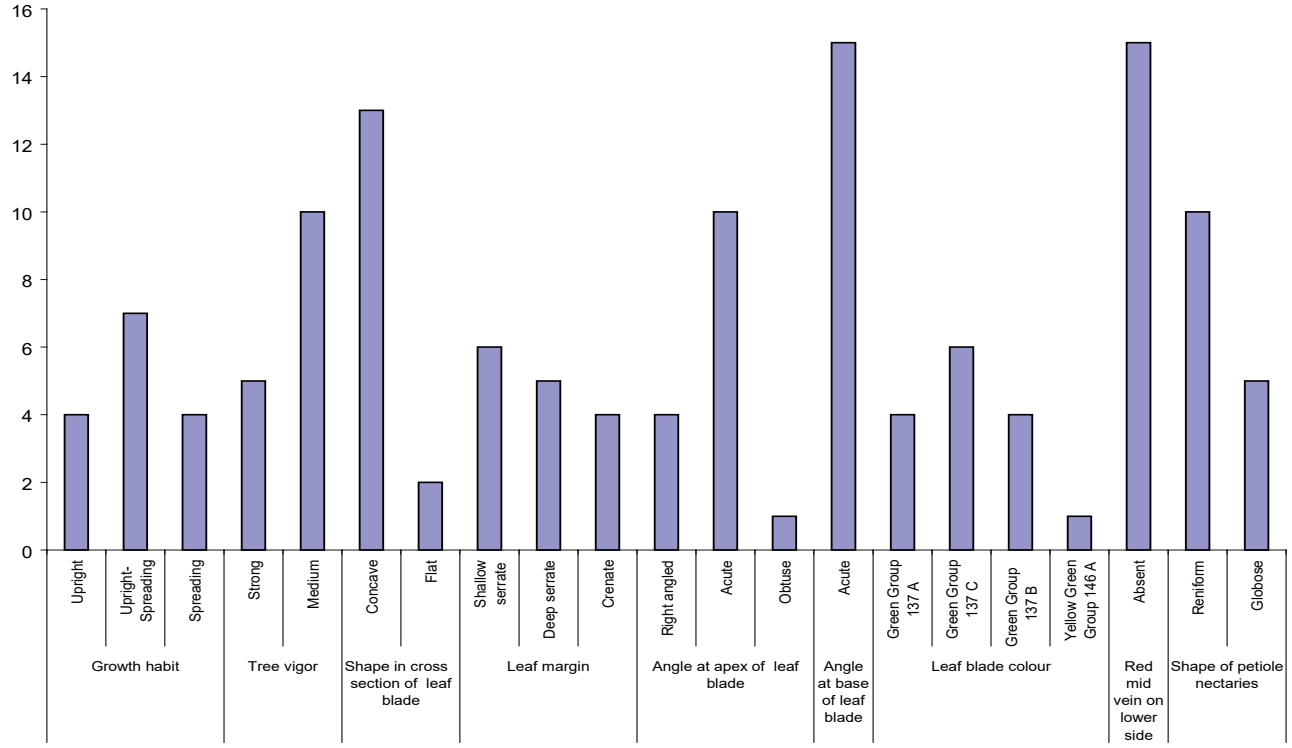

Fig. 1. Frequency distribution of 15 peach varieties based on tree and leaf characters

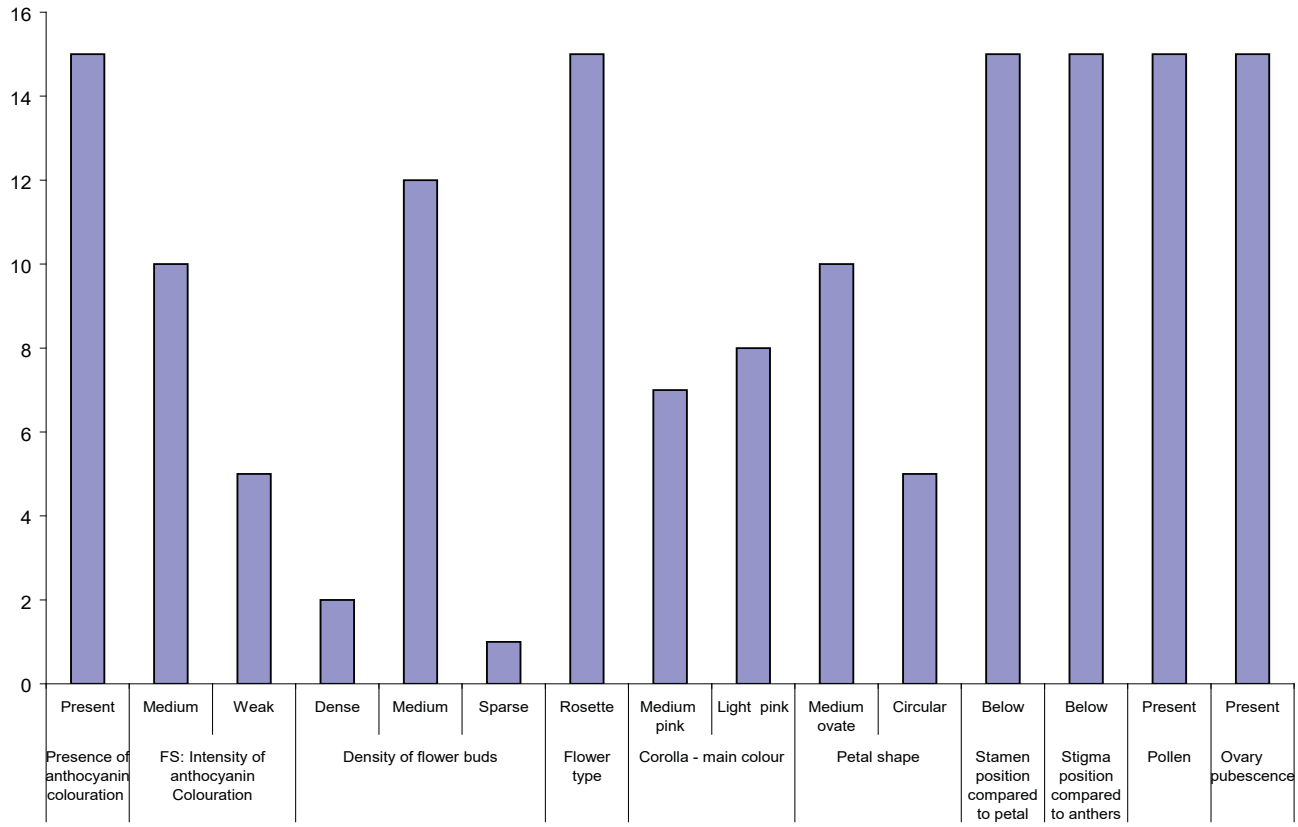

Fig. 2. Frequency distribution of 15 peach varieties based on floral characters

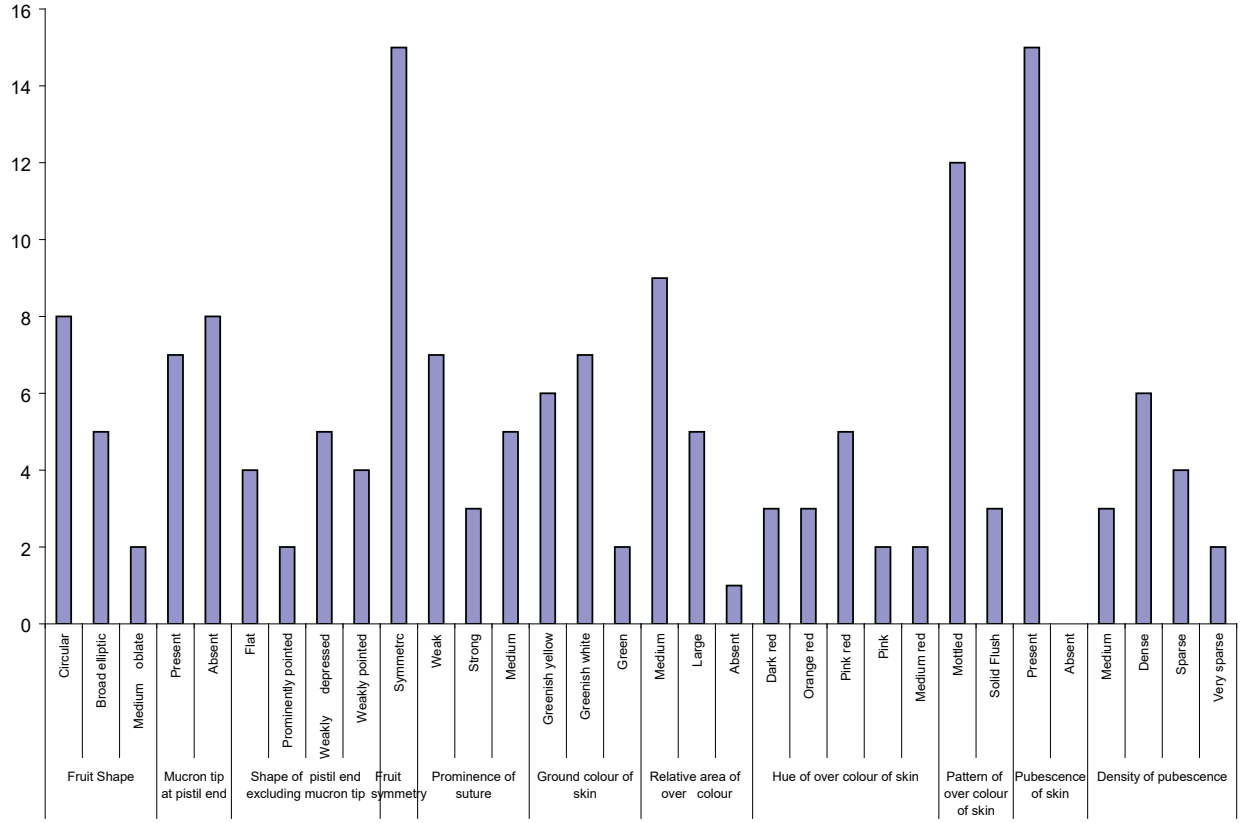

Fig. 3. Frequency distribution of 15 peach varieties based on fruit characters

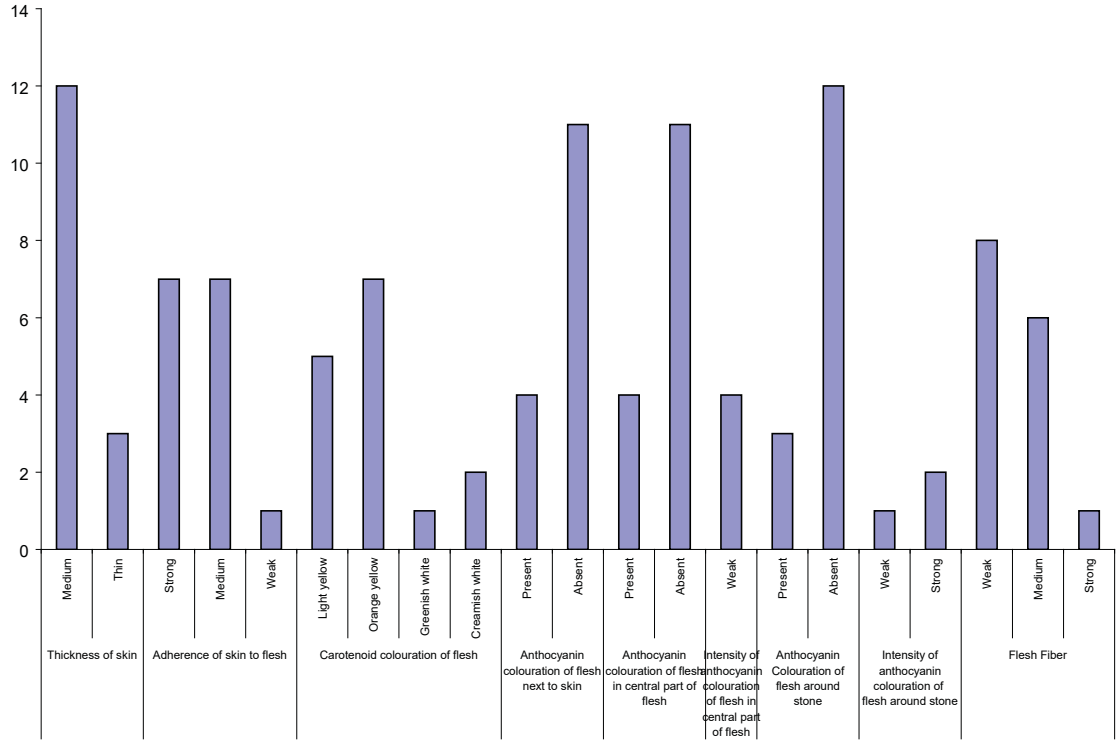

Fig. 4. Frequency distribution of 15 peach varieties based on fruit characters

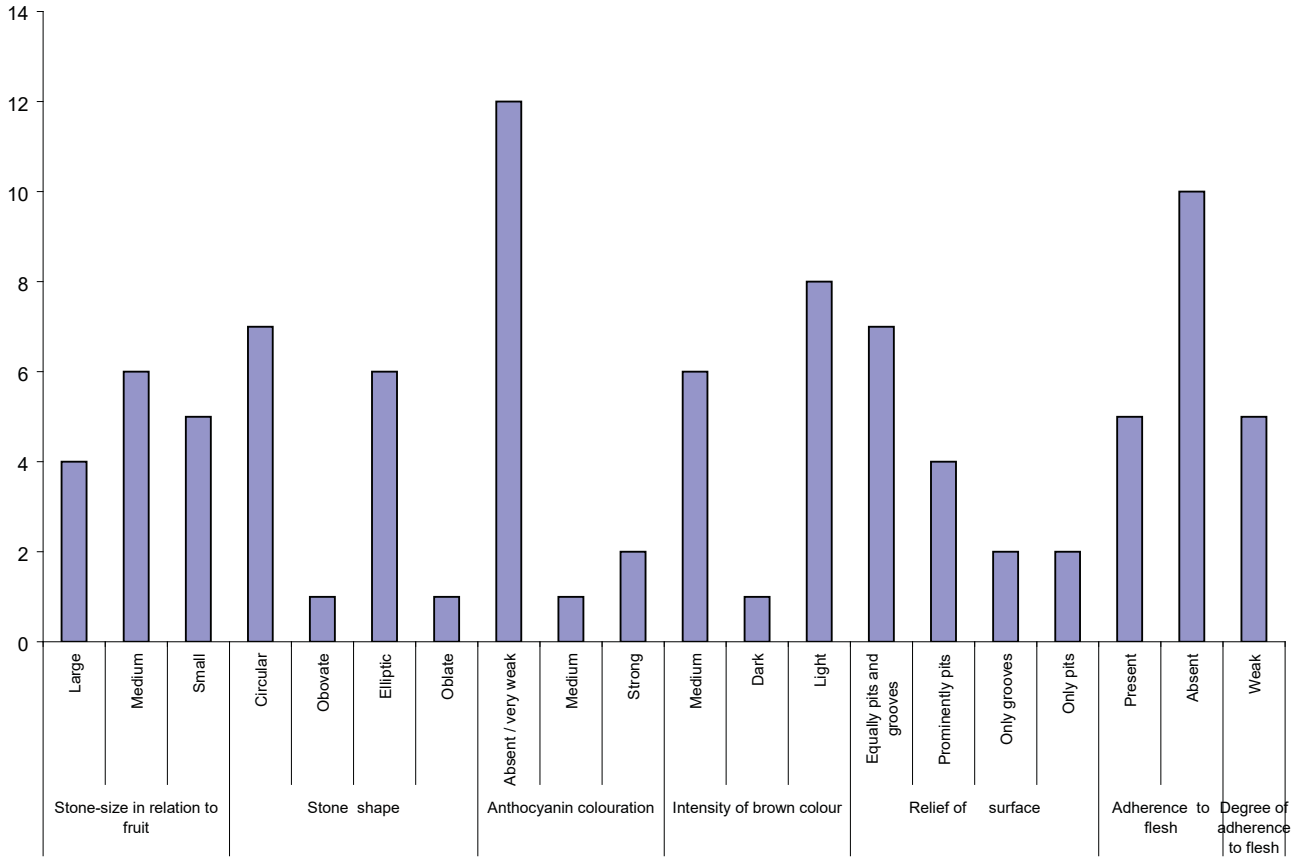

Fig. 5. Frequency distribution of 15 peach varieties based on stone characters

would also facilitate in multiplication of true-to-type planting material and help in checking bio-piracy besides authenticating the claims over newly developed peach genotypes for registration/protection.

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*Supplementary Table or Figure mentioned in the article are available in the online version.

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RESEARCH ARTICLE

Genetic Diversity, Character Association and Path Coefficient Analysis in Kalanamak Advanced Lines of Rice (*Oryza sativa* L.)

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Eighty-eight advanced lines of Kalanamak aromatic rice were studied to measure nature and magnitude of genetic divergence, character association and path analysis for 10 quantitative characters. The advanced lines have been generated from four different crosses of Kalanamak × Kalanamak accessions and Kalanamak × Basmati crosses. Pooled data analysis revealed, high order of divergence between cluster I and VII (252.04) followed by cluster I and VIII (219.35) and cluster III and VII (219.28). Maximum intra-cluster distance was observed among the advanced lines *viz.*, PMS-23, PMS-29, PMS-24, PMS-25, PMS-33, PMS-30 and these lines have been evolved from a cross of Kalanamak (3131-SN) and Basmati (PB-1). 1000-grain weight contributed maximum towards divergence (37.25%) followed by plant height (30.25%) and number of filled grains per panicle (10.66%). Character association studies revealed significant positive association of grain yield with number of effective tillers per plant, followed by number of filled grains per panicle, number of spikelets per panicle and 1000-grain weight. Path co-efficient analysis revealed the maximum direct and indirect effect towards grain yield with number of spikelets per panicle followed by 1000-grain weight and others. Number of spikelets per panicle and 1000-grain weight showed significant positive association and also had maximum positive direct effect on grain yield, indicating that direct selection through these traits would be greatly effective for the improvement of grain yield in high value Kalanamak aromatic rice background. Selection of yield component traits *viz*. spikelets per plant and 1000-grain weight significantly contribute towards the total genetic divergence as well as have positive significant association on grain yield and may be helpful in identifying genetically diverse Kalanamak aromatic advanced lines, particularly to be exploited in further hybridization to execute efficient selection in segregating generations.

Key Words: Aromatic, Correlation, Genetic divergence, Kalanamak, Path analysis

Introduction

Rice is the most important food crop in India contributing to more than 40 per cent of total food grain production and cultivated/consumed across the country (Anonymous, 2016). Aromatic rice is a special type of rice known for aroma, taste and fluffiness, and includes long grain basmati, jasmine rice and other short to medium grain scented rice of India and Pakistan. The biodiversity of long grain basmati and other short and medium grain scented rice in India is the largest in the world. The small and medium grain non-basmati scented rice has adapted to specific localities and are widely distributed in different parts of the country (Singh *et al*., 2000). Kalanamak is one of the most important non-basmati aromatic rice of India and exhibits wide range of variability in respect of grain quality, cooking quality and other economic traits. It is mostly grown in eastern Uttar Pradesh and has immense potential for domestic as well as international market. In recent years, Kalanamak area has declined sharply mainly due to its long duration growth cycle, tall stature, low yield deterioration in grain qualities, lack of domestic market support, policy interventions and others (Chaudhary *et al*., 2008; Kumar *et al*., 2018).

A dynamic breeding programme is essentially required to increase the production potential of Kalanamak rice suitable for specific agro-climatic regions. For scientific planning and execution of breeding programme, the most essential pre-requisite is the availability of sufficient desirable genetic variability for important economic traits in the elite germplasm or breeding lines (Pratap *et al.,* 2012). Although genetic variability for grain types and cooking quality traits are

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available in native germplasm of Kalanamak but genetic variability for yield, duration and plant types are not adequate enough to select a high yielding Kalanamak rice. Further, genetic variability could be generated though hybridization of Kalanamak rice accession to promising basmati rice.

Keeping the above in view, present investigation was undertaken to unravel the genetic divergence, determination of genotypic and phenotypic association among grain yield components and their direct as well as indirect effects on grain yield in 88 advanced lines of Kalanamak emanated from four different crosses (Kalanamak × Kalanamak and Kalanamak × Basmati aromatic accessions).

Materials and Methods

The experimental material consisted of 88 advanced lines of Kalanamak aromatic rice with two different checks (3131-SN and PB-1). Thes lines were evaluated in two consecutive *kharif* seasons (2013 and 2014) in a randomized block design (RBD) with three replications at the Organic Farm of Breeder Seed Production Centre, GB Pant University of Agriculture and Technology, Pantnagar, Uttarakhand. Advanced lines have been developed through varietal hybridization of Kalanamak × Kalanamak (3131-SN × 3327-SN, 3131-SN × 3216-SN & 3216-SN × 3327-SN) accessions and Kalanamak × Basmati (3131-SN × Pusa Basmati-1) crosses (Table 1**)**. The test plot consisted of 3 rows of 6 m length with row to row and plant to plant spacing of 20 cm and 15 cm, respectively. The recommended cultural practices were strictly followed under organic conditions. Data were recorded on various quantitative characters *viz*. plant height, days to 50% flowering, days to maturity, number of effective tillers per plant, panicle size (cm), number of filled grains per panicle, number of spikelets

Table 1. List of Kalanamak advanced lines of rice selected for the study along with their pedigree

S.No	Advanced Lines	Pedigree	S. No	Advanced Lines	Pedigree	S. No	Advanced Lines	Pedigree
1	PMS-1	3131-SN × 3327-SN	31	PMS-31	3131SN × PB-1	61	PMS-61	3131SN × PB-1
2	PMS-2	3131-SN × 3327-SN	32	PMS-32	3131SN × PB-1	62	PMS-62	3131SN × PB-1
3	PMS-3	3131-SN × 3327-SN	33	PMS-33	3131SN × PB-1	63	PMS-63	3131-SN × 3216-SN
4	PMS-4	3131-SN × 3327-SN	34	PMS-34	3131SN × PB-1	64	PMS-64	3131-SN × 3216-SN
5	PMS-5	3131-SN × 3327-SN	35	PMS-35	3216-SN × 3327-SN	65	PMS-65	3131SN × PB-1
6	PMS-6	3131-SN × 3327-SN	36	PMS-36	3131SN × PB-1	66	PMS-66	3131SN × PB-1
7	PMS-7	3216-SN × 3327-SN	37	PMS-37	3131SN × PB-1	67	PMS-67	3131-SN × 3216-SN
8	PMS-8	3131-SN × 3327-SN	38	PMS-38	3131SN × PB-1	68	PMS-68	3131-SN × 3216-SN
9	PMS-9	3216-SN × 3327-SN	39	PMS-39	3131SN × PB-1	69	PMS-69	3131-SN × 3216-SN
10	PMS-10	3216-SN × 3327-SN	40	PMS-40	3216-SN × 3327-SN	70	PMS-70	3131-SN × 3216-SN
11	PMS-11	3131-SN × 3327-SN	41	PMS-41	3216-SN × 3327-SN	71	PMS-71	3216-SN × 3327-SN
12	PMS-12	3131-SN × 3327-SN	42	PMS-42	3216-SN × 3327-SN	72	PMS-72	3216-SN × 3327-SN
13	PMS-13	3216-SN × 3327-SN	43	PMS-43	3216-SN × 3327-SN	73	PMS-73	3216-SN × 3327-SN
14	PMS-14	3216-SN × 3327-SN	44	PMS-44	3216-SN × 3327-SN	743	PMS-74	3216-SN × 3327-SN
15	PMS-15	3131-SN × 3327-SN	45	PMS-45	3131-SN × 3327-SN	75	PMS-75	3216-SN × 3327-SN
16	PMS-16	3131-SN × 3327-SN	46	PMS-46	3131-SN × 3327-SN	76	PMS-76	3216-SN × 3327-SN
17	PMS-17	3216-SN × 3327-SN	47	PMS-47	3131SN × PB-1	77	PMS-77	3216-SN × 3327-SN
18	PMS-18	3216-SN × 3327-SN	48	PMS-48	3131SN × PB-1	78	PMS-78	3216-SN × 3327-SN
19	PMS-19	3216-SN × 3327-SN	49	PMS-49	3131SN × PB-1	79	PMS-79	3216-SN × 3327-SN
20	PMS-20	3131SN × PB-1	50	PMS-50	3131SN × PB-1	80	PMS-80	3131-SN × 3327-SN
21	PMS-21	3131SN × PB-1	51	PMS-51	3131SN × PB-1	81	PMS-81	3131-SN × 3327-SN
22	PMS-22	3131SN × PB-1	52	PMS-52	3131SN × PB-1	82	PMS-82	3216-SN × 3327-SN
23	PMS-23	3131SN × PB-1	53	PMS-53	3216-SN × 3327-SN	83	PMS-83	3216-SN × 3327-SN
24	PMS-24	3131SN × PB-1	54	PMS-54	3216-SN × 3327-SN	84	PMS-84	3216-SN × 3327-SN
25	PMS-25	3131SN × PB-1	55	PMS-55	3216-SN × 3327-SN	85	PMS-85	3216-SN × 3327-SN
26	PMS-26	3131SN × PB-1	56	PMS-56	3216-SN × 3327-SN	86	PMS-86	3216-SN × 3327-SN
27	PMS-27	3131SN × PB-1	57	PMS-57	3216-SN × 3327-SN	87	PMS-87	3216-SN × 3327-SN
28	PMS-28	3131SN × PB-1	58	PMS-58	3216-SN × 3327-SN	88	PMS-88	3216-SN × 3327-SN
29	PMS-29	3131SN × PB-1	59	PMS-59	3216-SN × 3327-SN			
30	PMS-30	3131SN × PB-1	60	PMS-60	3131SN × PB-1			

Kalanamak Accessions: 3131-SN, 3216-SN and 3327-SN; Basmati: PB-1 (Pusa Basmati-1).

per panicle, spikelet fertility (%) and grain yield per plant (g). Ten randomly selected competitive plants in each row of each replication were recorded for all the traits under investigation except days to 50 per cent flowering and days to maturity which were recorded on whole plot basis. The pooled data of individual genotypes was used for statistical analysis. Data were subjected to multivariate analysis following Mahalanobis' D^2 statistics (Mahalanobis, 1928 & 1936) to measure the genetic divergence followed by the clustering of genotypes based on Ward's (1963) hierarchical grouping employing Windostat (Version 9.2, Indostat services) software. The estimates of phenotypic (rp) and genotypic (rg) correlation coefficients were estimated by the formula suggested by Miller *et al*. (1958). Path coefficient analysis for estimating the direct and indirect effect of independent traits on grain yield was performed as per Dewey and Lu (1959) for grain yield and its component characters.

Results and Discussion

Analysis of variance prepared by the pooled data of two years revealed high significant mean sum of square for all the characters used in this study. Based on relative magnitude of D^2 values, 88 advanced Kalanamak rice were grouped into ten distinct clusters (Table 2). Among these cluster IV was the largest with 20 genotypes, followed by cluster II with 16 entries and cluster III with 12 entries. Clusters V had only the genotypes evolved from cross of Kalanamak (3131-SN) × Pusa Basmati-1. Cluster VIII had only one genotype evolved from cross of Kalanamak accessions (3216-SN × 3327-SN), whereas remaining clusters contained advanced lines emanated from both Kalanamak x Kalanamak (3131-SN × 3327-SN, 3131-SN × 3216-SN & 3216-SN × 3327-SN) accessions and Kalanamak × Basmati (3131-SN × PB-1) crosses. There was a high degree of relative divergence of amongst the clusters indicating advanced lines are of genetically diverse in nature. The maximum inter-cluster distance was observed between cluster I and VII (252.04) followed by cluster I and VIII (219.35) and cluster III and VII (219.28). Minimum inter-cluster distance was observed between cluster I and II (25.22) followed by cluster I and III (27.66), cluster II and IV (32.89) and cluster II and III (34.79). The diverse genotypes could be used in hybridization programme for further improvement in yield and yield contributing traits. Crosses involving parents belonging to divergent clusters are expected to manifest maximum heterosis and also generate genetic variability for different yield and its component traits (Arunachalam, 1981). Maximum intra-cluster distance was observed in the cluster V, which had the advanced lines (PMS-23, PMS-29, PMS-24, PMS-25, PMS-33 and PMS-30) only emanated from Kalanamak × Pusa Basmati cross. Therefore, the present investigation indicates higher degree of divergence among the progenies derived from Kalanamak × Pusa Basamati-1 than Kalanamak × Kalanamak aromatic accessions cross.

Considerable variation in cluster mean values was observed for all the characters in the present study (Table 3). Genotypes included in clusters VIII showed earlier for days to 50% flowering, days to maturity and also recorded more number of effective tillers per plant, large panicle size and high grain yield per plant. Cluster III had more numbers of grains per panicle, number of spikelets per panicle and maximum spikelet fertility. Cluster IX and X were recorded for short plant height and cluster VII was recorded high for 1000-grain weight. Hence, these

Table 2. Cluster pattern, size and constituents of cluster involving 88 Kalanamak advanced lines of rice based on D^2-analysis

Clusters	No of genotypes	Name of genotypes
I	10	PMS-02, PMS-07, PMS-03, PMS-04, PMS-38, PMS-88, PMS-14, PMS-77, PMS-81, PMS-01
II	16	PMS-11, PMS-37, PMS-71, PMS-66, PMS-72, PMS-40, PMS-44, PMS-87, PMS-64, PMS-68, PMS-65, PMS-67, PMS-12, PMS-17, PMS-22, PMS-09
III	12	PMS-05, PMS-13, PMS-06, PMS-32, PMS-20, PMS-21, PMS-19, PMS-86, PMS-08, PMS-69, PMS-84, PMS-70
IV	20	PMS-16, PMS-76, PMS-10, PMS-82, PMS-73, PMS-75, PMS-18, PMS-83, PMS-15, PMS-57, PMS-85, PMS-78, PMS-54, PMS-31,PMS-41, PMS-51, PMS-55, PMS-58, PMS-80, PMS-63
V	6	PMS-23, PMS-29, PMS-24, PMS-25, PMS-33, PMS-30
VI	4	PMS-49, PMS-50, PMS-53, PMS-52
VII	3	PMS-28, PMS-36, PMS-35
VIII	1	PMS-56
IX	8	PMS-26, PMS-74, PMS-39, PMS-43, PMS-48, PMS-42, PMS-45, PMS-62
X	8	PMS-27, PMS-34, PMS-79, PMS-46, PMS-47, PMS-59, PMS-60, PMS-61

Table 3. Cluster mean for 10 quantitative characters in 88 Kalanamak advanced lines of rice

Cluster Number	Plant height (cm)	Days to 50% flowering	Days to maturity	No. of effective tillers/ plant	Panicle length (cm)	No. of filled grains/ panicle	No. of spikelets/ panicle	Spikelets fertility (%)	1000-grain weight (g)	Grain yield/ plant(g)
I	150.26	135.53	178.40	6.56	26.11	174.34	223.75	77.92	12.61	11.81
II	134.41	134.72	177.27	6.22	25.80	164.21	211.45	78.06	12.77	10.76
III	146.28	136.24	179.65	5.68	27.49	205.14	261.41	**78.51**	14.10	13.09
IV	135.19	135.30	176.83	7.14	26.16	161.70	208.59	77.64	14.94	14.01
V	131.92	132.53	174.50	5.47	30.12	132.45	177.24	75.62	19.00	12.85
VI	133.98	138.83	179.75	8.03	27.88	126.99	168.59	75.09	17.09	15.13
VII	112.24	124.94	172.44	6.04	26.44	98.09	131.27	74.77	19.97	9.78
VIII	127.72	118.00	158.67	9.90	30.10	118.80	161.97	73.32	19.02	15.44
IX	101.79	136.21	179.00	6.15	24.84	161.80	224.38	72.09	13.02	10.96
X	106.31	135.96	177.81	6.08	25.86	147.67	196.12	75.09	16.22	13.50
Mean	131.46	134.91	177.38	6.44	26.53	162.02	211.22	76.73	14.70	12.56

genotypes in the above concerned clusters hold great promise and could be used in hybridization programme to create further novel variability for selection.

The contribution of characters towards the total genetic divergence is important in deciding the characters for selection process. 1000-grain weight contributed maximum towards divergence (37.25%), followed by plant height (30.25%), number of filled grains per panicle (10.66%), number of effective tillers per plant (7.92%), panicle length (6.79%) and days to 50% flowering (5.43%). Remaining characters had very little or no contribution towards genetic divergence and hence were of less importance in this present materials. Similarly, Chakma *et al*. (2012) and Sandhya *et al*. (2014**)** also reported 1000-grain weight, number of spikelets per panicle and days to 50% flowering to have maximum contribution towards genetic divergence in rice genotypes.

Genotypic and phenotypic correlation coefficients of all the characters studied are presented in Table 4 In general, magnitude of genotypic correlations was higher than corresponding phenotypic correlations for

Table 4. Phenotypic and genotypic correlation coefficient among various yield and yield component traits of Kalanamak advanced lines of rice

Character		Plant height (cm)	Days to 50% flowering	Days to maturity	No. of effective tillers/ plant	Panicle length (cm)	No. of filled grains/ panicle	No. of spikelets/ panicle	Spikelets fertility (%)	1000-grain weight (g)
Plant height (cm)	P	1.000	0.110	0.117	0.020	0.265*	0.286**	0.216*	0.258*	-0.235*
	G	1.000	0.147	0.155	0.008	0.300**	0.360**	0.275**	0.451**	-0.264*
Days to 50% flowering	P		1.000	0.658**	-0.082	-0.212*	0.221*	0.216*	0.061	-0.263*
	G		1.000	0.896**	-0.127	-0.260*	0.285**	0.274**	0.121	-0.321**
Days to maturity	P			1.000	-0.149	-0.160	0.202	0.198	0.062	-0.285**
	G			1.000	-0.235*	-0.282*	0.288**	0.282**	0.107	-0.407**
No. of effective tillers/ plant	P				1.000	-0.091	-0.068	-0.101	0.053	0.039
	G				1.000	-0.113	-0.069	-0.114	0.128	0.048
Panicle length (cm)	P					1.000	-0.093	-0.067	-0.069	0.298**
	G					1.000	-0.126	-0.109	-0.064	0.368
No. of filled grains/ panicle	P						1.000	0.947**	0.286**	-0.411**
	G						1.000	0.974**	0.328**	-0.493**
No. of spikelets/ panicle	P							1.000	-0.027	-0.401**
	G							1.000	0.107	-0.482**
Spikelets fertility (%)	P								1.000	-0.115
	G								1.000	-0.196
1000-grain weight (g)	P									1.000
	G									1.000
Grain yield per plant	P	0.045	0.071	-0.031	0.471**	0.054	0.352**	0.334**	0.074	0.314**
	G	0.070	0.094	-0.053	0.535**	0.098	0.394**	0.372**	0.142	0.349**

(* & ** significant at 5% and 1% level respectively)

all the characters indicating strong inherent association among the characters. At phenotypic level, grain yield per plant had significant positive correlation with number of effective tillers per plant (0.471), number of filled grains per panicle (0.352) number of spikelets per panicle (0.334) and 1000-grain weight (0.314). These observations were in agreement with the finding of Naseem *et al*. (2014), Das (2015) and Pradhan *et al*. (2015). Plant height had a significant positive correlation with number of filled grains per panicle (0.286), panicle length (0.265) and number of spikelets per panicle (0.216). Similar results were reported by Nayak *et al*. (2001), Madhavilatha *et al*. (2005) and Chandra *et al*. (2009). Days to 50% flowering showed significant and positive association with days to maturity (0.658), number of filled grains per panicle (0.221) and number of spikelets per panicle (0.216). However, it showed significant and negative association with panicle length (-0.212) and 1000-grain weight (-0.263). The results are in consonance with the findings of Swain and Reddy (2006) and Dhurai *et al*. (2016). Number of filled grains per panicle showed positive association with number of spikelets per panicle (0.947) and spikelet fertility percentage (0.286) while it showed negative correlation with 1000-grain weight (-0.411). Similarly, Bhuvaneswari *et al*. (2016) also observed significant positive association of number of filled grain per panicle with spikelet fertility in F_2 populations of black rice.

An estimate of simple correlation does not provide the clear picture of the characters towards the yield, therefore, it's worthwhile to understand the direct as well indirect effects of various components traits on grain yield. Path coefficient analysis partitions the estimates of simple correlation coefficient into direct and indirect effects and gives an idea about actual contribution of each independent character on dependent characters i.e. yield. Relative contribution (direct or indirect) of components characters to yield helps in assigning appropriate weightage for the purpose of selection. The estimates of direct and indirect effect of component characters on grain yield are presented in Table 5. At the phenotypic level, number of spikelets per panicle (0.597) had the highest positive direct effect on grain yield per plant followed by 1000-grain weight (0.577), number of effective tillers per plant (0.513) and days to 50% flowering (0.130). At genotypic level also, the above mentioned characters had positive direct effect on grain yield per plant however, the magnitude of the effect was greater than at phenotypic level. Grain yield per plant also had positive correlation with number of spikelets per panicle, number of effective tillers per plant and 1000-grain weight, which indicates that direct selection through these traits would be effective for the improvement of grain yield per plant. Our results are

Table 5. Phenotypic (P) and genotypic (G) path coefficient among various yield and yield component traits of Kalanamak advanced lines of rice

Characters		Plant height (cm)	Days to 50% flowering	Days to maturity	No. of effective tillers/ plant	Panicle length (cm)	No. of filled grains/ panicle	No. of spikelets/ panicle	Spikelets fertility (%)	1000-grain weight (g)	Correlation with grain yield/plant
Plant height (cm)	P	-0.005	0.014	0.001	0.010	0.002	-0.001	0.128	0.032	-0.136	0.045
	G	0.019	0.008	0.021	0.006	-0.045	-4.955	3.825	1.458	-0.267	0.070
Days to 50% flowering	P	-0.001	0.130	0.001	-0.042	-0.001	-0.001	0.129	0.008	-0.152	0.071
	G	0.003	0.053	0.120	-0.092	0.039	-3.916	3.820	0.391	-0.324	0.094
Days to maturity	P	-0.001	0.086	0.001	-0.077	-0.001	-0.001	0.118	0.008	-0.165	-0.031
	G	0.003	0.048	0.134	-0.171	0.043	-3.967	3.921	0.346	-0.410	-0.053
No. of effective tillers/ plant	P	0.000	-0.011	0.000	0.513	-0.001	0.000	-0.061	0.007	0.023	0.471
	G	0.000	-0.007	-0.032	0.726	0.017	0.953	-1.586	0.415	0.048	0.535
Panicle length (cm)	P	-0.001	-0.028	0.000	-0.047	0.006	0.000	-0.040	-0.009	0.172	0.054
	G	0.006	-0.014	-0.038	-0.082	-0.151	1.729	-1.517	-0.207	0.371	0.098
No. of filled grains/ panicle	P	-0.002	0.029	0.000	-0.035	-0.001	-0.003	0.565	0.036	-0.237	0.352
	G	0.007	0.015	0.039	-0.050	0.019	-13.761	13.560	1.063	-0.497	0.394
No. of spikelets/ panicle	P	-0.002	0.028	0.001	-0.052	0.000	-0.003	0.597	-0.003	-0.231	0.334
	G	0.005	0.015	0.038	-0.083	0.016	-13.402	13.923	0.346	-0.487	0.372
Spikelets fertility (%)	P	-0.001	0.008	0.000	0.027	0.001	-0.001	-0.017	0.125	-0.066	0.074
	G	0.009	0.007	0.014	0.093	0.010	-4.519	1.489	3.237	-0.198	0.142
1000-grain weight (g)	P	0.001	-0.034	0.000	0.020	0.002	0.001	-0.239	-0.014	0.577	0.314
	G	-0.005	-0.017	-0.055	0.035	-0.055	6.785	-6.713	-0.634	1.009	0.349

Residual effect. Phenotypic: 0.599, Genotypic: 0.244

more or less similar with findings of Khedikar *et al*. (2004), Zia-ul-Qamar *et al.* (2005) and Mohammad *et al.* (2013). Number of spikelets per panicle also had the highest indirect effect on grain yield *via* number of filled grains per panicle (0.565, 13.56), days to 50% flowering (0.129, 3.820), plant height (0.128, 3.825) and days to maturity (0.118, 3.921). Therefore, it may be considered that number of spikelets per panicle has maximum direct as well as indirect influence on grain yield per plant. Similar result was also reported by Cheema *et al*. (1998) in basmati rice. 1000-grain weight had positive indirect effect on grain yield per plant *via* panicle length (0.172, 0.371) and number of effective tiller per plant (0.023, 0.048). Days to 50% flowering had positive indirect effect on grain yield *via* days to maturity (0.086, 0.048), number of filled grains per panicle (0.029, 0.015) and number of spikelet per panicle (0.028, 0.015), however it had negative indirect effect *via* 1000-grain weight (-0.034, -0.017) and panicle length (-0.028, -0.014). The present finding is in conformity with result of Patil and Sahu (2009) who similarly reported days to 50% flowering had positive indirect effect on grain yield via number of filled grain per panicle and negative indirect *via* 100-seed weight in rice accessions. Number of effective tillers per plant had positive indirect effect on grain yield per plant *via* spikelet fertility percentage (0.027, 0.093) and 1000-grain weight (0.020, 0.035) and it showed negative indirect effect *via* days to maturity (-0.077, -0.171), number of spikelets per panicle (-0.052, -0.083), panicle length (-0.047, -0.082), days to 50% flowering (-0.042, -0.092) and number of filled grains per panicle (-0.035, -0.050). Spikelet fertility percentage had positive indirect effect on grain yield per plant *via* number of filled grains per panicle (0.036, 1.063), plant height (0.032, 1.458) and negative indirect effect *via* 1000-grain weight (-0.014, -0.634).

Selection of yield component traits having high contribution towards the total genetic divergence as well as positive significant association on grain yield may be helpful in identifying genetically diverse Kalanamak aromatic advanced lines, particularly to be exploited in further hybridization to execute efficient selection in segregating generations.

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SHORT COMMUNICATION

Assessment of Genetic Variability in 1129 Accessions of Pigeonpea [*Cajanus cajan* (L.) Millsp.]

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A total of 1,129 pigeonpea germplasm lines conserved at ICAR-National Bureau of Plant Genetic Resources (NBPGR) under Consortium Research Project on Agro Biodiversity (CRP-AB) were evaluated during *kharif* 2017, 2018 and 2019 for 20 qualitative and quantitative traits for the purpose of genotypic characterization, evaluation and selection of cultivar potentially suitable for growing in Karnataka region. The experiments conducted out at Zonal Agricultural Research Station, Kalaburagi in Augmented Block Design. A wide range of variation was observed for 14 qualitative traits. Out of the total 1,129 genotypes 313 were early maturing (<140 days). The genotypes IC245540, IC245558 and IC245559 were earliest in their maturity (106 days). A total of 92 accessions recorded more than 11 g test weight, highest 100-seed weight 21.0 g was observed in IC525757 and highest number of pods per plant (740 pods/plant) was obtained in IC407203. Grain yield per plant was highest in IC527696 (266.67 g) as compared to best check PT-0012. Thus, morphological characterization led to genotype identification for different traits.

Key Words: Accessions, Genetic variability, Germplasm, Morphology, Pigeonpea

Introduction

Pigeonpea (*Cajanus cajan* L.) is the second most important pulse crop after chickpea in India. It plays an important role in food security, balanced diet and alleviation of poverty because of its diverse usages as a food, fodder and fuel (Rao *et al.,* 2002). It ranks sixth in global grain legume production and worldwide it is cultivated in about more than 5.0 m ha area. India is the largest producer and consumer of pigeonpea with an area of 4.4 m ha, with annual production of 3.68 m t and productivity of 832 kg/ha (Anon., 2019).

To develop high yielding varieties, the existing genetic variability in the crop needs to be assessed and quantified. Yield is a complex trait that is highly influenced by environment. The information on genetic variability for different characters of economic importance is a prerequisite for studies on any plant species (Rathaswammy *et al*., 1973). Pigeonpea is predominantly self-pollinated with occasional cross-pollination (6-7%) leading to genetic variability. The exploitation and maintenance of this variability results in evolution of better plants through selection.

So far, varietal improvement of pigeonpea has mainly been limited to selection. For further yield gains not only a large and diverse germplasm collection and preservation is necessary (Frankel and Bennett, 1970) but also its utilization in crop improvement programme in the country. The main aim of this study was categorization of part of the Indian base collection of germplasm conserved in National Genebank at National Bureau of Plant Genetic Resource (NBPGR) based on morphological and quantitative characters and selection of useful genotypes for further breeding programmes.

Materials and Methods

The experiment was conducted during *kharif* 2017, 2018 and 2019 at Zonal Agricultural Research Station (ZARS), Kalaburagi, which is situated in agro-climatic zone-2 (North Eastern Dry Zone) of Karnataka state with 17º 20' N latitude, 76º 49' E longitude, at an altitude of 443.88 MSL.

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A total of 1,129 accessions of pigeonpea germplasm were received from NBPGR, New Delhi along with three checks BSMR-736, Asha and PT-0012. A total of 391 genotypes were evaluated during 2017, 365 and 373 genotypes were evaluated during 2018 and 2019, respectively. Every year genotypes were sown in Augmented Block Design (ABD) with randomisation of checks in each blocks. Each genotype was sown in two rows of three metre length with spacing of 90 cm between rows and 20 cm between plants. Standard agronomic practices were followed and plant protection measures were taken as and when required by following the recommended package of practices (Anon., 2017). Observations were recorded on 10 quantitative traits in five randomly selected plants from each genotype, *viz.*, days to 50% flowering, days to maturity, plant height, number of primary branches, number of secondary branches, pod bearing length, number of pods per plant, seed yield per plant, seed yield per plot and 100-seed weight.

Observations on 14 morphological characters were recorded in the form of multiscale scores using standard pigeonpea descriptors (IBPGR and ICRISAT, 1993) these included seedling vigour, plant growth habit, plant habit, base flower colour, stem colour, leaf and pod pubescence, streak pattern, pod shape, pod colour, seed colour pattern, seed eye width, base seed colour and seed shape. Data recorded on five plants were averaged and were used to determine range and per cent values.

Results and Discussion

Ample variability was observed for various qualitative and quantitative traits. Based on the pooled data ten quantitative traits of pigeonpea germplasm are shown in the Table 1.

Wide variability was observed on quantitative traits in 391 pigeonpea germplasm during the year 2017. Days to 50% flowering ranged between 57 days (IC245519) to 143 days (IC254901), plant height varied between 99.0 (IC368861) to 257.3 cm (IC268784), primary and secondary branches were in the range of 4 (IC245495) to 26 (EC528321) and 0 (IC245471) to 35 (IC407203), respectively. Highest pod set was observed in IC407203 (740 pods/plant) and more pod bearing length was observed in IC407357 (90.7 cm). The variation observed with respect to days to 80% pod maturity was 106 (IC245540) to 195 days (IC254901), highest 100 - seed weight was observed in IC248845 (18.0 g), seed yield per plant (159.33 g) in IC407306 and seed yield per plot (1.98 kg) in EC528313.

A total of 365 accessions were evaluated during 2018, days to 80% pod maturity varied between 127 (IC468571) to 201 days (IC468581), plant height varied between 63 (IC468569) to 174 cm (IC523444), primary

Table 1. Range of variability for different quantitative traits assessed during 2017-2020.

S. No.	Traits	Minimum			Maximum		
		2017-18	2018-19	2019-20	2017-18	2018-19	2019-20
1	Days to 50% flowering	57 days (IC245519)	79 days (IC523385)	69 days (IC525855)	143 days (IC254901)	167 days (IC523140)	184 days (IC527697)
2	Days to 80% pod maturity	106 days (IC245540)	127 days (IC468571)	128 days (IC525855)	195 days (IC254901)	201 days (IC468581)	241 days (IC527477)
3	Plant height	99 (IC368861)	63 (IC468569)	138.3 (IC 525777)	257.3 (IC268784)	174 (IC523444)	292 (IC527657)
4	No of primary branches	4 (IC245495)	2 (IC523442)	4 (IC525825)	26 (EC528321)	18 (IC523445)	35 (IC 525759)
5	No of secondary branches	0 (IC245471)	0 (IC468572)	0 IC525811	35 (IC407203)	22 (IC523108)	25 (IC527705)
6	Pod bearing length (cm)	14.7 (IC268834)	9.3 (IC468570)	15.3 (IC525843)	90.7 (IC407357)	67.7 (IC523124)	83.7 (IC525812)
7	No. of pods per plant	42 (IC424214)	31 (IC523456)	37 (IC527473)	740 (IC407203)	306 (IC523446)	347 (IC527543)
8	100 Seed weight(g)	6.0g (IC268828)	4.5g (IC523266)	5.0g (IC 525818)	18.0g (IC248845).	16.0g (IC523369)	21.0g (IC525757)
9	Seed yield per plant(gm)	0.05g (IC424214)	3.3g (IC468592)	2.0g (IC525849)	159.33g (IC407306)	76.3g (IC523144)	266.67g (IC527696)
10	Seed yield per plot (kg)	0.11 kg (IC424217)	0.04 kg (IC523396)	0.06 kg (IC525785)	1.98 kg (EC528313).	1.79 kg (IC523171)	1.4 kg (IC468465)

Fig. 1. IC525757 genotype having 100-seed weight of 21.0 g (2019-20)

and secondary branches varied from 2 (IC523442) to 18 (IC523445) and 0 (IC468572) to 22 (IC523108), respectively. Highest pod set was observed in IC523446 (306 pods/plant) and more pod bearing length was observed in IC523124 (67.7 cm). Highest 100 seed weight was observed in IC523369 (16.0g) and seed yield per plot was highest in IC523171 (1.79 kg).

During 2019, a total of 373 pigeonpea germplasm were evaluated, number of primary and secondary branches varied from 4 (IC525825) to 35 (IC525759) and 0 (IC525811) to 25 (IC527705), respectively. Highest 100-seed weight was observed in IC525757 (21.0 g), Fig. 1. The variation observed with respect to days to 80% pod maturity was from 128 (IC525855) to 241 days (IC527477), highest seed yield per plant was obtained in IC527696 (266.67 g) and seed yield per plot in IC468465 (1.4 kg).

Based on pooled data of three years a wide range of variation observed for days to maturity, 100 - seed weight and grain yield per plant and is presented in Table 2 and 3. Out of 1,129 accessions a total of 313 germplasm showed early maturity (<140 days), 139 germplasm showed mid early (140-160 days), 326 germplasm showed medium duration maturity (160-180 days) and 351 genotypes were late maturing (>180 days). Similarly, a total of 790 germplasm showed small sized seeds (< 9 g), 247 germplasm showed optimum seed size (9-11 g) and 92 germplasm showed large seed size (> 11 g) 100-seed weight. Comparing three years of yield data with the check entries revealed that grain yield per plant was highest in IC527696 (266.67 g). On par yield with best check (PT-0012, 150 grams) was obtained in genotypes *viz*., IC407306 (159.33 g) followed by IC254905 (157.17 g) and IC407203 (151.3 g). Similar

Table 2. Categorization of germplasm based on variability parameters

Traits	2017-18	2018-19	2019-20	Total	Percentage
Days to maturity					
Early maturity (< 140 days)	105	189	19	313	27.72
Mid-early (140-160 days)	63	63	13	139	12.31
Medium (160-180 days)	182	55	89	326	28.87
Late maturity (>180 days)	41	58	257	351	31.08
100 seed weight (g)					
Small seed (< 9 grams)	243	300	247	790	69.97
Optimum seed (9-11 grams)	107	49	91	247	21.87
Large seed (>11 grams)	41	16	35	92	8.15

Table 3. Trait specific genotypes obtained after evaluating 1129 accession

S. No.	Trait	Genotypes
1	Early maturing genotypes	IC245540 (106 days), IC245558 (106), IC245559 (106), IC245501 (107) and IC245516 (107)
2	More number of pods per plants	IC407203 (740), IC406880 (715), EC528319 (656), EC13693 (653) and IC268837 (640)
3	Test weight (100 seed wt.)	IC525757 (21 g), IC525760 (18.1 g), IC248845 (18.0 g), IC268796 (17.0g) and IC268842 (17.0g)
4	More yield/plants compared to best check (PT-0012)	IC527696 (266.67g), IC407306 (159.33g), IC254905 (157.17g), IC407203 (151.33g)

(Values in the parenthesis indicate the values of genotype for each trait)

results were also obtained by Rangare *et al.* (2013), Singh *et al.* (2010), Kumara *et al. (*2013), Muniswamy *et al*. (2014), Chethana *et al.* (2015) Baldaniya *et al.* (2018) and Hariprasad (2018).

Results on trait specific genotypes are shown in Table 3. Emphasis should be given on these traits while selecting genotypes for desirable traits *viz.,* early maturity, 100-seed weight, more number of pods per plant and more yield per plant and such genotypes could be utilized in hybridization program for improvement in yield and its component traits in pigeonpea.

Morphological traits are important for varietal description. The percentage of variation for each morphological trait

Table 4. Morphological characterization of 10 characters of pigeonpea germplasm-pooled analysis (2017-2020)

S.No.	Trait	Phenotype	2017-18	2018-19	2019-20	Total	Percentage
1	Plant vigour	Good	372	270	220	862	76.35
		Very good	6	90	85	181	16.03
		Poor	13	5	68	86	7.62
2	Growth habit	Erect and compact	273	131	180	584	51.73
		Semi spreading	118	148	155	421	37.29
		Spreading	0	86	38	124	10.98
3	Base flower colour	Light Yellow	50	28	47	125	11.07
		Yellow	309	303	322	934	82.73
		Orange yellow	27	28	0	55	4.87
		Red	5	6	4	15	1.33
4	Stem colour	Green	351	293	351	995	88.13
		Purple	37	65	16	118	10.45
		Sun red	3	7	6	16	1.42
5	Streaks pattern	Sparse streaks	229	228	219	676	59.87
		Medium streaks	137	115	108	360	31.89
		Uniform streaks	0	0	18	18	1.59
		Dense streaks	25	22	28	75	6.64
6	Pod colour	Green	0	18	26	44	3.89
		Purple	23	31	40	94	8.32
		Green and Purple	368	316	306	991	87.78
7	Pod shape	Flat	391	354	348	1093	96.81
		Cylindrical	0	11	25	36	3.19
8	Seed colour pattern	Plain	359	348	339	1047	92.74
		Mottled	7	1	9	17	1.51
		Speckled	16	10	14	40	3.54
		Mottled and Speckled	9	5	11	25	2.21
9	Base seed colour	Cream	34	43	37	114	10.09
		White	20	12	11	43	3.81
		Orange	41	31	106	178	15.76
		Light brown	106	128	92	326	28.87
		Reddish brown	176	93	38	307	27.19
		Dark Purple	11	8	12	31	2.75
		Dark grey	3	0	6	9	0.79
		Mix	0	50	71	121	10.72
10	Seed shape	Oval	238	236	171	654	57.93
		Globular	109	80	130	319	28.26
		Square	41	39	62	142	12.58
		Elongate	3	1	10	14	1.24

is presented in Table 4. Morphological variation was not observed for plant growth habit, leaf pubescence and pod pubescence. Unique genotypes were observed for characters like dark purple stem colour, cylindrical pod shape, mottled, specked seeds and elongated seed shape. The traits like early plant vigour, branching pattern, base flower colour, stem colour, streak pattern of base petal, pod colour and seed characteristics exhibited lot of variation. Similarly, more variability was observed for plant vigour, growth habit, base flower colour, pattern of streaks, pod colour, pod form and seed colour pattern. Considering the individual trait a majority of genotypes i.e. 82.73 per cent showed yellow base flower colour, 51.73 per cent showed erect and compact growth habit, 87.78 per cent showed mixed green and purple pod colour, 96.81 per cent showed flat pod shape, 28.87 per cent showed light brown colour seeds and 59.87 per cent showed sparse streak pattern. Similar results were reported by Upadhyaya *et al*. (2007) and Manyasa *et al*. (2008) for growth habit, base flower colour, pattern of streaks, pod color, pod form and seed color pattern. These traits can be used for identifying individual germplasm. Similar findings for plant vigour and plant growth habit were observed by Muniswamy *et al*. (2014), Kumar *et al*. (2016) and Hariprasad (2018) found similar results for branching pattern and stem colour. Kallihal *et al*. (2016) observed similar morphology in case of streaks pattern on base petal, pod shape and pod colour.

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SHORT COMMUNICATION

Early Maturing Niger Germplasm Accessions

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In order to have wide variability in niger breeding, augmentation and evaluation of 1800 niger accessions, including indigenous and exotic collections, was conducted in *rabi* season at five different locations of India, *viz*., Hyderabad (Telangana) in *kharif* and *rabi* seasons, Ranchi (Jharkhand), Akola (Maharashtra), Delhi (New Delhi) and Chinthapally, Araku (Andhra Pradesh). In all the locations, accessions IC0268292, IC0268293, IC0268294, IC0268295, IC 412911, IC411511, and IC305117 were found to be early for 50% flowering (30-40 days) and days to maturity (58-75 days) compared to the standard checks (JNS-9 and JNS-28). Seed yield in *kharif* season varied between 1.87 g/plant to 2.96 g/plant and in *rabi* season, it was 1.31 g/plant to 1.83 g/plant in the early accessions. These accessions can be further utilized in crosses with long-duration Ethiopian lines (which are late-types coupled with high yield) to select early and high yielding lines in the transgressive segregants.

Key Words: Early maturing, Evalution, Germplasm, Niger

Introduction

Niger [*Guizotia abyssinica* (L.f.) Cass.] a lesser-known annual oilseed crop is valued primarily for its nutritive edible oil while it has a wide range of uses from its edible oil to medicinal purposes. India and Ethiopia are the two major producers of niger in the world. Though the crop is native of Tropical Africa, it is widespread and cultivated extensively in India in the 19th century and known from the broker's report in London. Globally, it is cultivated in tropical and subtropical countries like Ethiopia, India, East Africa, West Indies, and Zimbabwe. India ranks first in area, production, and export of the niger globally. It forms an integral part of the tribal agriculture economy and supports bird feed industries which have great demand for the crop to export as bird feed, supports the apiary industry, and also adds to tourism especially in the Araku valley region of Andhra Pradesh by its aesthetic value. Niger as an oilseed crop, is important with its 32 to 40% edible oil and 18 to 24% protein in the seed. This crop is mainly grown by tribals on marginal, unproductive wastelands without any production management under rainfed conditions in India. As the crop is cultivated largely by tribal farmers, it has thus far remained neglected. There is an ever-increasing demand for edible oil in the country, and the niger crop may contribute to increasing oil production in the country by expanding its area under rice fallows during the *rabi.* This study constitutes the first attempt at characterization and evaluation of entire niger genetic material available in India.

Materials and Methods

Collection

A total of 1807 germplasm accessions under long-term storage in NBPGR were subjected to characterization and evaluation. These accessions includes indigenous collections (IC) collected through exploration and exotic collections (EC).

Characterization and evaluation

Systematic efforts were made for seed multiplication, characterization, and evaluation of the entire germplasm of both indigenous and exotic accessions in augmented block design using two national checks JNS-9 and JNS-

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Table 1. Performance of early flowering accessions at four different locations

S.No.	Locations and season	Days to flowering and maturity (Early accessions*)			Days to flowering and maturity in Checks (JNS-9, JNS-28)		
		Flower initiation (No. days)	50% flowering time	Days to maturity	Flower initiation	50% flowering time	Days to maturity
1.	Hyderabad, Telangana State (2019 *rabi*)	28	30	58	44	52	95
2.	Hyderabad, Telangana State (2020 *kharif*)	28	32	67	46	55	98
3.	NBPGR, New Delhi (2020 *rabi*)	31	36	70	42	50	90
4	Akola, Maharashtra (2020 *rabi*)	35	40	75	45	54	94
5	Ranchi, Jharkhand (2020 *rabi*)	25	30	55	40	50	85
6	Chinthapally, Andhra Pradesh (2020 *rabi*)	36	40	75	46	53	98
Standard Deviation		4.32	4.68	8.50	2.40	2.07	5.05

*: IC0268292, IC0268293, IC0268294 and IC0268295 at all locations. IC412911, IC411511, IC305117 at Akola, Maharashtra; IC49568 and IC206246 at Ranchi, Jharkhand

28. The evaluation was carried out at six locations, i.e., Hyderabad (Telangana) (18.11° N, 79.01° E), Ranchi (Jharkhand) (23.61° N, 85.27° E), Akola (Maharashtra) (20.20°N, 77.00° E), Delhi (New Delhi) (28.70°N, 77.00 °E) and Chinthapally, Araku (Andhra Pradesh) (18.3273° N, 82.8775° E). The crop was evaluated during 2019 *rabi* season at all locations, except Hyderabad, where it was assessed in 2020 *kharif* and *rabi* seasons. Flower initiation was recorded when heads started to open in each line; after attaining a minimum of 50% flowering in each line, data on 50% flowering was noted down. Physiological maturity in niger is considered as days to maturity and recorded the observation once heads started to turn a brown color. Data on seed yield per plant and percent oil content were also recored.

Results and Discussion

Among the 1807 accessions characterized, 50% flowering duration varied from 30-40 days, and four accessions *viz*., IC0268292, IC0268293, IC0268294 and IC0268295 (Fig 1) were found early for 50% flowering compared to the checks in all locations (Table 1). Additionally, IC412911, IC411511, and IC305117 also flowered early at Akola, Maharashtra; and IC 49568, IC 206246 at Ranchi, Jharkhand. All these accessions are mainly indigenous landraces from Maharashtra except one each from Madhya Pradesh (IC305117) and Jharkhand (IC206296). Early maturing accessions were found to be low seed yield and have low oil content. Hence, these accessions can be used as donors for early maturing trait.

The characterization and evaluation of niger genetic resources have resulted in identification of early maturing accessions which was confirmed at different locations. Variability for 50% flowering ranged from 73 to 83 days and for days to maturity ranged from 128 to 144 days in a study conducted by Jagadev and Salman (1991) in 14 cultivars which indicates that there are no early maturing cultivars. A study conducted by Jadhavar (2014) for genetic diversity in 40 niger genotypes found that range for 50% flowering varied from 56.5 days to 68 days and days to maturity was 96 days to 106 which indicated that there were no early maturing genotypes

Table 2. Performance of early flowering accessions for seed yield at Hyderabad, Telangana State (*Rabi* and *kharif* 2019)

S. No.	Accessions	Rabi		Kharif	
		Seed yield (g/plant)	Oil content (%)	Seed yield (g/plant)	Oil content (%)
1.	IC0268292	1.83	25.24	2.53	26.35
2.	IC0268293	1.31	23.61	2.11	24.72
3.	IC0268294	1.43	28.67	1.87	29.31
4.	IC0268295	1.36	27.36	2.96	27.34
5.	JNS-9 ©	3.99	35.12	5.26	35.61
6.	JNS-28©	4.16	33.95	4.71	34.29
Standard Deviation		0.24	2.24	0.48	1.92

Fig. 1. Early accessions IC0268292, IC0268293, IC268294 and IC268295

and similar results were observed by Singh and Patra (1989), Alemaw and Teklewold (1995), Pradhan *et al.* (1995), Sreedhar *et al*. (2005). Studies conducted in Ethiopian lines recorded three maturity groups in the niger germplasm – early maturing group (120-130 d), mid maturity group (140-150 d), and late maturity group (175-185 d) (Getinet and Sharma, 1996). Hence, this constitutes the first report of screening a large number of accessions for variability and identified early maturing niger accessions.

Conclusion

Early maturing accessions identified in the present study can be used as a source for pyramiding high seed yielding genotypes with early maturity by crossing them with late types having high yield. This is the first report of evaluating niger genetic resources for early flowering, and information generated would be useful to breeders in selecting transgressive segregants for high yielding coupled with early maturing breeding lines.

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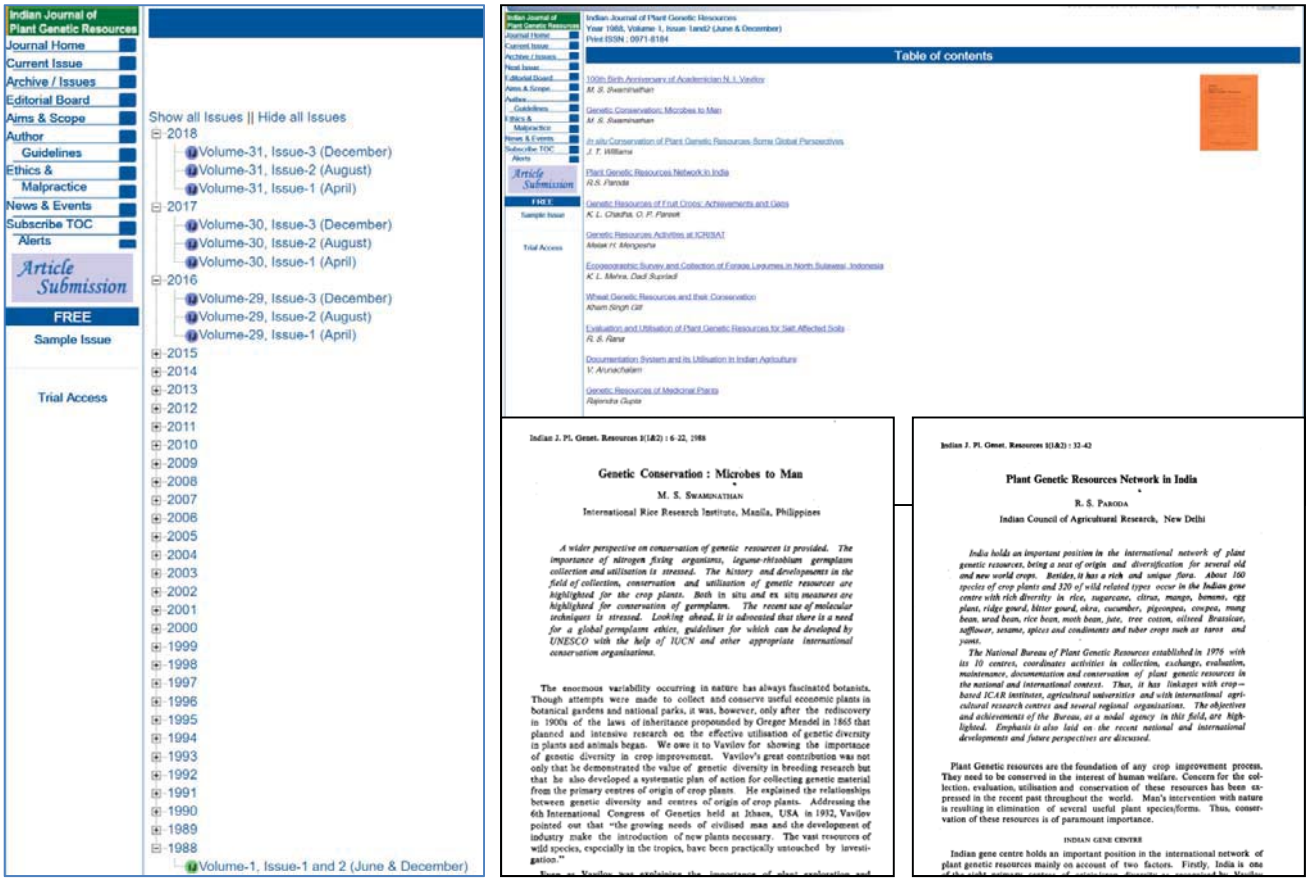

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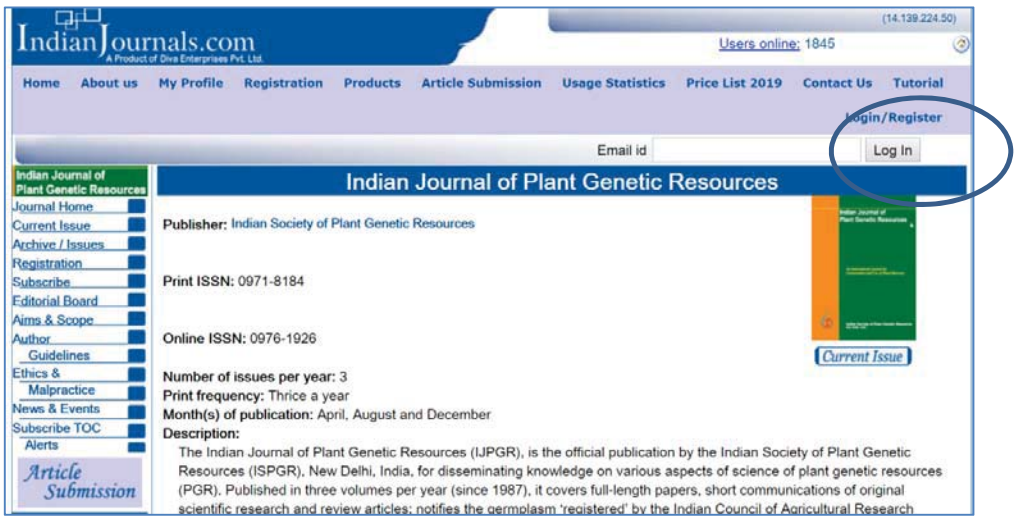

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