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PGR NOTE

Site Identification Survey in Garhwal Himalayas to Establish Safety Duplicate of National Genebank

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A survey was conducted in the Garhwal Himalayas, to identify a safety duplicate site for the National Genebank, which could be maintained with minimum energy investment. The survey covered Bhyundar Valley- Hemkund, Mana-Badrinath and Joshimath-Auli areas of Uttarakhand. The feasibility of each area was assessed based on the accessibility, persistence of sub-zero temperature for maximum duration, geological safety and cost-effectiveness. Auli, which lies at an elevation of 3000m, surrounded by snowcapped peaks of Nanda Devi and Mana Parvat ranges, was found to be a suitable zone for exploring in detail, the geological possibilities for establishing the safety duplicate site.

Key Words: Garhwal Himalayas, Genebank, Safety duplicate, Survey, Uttarakhand

Introduction

During the last few decades, *ex situ* conservation of plant genetic resources (PGR) has been accorded the highest priority in the global agenda for conservation and sustainable use of crop resources. The mandate on conservation is executed by an extensive international network which involves around 1750 genebanks. The Indian National Genebank (NGB) is the second largest genebank in the world, holding a collection of more than 4.4 lakh accessions belonging to 1991 species. Thus, our Indian germplasm collection stands as a colossus in the global agricultural gene pool, with immense ethnic diversity that has been richly complemented by exotic introductions made since centuries from all over the world. Currently, the NGB collection is maintained at a single location, within the premises of ICAR-National Bureau of Plant Genetic Resources (NBPGR), New Delhi. But as per the global conservation strategy, it is recommended that a duplicate set of unique genetic resources should be maintained at an alternate site. The issue of developing alternate sites for genebank collections mainly arose due to the international concern over the skewed funding and management capacity of individual genebanks, which might lead to permanent loss of precious genetic resources. The vulnerability to man-made and natural calamities was also a major concern. The first initiative in developing safety duplicate site was made through the establishment of the Svalbard Global Seed Vault, at Norway, under the management of Government of Norway, the Nordic Genetic Resource Center (NordGen) and the Global Crop Diversity Trust (CROPTRUST). The major objective of Svalbard Seed Vault is to provide a safety net for the international conservation system of plant genetic resources, which is maintained under natural permafrost conditions. The relevance of such a global facility can never be undermined, especially when there are unprecedented incidents of catastrophe, leading to large scale, permanent destruction of genetic resources. ICARDA, Syria could re-establish almost all their conserved genetic resources which they had lost during the on-going political conflict, using their duplicate set conserved at Svalbard (Croptrust, 2015).

But, as we continue our support for this noble cause, it is equally important that we implement this concept within individual countries also. Though global policy regimes are striving to attain convergence on conservation objectives, it is quite apparent that the amalgamation of international and national policies on germplasm management is not seamless. Even with the implementation of the ITPGRFA, political boundaries continue to dominate in the negotiations pertaining to a major share of PGR which are outside the ambit of the multilateral system. Hence, countries should make

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independent efforts to secure their indigenous genetic resources at duplicate sites, using energy efficient measures. India has a large area with permanent permafrost features, which can serve as a suitable alternate site for genebank. At national level, efforts were initiated in 2001, for establishing a National Permafrost Facility (NPF) for long term conservation of plant genetic resources, under energy-efficient and cost-effective conditions. For finalizing the location, a team of engineers and scientists surveyed Chang La, Leh-Ladakh, between Drass and Zozila, Khardung La and vicinity of Siachin Base Camp including areas such as Kargil and Sasre La. Due to several advantages of Khardung La and Chang La region, emphasis was given to these sites and a joint conservation experiment was initiated by ICAR-National Bureau of Plant Genetic Resources, New Delhi and Defence Institute of High Altitude Research (DIHAR), Leh-Ladakh (Singh *et al.,* 2008), which continued upto 2015. Subsequently, the project could not be taken ahead further since the area became a sensitive zone, due to the Dokhlam standoff. Thus, it was imperative to identify a new location for the NPF, for which surveys had to be conducted. For this second phase of survey, Uttarakhand state was selected for exploration and site identification.

Objective of Uttarakhand survey: The exploration was conducted with the objective to identify a safety duplicate site for National Genebank, in the Garhwal zone of the Western Himalayan region, in Uttarakhand.

Sites that were surveyed (Fig. 1):

- Bhyundar Valley- Hemkund
- Mana-Badrinath
- Joshimath-Auli

Period of survey: 5-11 October, 2018

Method of Site Selection

We charted out five major points based on which the locations were to be explored and prioritized.

1. Subzero temperatures below -10°C for more than six months
2. Minimum running cost
3. Motorable road access
4. Safe from security point of view, complemented with presence of Army unit in its neighbourhood
5. Geological safety

Fig. 1. Route map of the surveyed area

The Garhwal-Kumaun Himalayan region in the state of Uttarakhand lies between 28.0 to 30.5°N, and 79.0 to 82.0°E. The mountain ranges stretching from the North-West to South-East part of the state are geologically classified as

1. The Greater or Inner Himalayas, with elevations ranging roughly from 3,000 to 7,600 metres
2. The Middle or Lesser Himalayas with elevation ranging from 2000 to 3000 metres
3. Sub-Himalayas or Outer Himalayas or Siwalik ranges, which merges at the southern part with the alluvial Bhabhar tract and further, with the marshy Tarai area. The combined Siwalik-Bhabar-Tarai area ranges in elevation from 300 to 3,000 metres.

The Uttarakhand Himalaya has a total of 1439 glaciers covering an area of 4060 km^2, which is sub-divided across the various mountain ranges (Sah *et al*., 2005). But studies indicate that glaciers in the Himalayan region are in a general state of recession since 1850's (Dobhal *et al*., 2004; Kulkarni *et al*., 2007), though glaciers beyond 4000m were found to be not affected by the global warming phenomenon. The permanent snow line altitude for Garhwal is reported to be 5050m (GSI, 2001).

These geologic characteristics theoretically offer several ideal sites for our genebank, especially in the Garhwal zone.

The Uttarakhand Himalayas is a seismically sensitive zone due to the Northward movement of the Indian tectonic plate against the Tibetan block of

the Eurasian plate, at a geologically significant rate. Earthquakes are routinely reported and Chamoli was the epicenter for the major earthquake that rocked Uttarakhand in 1999, causing wide scale destruction. During the peak monsoon period between June-October, there are frequent landslides in the steep and unstable mountain slopes, made vulnerable by the low grade metamorphosed sediments. Hence, expert opinion on the safety of the site by geologists based on seismic data, the occurrence of active geo-morphological features and the possibility of tectonic rejuvenation were also points to be considered.

Results of the Site Survey

Bhyundar Valley- Hemkund Survey

We started the survey of this zone from Govind Ghat, which is around 20 kms ahead of Joshimath. From Govind Ghat, we trekked over 13 Km, along the steep mountain trail, crossed Bhyundar valley and reached Ghangharia village or Govind Dham, where the trekkers usually break for the day. The entire route is along the Laxman Ganga River and during this trek we cross over from an altitude of 5500 feet (Govind Ghat) to 10200 feet (Ghangharia). This part of Himalayas is an explorer's delight, with huge diversity in plant species that shifts with change in altitude. The entire landscape envelopes a true microcosm of mountain biodiversity. At the beginning of the trail, there is an abundance of *Alnus nepalensis, Aesculus indica* and *Celtis australis*. As we moved along, the other tree species found were *Betula alnoides*, *Quercus floribunda*, *Carpinus viminea*, *Lyonia ovalifolia,* Willow/*Salix fragilis* (near streams), Poplars, *Taxus baccata* (locals used the powdered bark as a substitute for tea powder; has carcinogenic properties) and Rhododendrons. The Garhwal Himalayas has very rich diversity of wild edible fruits and we could find numerous species at various stages of fruit maturity. The common ones were Walnut/*Juglans regia*, *Sorbus cuspidata*, *Cornus capitata, Cornus macrophylla*, *Prunus jacquemontii, P. cornata, P. armeniaca, Rosa webbiana, R. macrophylla* and wild *Vitis.* Indian barberry (*Berberis aristata*) was aplenty and fully laden with purplish berries. Similarly *Cotoneaster microphyllus* (preferred root stock for pear) and *Prinsepia utilis* (Himalayan cherry) bushes were found thriving over a wide altitudinal range.

The entire mountain belt is a half-yearly dwelling place for the locals, who shift to Pulna area after mid-October. We could see few abandoned crops in the back yard of several homesteads, since shifting had already started. Halfway through the trek, we had to cross the river over an improvised bridge. The scars of the 2009 landslide were visible at this site. Clusters of houses were still lying submerged beneath the rubbles and boulders. At Kanjila, 1 Km before Ghangharia, the valley substantially widens. The helicopter service operating from Govindghat, brings visitors up till the Kanjila Helipad. There is also one unit of the State Disaster Management Organization functioning here. Ghangharia is the last habituated village in the Hemkund route and is open only from May to Mid-October. It is the confluence point where the Pushpawati River meets Bhyundar Ganga and continues down as Lakshman Ganga. The whole of Ghangharia is centred upon a radius of few kilometers and is surrounded on all sides by the characteristic steep and rocky upper mountain ranges. This mountain terrain at above 3000m altitude was having the advantage of permanent snow cover for a major part of the year. But the high susceptibility to landslides was a prominent disadvantage.

From Ghangharia, we proceeded for the steep climb that would take us to a further height of 3600 feet, to be covered in a 6 km trek. The trekking path has been laid out in late 1930s and the route has been changed on several occasions due to landslides. *Abies pindrow* or Himalayan fir tree is the major pine species in these higher mountain slopes. Lichens, mosses and several resilient flowering annuals, *viz., Polygonum polystachyum*, *Rheum emodi* (rhubarb), *Aconitum violaceum*, *Angelica glauca*, *Ribes alpestre*, *Morina longifolia, Jurinea dolomiaea, Rumex nepalensis, Sedum ewersii, Pleurospermum angelicoides, Selinum wallichianum, Thymus sp.* and *Oxyria digyna* (leaves are chewed to quench thirst) thrive on the cliffy terrain. The mountains host a huge population of *Betula utilis* or Bhoj Patra, revered for its religious and historical significance. In ancient India, many of the sanskrit texts were written on the papery bark peels of this tree. The silvery white tree trunks make them stand out even amongst the most brightly coloured autumn foliage of other temperate deciduous species and they persist even upto 4200m, thus forming the final boundary of the tree line. Beyond that it is just rocky terrain and rough boulders, with few intermittent patches of *Rhododendron lepidotum*. Ice sheets and dripping icicles, that were slowly melting away as the day advanced, covered the barren cliffs. The Hemkund glacier has significantly receded over the years and

the glacier cover has now totally withdrawn from the trekking route at this period of the year. But the moraine soil formed as a result of glacier erosion is apparently a boon to the alpine vegetation that thrives here with rich diversity. At the pinnacle, there is the Hemkund glacial lake (Fig. 2), which is surrounded by seven snow crested peaks. We explored the areas within permissible limits. There was no visible damage from geological hazards and at this altitude; the micro-climate too was congenial for safe seed storage. The absence of permanent glacier was compensated by deep snow cover for more than 7 months. But the location had a major disadvantage of being not accessible for several months due to snow-choked routes. Our discussion with the army unit revealed possibility of extending motorable road access till Ghangharia, but not beyond that. Hence due to these major lacunae, we had to rule out this site from our list.

Fig. 2. The Hemkund glacial lake

Mana-Badrinath Survey: Our next survey zone was the mountain ranges located towards the Mana village, located at more than 3000m altitude. Mana is the last Indian village before the Indo-Tibetan border and is just 3 Km from Badrinath. On our trip to Mana, we explored all areas adjoining Badrinath and discussed with the locals about the geological and demographic status of the locality. The area is dominated by the Bhotiya tribes who live off the resources in the region. They initially used to trade with the Tibetans which is now not permitted. They are highly dependent on the natural resources of the region and they are conscious of the need to sustain these resources, due to which an effective ecological balance is maintained. Sheep rearing and farming is the major occupation. They stay at Mana only for six months and the whole village remains uninhabited from November to March due to heavy snow fall. During their stay of six months, the Bhotiya people grow several underutilized crops which is part of their traditional cuisine, the major being *Fagopyrum esculentum, F. tataricum, Hordeum himalayense* (naked barley) and certain unique landraces of *Phaseolus vulgaris* and *Amaranthus hypochondriacus* (Fig.3). We collected landraces of barley & french bean, *Elymus* sp. (crop wild relative of wheat & barley) and *Cicer microphyllum* (rarely available close wild relative of chick pea). The villagers collect rare material having unique traits, like *Allium tuberosum* (wild), *Brassica* sp. (Toria type), potato (local type), *Prunus jacquemontii* (bold fruits), and *Origanum vulgare* (offered to lord Badrinath and has high medicinal and aromatic value) from surrounding wild habitat and grow in their homesteads. Their major source of income is obtained from the sale of handmade woolen products that are unique to Mana. In addition to the woolen items, the local shops sell packed products of *Thymus serpyllum* (dried whole plant used as tea leaves), *Allium stracheyi*/ pharan (whole stalk), *Onosma hispidum* /baal chad, and shilajeeth resin to tourists. A large stretch of the river embankment in this area had a considerable population of Sea buckthorn / Leh berry *(Hippophae salicifolia)*, which, surprisingly, was nearly thorn-less. Our survey revealed that the area had high susceptibility to landslides and snow cover was also receding every year at a rapid pace.

Fig. 3. *Amaranthus hypochondriacus* crop in Mana

Joshimath-Auli Survey: Auli, which is 12 Kms from Joshimath is a major winter tourism destination of Uttarakhand. It is at 3000m altitude and is surrounded

by snow capped peaks of Nanda Devi and Mana Parvat ranges (Fig. 4). The Mountaineering and Skiing Training Institute of ITBP is located here and the Army has also started its unit, recently. There were well maintained motorable roads used for transport of heavy machinery and stable power supply, supported with state-of- the- art infrastructure. The presence of ITBP and Army units ensured proper security as well. The exploration team held a meeting with Sh. GS Chauhan, Deputy Inspector General (DIG), and Director of the Training Institute, and discussed about the geological and meteorological characteristics of the area. Amongst the various zones that were surveyed, Auli was marked as a suitable area, which met the basic criteria for a duplicate genebank site.

Fig. 4. Surveying the area adjoining Nanda Devi and Mana Parvat ranges

Conclusion

This preliminary survey conducted in the Garhwal Himalayas was aimed at identifying a suitable safety duplicate site for the National Genebank, based on a defined set of criteria. The three major areas covered in the survey were – 1. Bhyundar valley-Hemkund 2. Mana- Badrinath and 3. Joshimath-Auli. The Bhyundar valley-Hemkund region had the advantage of having suitable temperature for a major period of the year, but it could not be recommended due to the absence of motorable roads. The Mana- Badrinath area had better accessibility, but was highly prone to landslides. Joshimath-Auli area (specifically, Auli and its adjoining snow covered mountain ranges) was found to have distinct advantage over the other surveyed regions. As per the criteria that were set for site-selection, Auli was having good accessibility, safe from security point of view and good infrastructure facilities. Any further decision on identification of a specific site within this region requires a detailed exploration with additional expertise, by a team involving geologists, engineers, disaster management personnel and environmental scientists.

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REVIEW ARTICLE

Logistics Planning for Plant Genetic Resources Collecting from Nicobar Islands of India

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The Nicobar group of Islands forming a part of Sundaland biodiversity hotspot were explored for plant genetic resources during three consecutive years, 2017, 2018 and 2019. Out of the total 22 Islands in Nicobar district 12 are inhabited. Considering the remoteness, communication and transport problems, hurdles in getting clearance from forest and tribal welfare departments, impenetrable forest, unpredictable sea voyage conditions, unavailability of camping sites, scare of dangerous reptiles and many such issues, these explorations were really challenging. Also, equally rewarding as over 370 germplasm collections belonging to 180 species of mostly crop wild relatives including several endemics (not available in the genebanks in mainland) could be collected and conserved in the Long Term Storage/established in the Field Gene Banks. The planning and implementation of exploration missions to Nicobar was an entirely different experience for us than that followed in mainland. Hence, various aspects of logistics while undertaking an exploration in Nicobar Islands is dealt in detail in this narrative in order to serve as a guide to future explorers. Based on observations and experience gained during these trips spanning over 48 working days, future strategies for conservation and utilization are also briefly outlined.

Key Words: Agrobiodiversity, Andaman & Nicobar, Crop wild relatives, Germplasm, Sundaland biodiversity hotspot

Introduction

The Nicobar group of Islands of India is endowed with rich plant diversity and endemism; however, less studied and least explored by plant genetic resources (PGR) collectors mainly due to its remoteness causing difficulties in access. It is a part of Sundaland biodiversity hotspot in continuation with Sumatra (Indonesia). During the summer seasons of 2017, 2018 and 2019, the ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR), New Delhi in collaboration with ICAR-Central Island Agriculture Research Institute (ICAR-CIARI), Port Blair and ICAR-Indian Institute of Spices Research (ICAR-IISR), Kozhikode undertook explorations to these islands (Fig. 1) stretching over 48 working days. This narrative deals with various aspects of PGR exploration and collection in the Nicobar Islands, as experienced by the authors, with emphasis on logistics and future plan of action, serving as a guideline for future explorers to the region.

The Nicobar division/district comprised of 22 islands with a total land area of 1841 Km^2. The Ten Degree Channel separates Andaman group of islands from Nicobar group and plays a major role in the geographical isolation of the flora. The Great Nicobar Biosphere Reserve (GNBR) including Little Nicobar and Kondul, the Nancowry group of islands (Bampuka, Champin, Chowra, Hithui, Kamorta, Katchal, Nancowrie, Teressa,Tillangchong and Trinket) and Car Nicobar are the three major divisions in Nicobar. The Nicobar group of islands is reported as peaks of a submarine mountain range standing above sea level extending from ArakanYoma ranges in Myanmar (continuity of Mizoram-Nagaland hill ranges) to Mettawai Island in Sumatra. Mount Thullier near Campbell Bay of about 670m is the highest peak in Nicobar. Five perennial rivers, namely, Galathea, Dogmar, Amrit Kaur, Jubilee and Alexandra with their tributaries constitute the drainage system in Great Nicobar, while other islands

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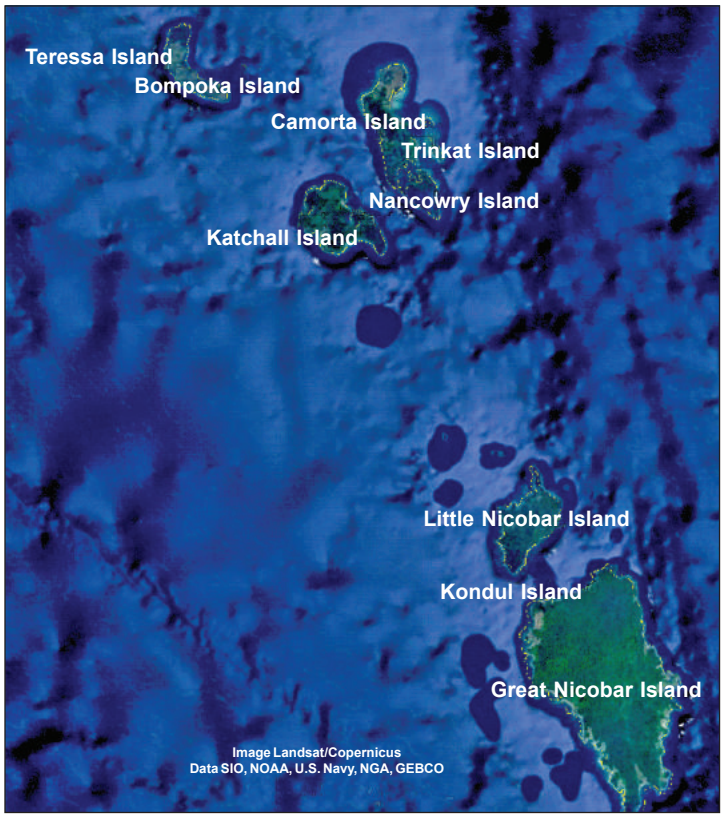

Fig. 1. Location map of explored islands in Nicobar

in Nicobar do not have major rivers except streams and forest *nallahs*. Car Nicobar is mostly plain land, while all other islands have rugged terrain. Car Nicobar has the highest density of population (140/km^2).

Historical Perspective

The first definite reference to the Nicobar Islands is found in great Tanjore Inscription of Chola kings (around 1050 CE), who with their strong navy had contacts with these islands have described them in the inscription as '*Nakkavaram*' which means the land of naked people (GOI, 1908). In the 16th century, these islands attracted attention of Christian missionaries. In 1750, the Danish took possession of these islands and established their headquarters in Kamorta Island. In 1869, after negotiations with the Danish government, the British took possession of the islands.

The British made the Nicobar Islands a part of A&N Islands under Chief Commissioner of A&N Islands, Port Blair, and a government agent was put up at Car Nicobar to maintain law and order between natives and the Indian traders. Car Nicobar was selected as the seat of agent as it was more populated than the other islands and the attitude of the British was non-interference in the life of people. The contact with the mainland grew over the years with the occupation of the Islands by the British. Many traders arrived especially from Sumatra (Indonesia) and also from Minicoy and Lakshadweep islands for coconut and betel nuts. Currency was not in use in Nicobar as they followed the barter system, exchanging coconuts to buy things.

The Second World War and the consequent Japanese occupation of Car Nicobar and other Islands during 1942 to 1945 brought many changes in the history of these islands. Many coconut trees were cut down and the Nicobarese were forced to work as labour for construction of roads around the island, runways and jetties. After independence from British, the Indian government has enacted the Protection of Aboriginal Tribes Regulation Act in 1956 to protect the interests of native people of Andaman and Nicobar Islands from exploitation under which entry to the tribal area of Nicobar Islands is restricted.

Demographic Profile

The native population consists mostly of two ethnic groups, the Nicobarese and the aboriginal Shompens, one of the least known particularly vulnerable tribal groups (PVTGs) in the Andaman and Nicobar Islands. As per the 2011 Census, Nicobarese population numbers around 30,000 and literacy rate for the entire Nicobar district is about 84 per cent. Vast majority of them have embraced Christianity, and pastors are treated with great respect. A small fraction of Nicobarese mainly at Kamorta follows Islam. Shompen population could be between 200 and 300 as per the demographic database of Anthropological Survey of India (AnSI).

Linguistic and Cultural Milieu

People in Nicobar Islands speak different languages. Car Nicobar people speak Nicobarese (part of the Austro-Asiatic language family). For centuries in the past they had no script; however in 1912, George Whitehead who lived some years in the Nicobar Islands as agent and missionary has evolved a script. Nicobarese can be taken to be the *lingua franca* of most of these islands.

Nicobarese people have good health. It is commonly said that the visitors to these islands get struck by the welcome sight of muscular bodies, the glow of health and the happiness on most faces. People of Nicobar are known to possess considerable practical skills and all men have been known to have some basic carpentry skill. They make good canoes of varying size from very small (which can carry 2 or 3 persons) to bigger ones which can easily accommodate about 40 persons. It is also reported that they use no nails for joining their outrigger.

Nicobarese are not much interested in money and in general are content with what they have. However, unlike other aboriginal tribes of A&N, they handle cash but wage earning for labour is less and voluntary labour donation or exchange is in vogue. There is no title deed for cultivated lands of Nicobarese, which is community-owned and inherited by families.

The Tsunami in 2004 had played havoc with their lives. Loss of human life was very high. The pain of bereavement still haunts the survivors. Monuments adorned with marble plaques with names of dear departed engraved, erected at entry points or prominent places in all Nicobari villages, stand a grim reminder to the loss (Fig. 2). Countless have been orphaned; however, nobody is rendered homeless and every orphan child is adopted by one family or the other.

Nicobarese are not hostile, mostly reserved and shy in socializing, in general, and often friendly and helpful. Generally, they don't greet strangers unless introduced by captain (village chieftain). A smile or a raised hand may be taken as reciprocation of greetings. Shaking hands and offering food/ Nicobarese delicacies may be taken as goodwill of the community. If we need any help, we have to clearly ask for nonpaid services and even for food and other requirements. Those Nicobarese, who are exposed to public contact extend hospitality and offer tea, tender coconuts etc. They do not generally express their emotions, and communication with outsiders is very less. They are considered very sensitive people and liable to get hurt easily and feel deeply. Thus, it is said that the greatest of tact, consideration, understanding as well as sympathy and patience are required for dealing with the Nicobarese, and without these qualities anybody wanting to work amongst these people must never ever hope to succeed.

Gender Mainstreaming

Men treat women with great respect; domestic violence is not heard off and women enjoy privileges in the society. Occasionally women hold the elected post of captaincy. There are quite a few Nicobari women in the Government services as doctors, nurses, teachers, police personnel, forest staff etc. However, unlike other Mongolian societies, women do not engage in any business like running petty shops and are confined to homes as agriculturists and home makers.

Agricultural Systems and Practices

The Nicobari system of crop cultivation is basically of sustenance agriculture. After the Tsunami, people moved from the traditional huts (Fig. 3) to Govt. provided Tsunami shelters which are in upper terrain away from sea coast. Agro-ecosystems are of three types – coconut monocrop in the coastal plains, *tuhets*, and backyard gardens in and around shelters. The coconut plantations are mostly monocrop with an occasional jackfruit, bread fruit or mango trees. Occasionally coconuts are planted near settlements. Ownership of bearing coconut tree is given to individuals and nobody encroaches on neighbour's property even for a fallen coconut. *Tuhet* is the traditional agro-ecosystem jointly managed by extended family members. The labour is contributed by members and harvest also shared between member families. Men do only a few limited activities like fencing and generally women tend to the crops. Agronomical practices are bare minimum in *tuhets*. No scientific practices of spacing, digging or earthing up are followed. An assorted mix of various crops like taro, cocoyam, giant taro, potato yam, greater yam, lesser yam, sweet potato, sugarcane, vegetable banana, fruit banana, pineapple, tobacco etc. are planted. Soil fertility is maintained by adding crop residues and pig dung. So far, no improved varieties have reached Nicobar Islands. We could not come across any rice cultivation except in Great Nicobar by settlers. *Tuhets* are protected from domestic pigs by meticulously built, beautifully designed wooden fences (Fig. 4). This zig-zag fence is an icon of the islands, especially in Teressa. Poles and logs of regular length are cut and placed in perfect geometric designs of waist height by tying with cordage fibre. Peculiar triangular wooden log tree guards are also seen in Little Nicobar to protect the individual plants from pigs (Fig. 5). Backyard gardens in and around shelters are common across Nicobar. An assorted mix of various vegetables and fruit trees is raised in and around the

dwellings. Ornamentals like various types of crotons, shoe flower, bougainvillea, jasmine, marigold, and many other annuals and shrubs are maintained in the vicinity of shelters. Trailing a betel vine to overhead water tank is a common practice in almost all Tsunami shelters. Nicobarese use only very few agricultural implements such as axe, crowbar and cutting knife. Even though State Agriculture Department is encouraging mechanized scientific agriculture, adoption is very low. Coconut climber is the only machine widely accepted.

Using *Imperata cylindrica* for thatching houses and giving fire to grasslands for promoting abundant fresh growth were common practices. However, after Tsunami, almost every dwelling is now roofed with tin sheets and there is no use for grasses either as thatching material or as fodder, as Nicobarese do not rear cattle. However, the annual ritual of lighting fire to the grasslands still continues. The ecological impact of this practice is worth studying.

Nicobarese live as a natural component of the predominantly forest ecosystem. There are no industries, hotels, tourism, public houses or anything of that sort. Due to strategic reasons, from 1960 to 1980, under the Great Nicobar Southern Frontier Rehabilitation Scheme of Govt. of India, 330 odd ex-servicemen families were allotted between 11-15 acres of cultivable, cleared forest land. Few makeshift shops and eateries at Kamorta and Campbell Bay by settler communities cater to floating populations arriving in ships mostly for Govt. work. Concrete constructions and double storey buildings are very few and restricted to Government buildings and defence enclaves. Roof-top rain water harvesting for drinking purpose is often practiced among Nicobari households.

Crop, Species and Genetic Diversity

Good diversity in greater yam and taro was observed in *tuhet* agro-ecosystem. Even though species diversity is high in *tuhets*, genetic diversity is less, probably because of less population density and recent agricultural history (except coconut). Species diversity comprises lime, lemon, citron, cotton, ginger, turmeric, lemon grass, *Alpinia nigra,* pineapple, bullock heart, soursop and banana. Altogether poor genetic variability was observed, probably due to people not keen in selecting, and whatever observed by us, are primarily due to natural selection. In riverine forests and marshy lowland forests, arecanuts are planted; however, no agronomic care or scientific cultivation is practiced, it becoming self-sown thereafter.

The colonial history of the islands especially under Danish and British rule helped to augment genetic resources of crops like citrus. It was informed that seafarers from Minicoy (Lakshadweep) also brought some crops, especially in Kamorta.

Food Sources and Culinary Traditions

Rice supplied through public distribution system, few tubers, fish, banana and coconut constitute the major intake. Bread fruit, sweet potato, giant taro, greater yam, cocoyam and taro are the important carbohydrate source. Consumption of cultivated vegetables is very less in Nicobar society. Wild gathered fruits and vegetables form a regular part of their diet, which includes *Gnetum gnemon*, *Champereia manillana, Cissus repens, Gymnema latifolium* (all leafy vegetables), *Mangifera nicobarica, Artocarpus chama, Pandanus leram* (all fruits) and *Cycas zeylanica* (starch).

Every household rears pigs and chickens. Nicobari fowl is an indigenous breed with high population diversity. However, egg-laying hens are not generally slaughtered, as they are intended for maintaining higher flock size. Milking cows are seldom seen in Nicobari households. Goats were also spotted in very less numbers, even though Teressa Island is known for a unique breed of goat. Aromatic herbs like lemon grass, *Pandanus amarylliifolius, Ocimum tenuiflorus, Eryngium foetidum*, *Alpinia nigra*, *Kaempferia galanga*, ginger, turmeric, etc. are grown in almost all home gardens, which are well protected by fencing with used fish net, tin roofing sheets etc.

In fact, *Pandanus leram,* growing abundantly in the littoral forests and coastal strands, is an important food item for the Nicobarese. It used to be the staple food before free rations started. Now Nicobarese depend on rice and *dal* supplied by the Department of Tribal Welfare. During scarcity of food due to disruption of sea transport, people dig out various wild yam species like *Dioscorea glabra* (vern. *kokila*) and *D. piscatorum* (*okkav*) from the wild and eat the cooked tubers with fish and coconut. *Dioscorea piscatorum* is in early domestication phase. Wild stands are dug out and planted near home gardens for easy harvest (Pradheep *et al*., 2019a).

We were fortunate to witness various stages of *Pandanus* meal preparation. Mature bunches are harvested from the forest by men and transported to

habitation. Women take care of the processing. Individual fruits are separated, washed and steam cooked before extraction of pulp from the mesocarp of the fruit. The fruit pulp is scraped and separated from fibre manually. The mealy mass /dough is mixed with grated coconut, salt and cooked as steamed cakes, which formed staple food for Nicobarese till few decades back.

As whole Great Nicobar and Little Nicobar Islands are protected as Biosphere Reserve and human activities confined to only a small tract from Campbell Bay to down south up to Sastry Nagar, this wild food source is protected. However, *Pandanus* is worth studying for their natural populations for genetic diversity and clonal selection of superior trees, further promote it as a crop in Nicobari gardens. Nutritional evaluation of the product, preparation of value-added products, promoting as health food etc., and linking this ethnic food tradition with ecotourism would offer greater income avenues for the tribals. This tree can be acclimatized elsewhere in mainland (Sundarbans and West Coast) also as food resource, also holding promise as a good source of leaf fibre for mat weaving – *kewda Pandanus*. Bigger the leaf size, more the benefit to the weaving industry.

Soil, Climate and Forests

Soils are primarily of sand stone, silt stone and clay beds. Alluvial soils predominate in river beds, silt sands in river mouth and tidal swamps and calcareous soils in beach forests. Forest soils are rich in humus, and are mildly to moderately acidic and heavily leached. *Areca* swamps and *Myristica* swamps are seen in Little Nicobar. Soils of coral origin are found in parts of Kamorta and Katchal islands.

Climate is warm tropical and humid throughout the year. Temperature ranges between 25 to 30°C. March-May is the summer period, even though 5-6 rainy days are expected during this period also. There is no winter even though it is mildly cool in December-February. Average relative humidity is 80 per cent. An average annual rainfall of 300cm is received, almost well distributed with South-West monsoon contributing the major share.

Forests can be broadly classified into mangrove forest, beach forest, littoral forest, riverine forest and tropical evergreen forest. According to Porwal *et al*. (2012), mixed mangroves (>89%) and littoral beach forests (86.3%)in Great Nicobar and Little Nicobar were either submerged or severely damaged by Tsunami (Fig. 6).Stretches of grasslands dominated by *Imperata cylindrical* are found in Teressa (Fig.7), Kamorta and Champin islands. Vegetation also varies in different forest ecosystems; however, majority of the crop wild relative (CWR) species are found in the tropical evergreen forests (Fig. 8). *Vigna glabrescens* is found in grasslands of Teressa Island.

Logistics Planning

Consulting flora, herbarium and other associated resources

Agro-biodiversity in Nicobar is largely centered on CWR and useful economic plants with cultivation and domestication potential. Nevertheless, scope for collecting variability in few crops like jack fruit, citrus, mango, banana, coconut, arecanut, greater yam and taro does exist (Abraham *et al*., 2008). For planning areas to be explored and target taxa, floristic accounts (Thothathri, 1960; Chakraborty, 1970; Thothathri *et al*., 1973; Dagar and Singh, 1999; Pandey and Diwakar, 2008), botanical memoirs of explorers, relevant floras of A&N (Hajra *et al*., 1999; Sinha, 1999) and adjoining Malesia (http://portal.cybertaxonomy.org/flora-malesiana/node/1) may be consulted. Herbarium consultation at Botanical Survey of India, Kolkata and Port Blair and online herbarium collections (at Leiden herbarium) from nearby islands of Indonesia (Sumatra, Java) would help in field identification of species of interest, besides their collection sites. Accordingly, a checklist of expected economically important species and tentative itinerary may be prepared.

Nicobar has three entry points from Port Blair by voyage/passenger ships: Campbell Bay in Great Nicobar, Kamorta in Nancowrie and Car Nicobar port. From Port Blair it takes about 24 h to reach Kamorta, 36 h to reach Campbell Bay and 24 h to reach Car Nicobar. Ship schedule is published in local dailies and also available in A&N Administration website 3-7 days in advance and tickets can be booked in advance. However, there is no online booking facility available for ships. In general, one can expect sailing of 1-2 ships to Campbell Bay (via Kamorta) and 2-3 to Kamorta in a week. Three classes, Bunk (Lower), Cabin and Deluxe accommodations are available with berth facilities in all. For booking ship tickets a valid pass for entering restricted area is necessary for any destination in Nicobar other than Campbell Bay.

Getting Forest Department Permission and Tribal Area Landing Permit

For entering into any protected area and collecting any biological material, a researcher must obtain a valid permission from the A&N Forest Department. The team leader has to apply in writing specifying list of species to be collected, period of time to be spent in the forest, forest ranges and divisions to be covered along with personal details of the team members. After scrutiny of the proposal, the A&N Forest Department will ask the team leader to make a brief presentation of the research proposal before the Research Advisory Committee (RAC) of the Department, usually at their Headquarters in Haddo, Port Blair. Upon recommendations of RAC of the A&N Forest Department, Principal Chief Conservator of Forests (PCCF) will issue a permit to the team for a specific period with detailed instructions on do's and don'ts. However, this permission for collection of germplasm does not entitle the applicant to visit Biological Park, and similar kinds, for which special permission from PCCF Wildlife (Chief Wildlife Warden) is required.

Further, in order to land at any of the ports in Nicobar other than Campbell Bay and also to visit Nicobari tribal areas, a pass for entering restricted areas, i.e. Tribal Area Landing Permit (TALP) is required to be issued from the Deputy Commissioner headquartered at Port Blair or Car Nicobar. It is also possible to get TALP issued at Campbell Bay, Kamorta and Car Nicobar by Sub-Divisional Magistrate (SDM) or Assistant Commissioner but the process is too cumbersome and time consuming. Moreover, for booking ship tickets it is mandatory to show the TALP. Hence, it is advisable to file the papers at the Deputy Commissioner's office at Port Blair well in advance after forest entry permit is obtained and itinerary is finalized. All team members have to apply individually in prescribed format through online portal (including getting attestation from gazetted officer working in A&N) and after police verification, photo-affixed pass for restricted areas entry is issued which contains all details like areas permitted, period of stay permitted along with instructions regarding do's and don'ts which the pass holder is to strictly comply with. Upon disembarkment from the ship, the pass holder is bound to report to the nearest police outpost with the pass and identity card and to appraise the police officer about halt details of the team. Sufficient number of photocopies of entry permits and Identity cards needs to be kept by the pass holders to be deposited at police stations and forest check posts.

Liaisoning with Line Departments

For any PGR related explorations in A&N Islands, Director, ICAR-CIARI, Port Blair is the nodal person capable of providing necessary logistic support. Right from transit accommodation at CIARI guest house to local transport, hiring vehicles, *dungies* (country boat) etc. support and guidance from CIARI is required. The Directorate of Agriculture, Port Blair has their staff and offices and farms located in almost all inhabited islands. Logistic support from Forest Department, especially range officer is also crucial in almost all remote localities. Upon arrival at a port, the exploration team should meet and discuss with the concerned officials of Agriculture and Forest Departments for finalizing sites to be visited, transportation, accommodation, arranging local guides etc. A&N Public Works Department has guesthouses at Campbell Bay, Kamorta, Teressa, Katchal, Chowra and Car Nicobar. Rooms can be booked in advance on behalf of the team by line department officials. The SDMs at Campbell Bay, Kamorta and Car Nicobar are the designated officials for allotment of A&N Public Work Department guesthouse accommodation and the Special Relief Officer (SRO) for the other islands.

Inter-island and Road Transport

Passenger ships ply at regular intervals connecting Port Blair with Campbell Bay, Kamorta and Car Nicobar and other important jetties in Nicobar. Kamorta Jetty is the transit point for passenger boats to Nancowrie group of islands like Teressa, Katchal, Champin, Trinket and Hithui. Schedule of passenger boats are decided by Directorate of Shipping Service, A&N administration and can be known one week in advance from the jetty/ port office. Just like taxis are hired for road journey, nearby islands in Nancowrie can be reached through hired *dungies* (motorized country boats) (Fig. 9). For exploration purpose to connect various destinations *dungies* can be hired. Well-built jetties are in place at most of the inhabited island destinations like Afra Bay, Hithui, Teressa, Katchal and Champin. However, at destinations like Kondul, Macachua, Pilobao, Pilopanja, Pilomilo and Kuiinoh, no proper landing facilities are available. Hence, the *dungies* will have to be anchored in waist-deep water. In such cases, either one has to wade through shallow waters or transfer to the *oddies*

(single seated locally designed push boats) one by one to reach the shore (Fig. 10).

Good roads with asphalt (tar) top connecting diagonal points in the islands are laid out in Great Nicobar and single long roads in Kamorta, Katchal and Teressa. Campbell Bay has a fairly good network of roads and the North-South Road extending about 60 km connects Campbell Bay with Indira Point. Incidentally, this road had collapsed in Tsunami of 2004 and now construction has been completed up to 37th km, i.e. near Galathea Wild Life Sanctuary, the southernmost tip of India. Limited private taxis and auto rickshaws are available for hire at Kamorta, Campbell Bay and Katchal. At Teressa and Champin Islands, there is an option to hire motor bikes. However, Munak and Hithui Islands have no roads and trekking is the only option. In all the forest tracts like Dering, Mount Thullier, etc. no vehicular traffic is possible and trekking is the only option (Fig. 11). Table 1 provides information on the major trekking areas in various parts of Nicobar.

Efficient Management of Time

Sunrise and sunset in A&N Islands are at least two hours earlier than the mainland (barring eastern and north-east India), hence day starts early. Offices in the Nicobar Islands appear to have an extended lunch break. Regular electricity supply is available only at Kamorta and Campbell Bay guesthouses and at the remaining places diesel powered generator will run for only limited hours in the evening. Hence, planning of activities so as to use maximum day light is very important in Nicobar Islands. As the solar and lunar forces affect sea wave movements (Fig. 12), which in turn will affect smooth sailing of *dungies*, it is always advisable to start sailing early in the morning hours, preferably by 4-5 AM. Seasonal winds are also a deciding factor for calm sea. In certain periods of the year, landing at sea shore destinations is very difficult and risky. Hence, the exploration trip has to be planned preferably during January-April, and one should follow advice of port officials/ boat crew rather than taking own decisions regarding sailing and landing destinations.

Arrival at Island Port/Village

Upon disembarking, the team members are bound to report to the police station in person, enter details of visit in the register and deposit a photocopy of the permit. At Kamorta Jetty, police verify the documents before permitting entry in to the island and there is no need for further verification in the police station. In other islands, non-tribal visitor is bound to report in person at the police outpost upon arrival (Fig. 13). Also, it is advised to inform Tribal Council Chairperson/Secretary for various group of Islands (located at Campbell Bay, Kamorta etc.) the purpose of collection team, so that they would instruct the respective captains of villages well in advance to cooperate with the team and provide essential support. Explorers should keep sufficient money with them avoiding last minute withdrawal option, as currently there are only one or two ATMs available, that too only at Kamorta, Campbell Bay and Malacca.

As the administrative hierarchy is very strong in Nicobari tribal society, the captain (elected chieftain) of the village has to be consulted before venturing into any action in his/her territory. In the absence of 1st captain next one (2nd and 3rd captain) in the order has to be consulted for any activities like collection or engaging people or anything like that. For requirements like temporary accommodations and food in places where no APWD guest houses are available, the captain usually makes arrangements. The unoccupied Tsunami shelters were provided in a few occasions for our stay. In Macachua and Afra Bay, as the police outpost is spacious, police officials usually provide halting facility, upon prior intimation. In Nicobar, the police force is very friendly and they maintain good rapport with Nicobari youth. Often youth assemble at the station for a game of volley ball and also share meals with the police. Criminal offences are seldom heard in Nicobari society and the rare cases are that of poaching, often involving Myanmarese and Thai nationals illegally entering in to Indian Territory. In Galathea WLS, only a camp shed is made by Forest Department, which is to be shared with their staff (Fig. 14). Therefore, while planning for exploration in Galathea WLS, Afra Bay and Little Nicobar where no regular lodging facilities are available, it is advisable to carry light weight sleeping bags.

Many Nicobari field staff are presently employed in Agriculture and Forest departments. It is always advisable to have a Nicobari guide from the department along with the team who will help to build rapport and also act as your interpreter with local population. Majority of the Nicobari men understand and speak Hindi. Getting the service of a good guide, preferably a field staff of the Forest Department, is important in successful collection of economic plants. The A&N Forest Department's labour staff mostly are 'Ranchi' tribals, Karens and

Fig. 2. A memorial for the dear departed in Tsunami

Fig. 3. A traditional Nicobari dwelling

Fig. 4. Zigzag fenced tuhet garden

Fig. 5. Tree guard for protection from domestic pigs

Fig. 6. Tsunami devastated inland forest

Fig. 7. Grasslands of Teressa

Fig. 8. A distant view of virgin evergreen forests in Great Nicobar

Fig. 9. Exploration team availing hired dungies for inter-island travel

Table 1. **Sites explored by our team in Nicobar Islands**

	Exploration sites	Island	Days (No.)	Halt
1	Dering forest	Kamorta	1	APWD, Kamorta
2	19th km (in E-W Road) to Kopperheat	Great Nicobar	2	APWD, Camp bell Bay
3	Pilopanja to Macachua	Little Nicobar	1	Macachua
4	Laful to Mt. Thullier	Great Nicobar	2	APWD, Campbell Bay
5	Navy Dera to Mt. Thullier	Great Nicobar	2	APWD, Campbell Bay
6	Trinket Island walk	Trinket	1	APWD Kamorta
7	Galathea mouth point to Indira point	Great Nicobar	1	APWD, Campbell Bay
8	E-Wall to Jetty point	Katchal	1	APWD, Katchal
9	Vikas Nagar to Pilpilow	Kamorta	1	APWD, Kamorta

Nicobarese. Commonly called 'Ranchis', the descendants of Chottanagpur tribes, Uraon and Khadia brought by colonial administrators, have good knowledge of flora and fauna. Karens, the settlers from Myanmar origin are also equally good in their knowledge of ground realities of their forest beat.

Sea Security - Lifeboat, Coast Guard

Getting into and stepping out of the *dungies* needs to be managed carefully to avoid falling into the deep water. It is preferable that the team members know swimming. It is mandatory to keep life guards for every individual passenger and the team leader should ensure personal safety gear availability and also to hire only fit-to-sail *dungies* from service providers. Both at Kamorta and Campbell Bay jetties, Indian Coast Guard boats do keep surveillance and the team leader should be ready with all relevant documents and identity cards for verification by the Coast Guard officials. All *dungies* sail with Indian flag mast on small poles (Fig. 9).

Dungie travel from Campbell Bay to Macachua in Little Nicobar takes around 6.5 h in normal sea weather. Hired *dungies* have no seating arrangements and people have to sit for the whole time with cramped legs and those with sea sickness will often have bouts of vomiting. It is advisable to store sufficient drinking water, oral rehydration solution and lime juice, sugar and salt for rehydration. Exposure to open sun in no-roof *dungies* makes things worse. Umbrellas, hats, clothes etc. for covering whole body may be kept handy while embarking in *dungies*. Camera, mobile phones and other electronic gadgets should be kept covered safely as the sea water often splashes inside the boat. Toilet services are not available once the *dungie* set sail till it reaches shore. At Campbell Bay and Kamorta, shops selling groceries, bakery items, vegetables and banana are available, which may be bought and stored for use at camping sites. Every member has to carry their own ration at Little Nicobar and forest areas of Great Nicobar as there are no shops there.

Mudflats, estuaries, fresh water streams joining sea, river mouths and swamps are highly prone to crocodile attack. Forest Department has placed signage at most of the known localities warning visitors of crocodile attack (Fig. 15). One has to be very careful and cautious while venturing for collection in crocodile infested areas. Stepping in to sea water in Little Nicobar during evening and wee hours may invite crocodile attack.

Clothing, Protective Gear and First Aid

Water swells during high tides in the rivers and crossing the river and embarking the *dungies* may be risky during dusk; invariably, valuable papers and currency notes need to be properly packed in water-proof envelopes. Getting into the *dungies*, transferring to *oddies* and travelling through open sea may expose travelers to lashing sea waves. So, it is always advisable to wear shorts as often due to rough weather, one may have to disembark from boat in to knee-deep or may be waist-high water. However, after reaching the shore, before venturing in to the forest, one has to wear full pants, socks and hunter shoes to avoid many dangers like slippery path, pit viper bite, leech bite, etc. Ready-to-use powder salt in cloth bag may be always kept handy in the pocket to periodically wipe off blood sucking leeches. Leeches are serious problem in rainy season.

Since ring worm infection is a common problem in Great Nicobar, broad spectrum anti-microbial medicine (such as candid cream) needs to be kept handy. Pin worm infection through fruits and salad vegetables are also a common problem. Tick bite may occur in forests, especially in the areas where wild boars are common, which may linger for days together causing itching and skin infection. Mosquito and sand fly bites are extremely high in Great Nicobar, hence mosquito repellant creams, anti-allergic drugs, full body covering clothes, mosquito nets, etc. need to be carried during the trip (Fig. 16). In Little Nicobar, safe drinking water availability is a problem; therefore, it is advised to carry alum/germicide tablets/water-purification tablets to purify raw water. Weil's disease spread by rats is very high in A&N, and one has to be very careful in stepping into stagnant pools, ditches while negotiating marshy areas.

Even though January-March is considered summer season in Nicobar, cyclonic spells of rain may occur and, as trekking in narrow forest path with opened umbrella will be difficult, it is desirable to wear rain coat and cap. All clothes and collected germplasm should be properly packed in water proof polybags before placing in *dungies*. As sharp corals may injure the feet, it is not advised to set bare foot in to water while disembarking from *dungies*/*oddies*.

A strong but light weight wooden walking stick of about 6 feet height will be ideal for negotiating steep climb and narrow paths through the ridges of gorges. Local guides can help in selecting good material for walking stick. Leaning forward, in order to keep feet straight below the centre of gravity, would ease uphill movement. One is expected to cover maximum area on foot and on an average 10-15 km may have to be covered in a day. Descending down the steep slopes may cause muscular pain and necessary painkiller sprays and muscle relaxants may be kept in the first aid box. Due to remoteness of the sites in Great Nicobar and Little Nicobar, first aid kit comprising of all essential medicine may be carried. Due to non-availability of power supply and limited hours of generator running, it is essential to carry torch, candle and such lighting arrangements. As it gets dark by 4 pm in evergreen dense forests, it is always advisable to wear a head light or carry handy night vision devices. No woolens are required during any time of the year. Carrying threads and ropes is also advised for tying and packing.

Even though Campbell Bay and Kamorta are with mobile network now, most of the interior areas and forest tracts are not in network range. However, all police stations have wireless communication through which emergency messages can be sent and received. BSNL is the only service provider in these islands, and internet connections are available in Government Department Offices at Campbell Bay and Kamorta.

It is very important to respect local customs and traditions. Nicobarese usually do not tolerate encroaching in to their property without proper permission. Any movement in the village has to be with the permission of senior most available village captain. Pig is one of the most precious resources of Nicobarese and pigs and chickens are omnipresent near habitations. One should be very careful not to hit or kill any animals. The penalty for such mishaps imposed by the village council is said to be hefty, often calculating the benefits accrued, had the animal been alive, for 2-3 generations.

Apart from Nicobarese, an aboriginal tribe, 'Shompens' inhabit the interior forests of Great Nicobar. Exploration activity is not permitted in Shompen territory, for which special permission from the District Administration and the Tribal Welfare Department is required, which is very difficult, if not impossible. Shompen men occasionally come out of the forest and their outside visit is mainly restricted for procuring the provisions supplied through public distribution system from Campbell Bay. Explorers may encounter Shompen men, especially in East-West Road (here exploration up to 20 km length from Campbell Bay is permissible), and are advised neither to give lift to them in the vehicle nor share food or even interact with them. Also, explorers should strictly refrain from photography or videography in the area as it is an offense inviting fine or imprisonment or both.

Exploration and Collection

Post-harvest processing of germplasm

General guidelines on how to sample, collect germplasm from farm and forest areas and process them for *ex situ* conservation are available in literature like Guarino *et al*. (1995) and NBPGR (2014). However, unlike exploration in mainland, arrangements for handling collected germplasm need to be planned carefully for two reasons. Collected germplasm is mostly vegetative propagules, live cuttings or pulled out seedlings as it is a compromise of what is available at the time

of visit. Secondly, the time required for reaching the laboratory or planting in field may be lengthy and is often unpredictable due to uncertainty in transportation to Port Blair Airport. Hence, the collected vegetative propagules need to be processed properly to ensure extended viability. Desiccation-free storage is the most important aspect combined with fungus-free environment. The collected cuttings/ seedlings may be sprinkled with water and stored in an improvised vasculum. Extra-large transparent polybags of 45 × 15 cm or bigger may be used for this purpose. After sprinkling water, the mouth of the polybag may be closed with a rubber band and kept away from hot sun areas (Fig. 17). Upon reaching campsite, the surplus foliage may be trimmed to reduce transpiration loss and dressed twigs given a drenching with 0.2% Mancozeb. The fungicide may be brought as convenient 2g packets for use in 10 L water. The basal part of the cuttings may be dipped in rooting hormone as per requirement and wrapped with small quantity of 1:3 moist vermiculate-perlite mixture and again stored in closed medium sachet, keeping the mouth of the sachet open for 1-2 hours during night to prevent fungal infection. Roots of the pulled-out seedlings should also be covered with the above mixture, wrapped with a cotton cloth and then to be kept inside a transparent polythene cover to prevent transpiration loss. If the exploration trip extends for a long period, the live plants have to be taken out of the cover and monitored periodically to salvage the infected, if any. Extra-large poly bags can also be prepared as ready-to-use mist chamber for planting the vegetative propagules during transit. Lemon grass, curry leaf, wild *Mangifera* seedlings, *Garcinia* seedlings, wild banana seedlings, *Alpinia* suckers were all preserved by us like this up to six weeks. Seeds with recalcitrant/intermediate storage behavior like wild *Artocarpus*, *Citrus*, etc. also need a coating of vermiculate-perlite mixture and wrapping in polybags with provision for aeration. Orthodox seeds need to be threshed, and extracted seeds are to be packed in aerated cloth bags and spread on the floor to prevent fungal growth. Monkey menace is very high in Great Nicobar Island and explorers should be very careful while keeping the fresh fruits/seeds outside for drying. Wet samples may be labeled with aluminum foil and paper tags may be restricted for dry seed packets only. In case where both seeds and vegetative propagules are equally viable (e.g. *Amorphophallus*), collectors can prefer seeds in order to reduce the bulkiness.

Finally, the collected germplasm needs to be neatly packed for transportation in the ship. Weight reduction by trimming off undesirable parts and removing soil, and moisture-proof packing are very important for safe shipping of 36-40 hours. All plant materials are to be packed by sorting and keeping dry seeds, vegetative propagules, delicate materials etc. carefully in separate bags, all in decently looking zip lock bags. Carrying heavy luggage through negotiating narrow ladder while embarking is a cumbersome process; therefore, it is advised to divide the weight accordingly. Poorly packed and shabby looking luggage and gunny bags may warrant ship crew to put them into luggage cabin, which is an open space, not air-conditioned and exposed to wind. After embarking the ship and settling in one's cabin, the bag containing perishable vegetative materials may be kept open to prevent possible mould growth and decay.

In each forest range after completion of the collection, the permit holder should submit a list of live materials collected and approximate sample size to facilitate the Range Officer to issue a Transit Permit (TP). The TP is a must for transferring any germplasm within A&N and also from the island to mainland. At the airport, luggage screening counter also one is supposed to produce the TP in case asked by forest officials. In addition to the TP it is always advisable to keep an authorization certificate issued by Director, ICAR-CIARI or Director of Agriculture, stating that the material transported is of no commercial value/ not for commercial purpose and is meant for research/ conservation. Placing a perishable commodity 'fragile' label is important for live materials when sent as unaccompanied luggage. It is also possible to send neatly packed live specimens through speed post and private air cargo. At Kamorta and Campbell Bay, Postal Department has speed post offices. Air cargo courier services are available at Port Blair. From remote islands, it is possible to send small samples free of cost by helicopter service to Port Blair for which one has to contact SRO. From Port Blair, the agent can send the seeds to mainland through courier/ speed post, thus saving transportation time for perishable germplasm.

Herbarium processing

Collecting and preserving herbarium specimens of first-time collections are very important for authentication. As carrying FAA (Formalin Acetic Alcohol) and such preservative liquids are not permissible by air,

cumbersome process of pressing between blotter sheets (or more practically old newspapers) and occasionally pressing the semi-dried specimens within blotters using an electric iron is the only viable alternative.

Other Considerations

Return flights to mainland may be booked only after reaching Port Blair as return journey to Port Blair from remote destinations cannot be planned well in advance. For many reasons, ship schedule change at short notice or tickets may exhaust in quick time leading to disruption of travel plan. Even though helicopter services are available connecting Port Blair to various islands in Nicobar, it may not be feasible for an explorer due to obvious reasons. Only four passengers are permitted in helicopter and maximum luggage permitted is five kg per person. Also, patients waiting for airlifting and A&N administration officials on Govt. duty are given preference. Further, fares are very high for non-islanders.

Upon completion of the trip and arrival at headquarters, a report needs to be submitted to the Forest Department with details of collections made, photographs, new findings, observations and any such information which would strengthen the research and development wing of the Forest Department. The Forest Department must be acknowledged in all publications. It is very important to maintain rapport with few influential people in the community, preferably captains so that some precious germplasm, which were not physiologically mature at the time of exploration, can be procured at a later time/season through locals and delivered to mainland through speed post.

Future Line of Work

By and large, forests in Great Nicobar and Little Nicobar are dense and undisturbed. Unexplored and underexplored diversity rich pockets in Great Nicobar like Mount Thullier needs special permission and facilitation from Departments of Environment & Forests, and Tribal Welfare, as reaching the top, making collections and returning to Navy Dera shore the same day is not possible. Managing day long trekking to reach the top and then returning besides time required for collection of samples needs to be kept in mind. It may take around 8 hours to reach the top and scaling down the same day is not possible. At present the Department doesn't permit overnight stay in the forest due to administrative issues. Same is the case with Laful (on north-eastern side) and Kopenheat (on western side) of Great Nicobar. Out of the five perennial rivers in Great Nicobar, we could only explore in part Galathea riverine forests, the other accessible areas also need to be explored for CWR.

ICAR-Central Island Agriculture Research Institute (ICAR-CIARI), Port Blair maintains a good collection of coconut germplasm of islands partnering with ICAR-Central Plantation Crops Research Institute (ICAR-CPCRI), Kasaragod, whereas, all the remaining crops and their wild relatives need suitable *ex*-situ regeneration in near natural habitat. In this regard, one of the Agriculture Department farms at Katchal or Kamorta are ideal maintenance site for representative economic species of Sundaland Biodiversity hotspot, from connectivity point of view. While implementing the proposed conversion of Katchal rubber plantation to natural forest, the forest department may consider developing a demonstration plot of tree species, CWR, wild economic species under the technical support of ICAR-CIARI and ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR).

Updating of Nicobar flora through publishing new reports of distribution is currently underway (e.g. Pradheep *et al*., 2019b, 2020), which will also help in updating CWR database of India. A detailed documentation of all ethno-botanically important species used by native tribals needs to be undertaken, although interacting with Shompens may be difficult due to Govt. restrictions. The sustainable forest management plans adopted by Nicobarese in Great Nicobar and Little Nicobar also need proper documentation.

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Fig. 10. Transferring into oddie boat to reach shore

Fig. 11. A view of forest trekking path

Fig. 12. Anchoring near muddy shore during low tide

Fig. 13. Reporting at Nicobari police outpost

Fig. 14. The only camping site at Galathea WLS

Fig. 15. Signage by Forest Dept. in crocodile prone areas

Fig. 16. Night halt at camp site

Fig. 17. Processing collected seedlings

APWD hospitality staff and Nicobari tribal farmers for their hospitality, generosity and support.

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RESEARCH ARTICLE

Seed Cryopreservation of Orchid *Coelogyne nitida* (Wall. ex Don) Lindl. Using Air Desiccation and Vitrification Techniques

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Orchid seeds are short-lived and difficult to conserve using conventional methods. Diverse germplasm of orchids occurring in North-eastern parts of India need attention for their long-term conservation. In this study, seed cryopreservation in *Coelogyne nitida* (Wall. ex Don) Lindl. was attempted. A modified TTC test was standardised as a quick estimate of seed viability. *In vitro* asymbiotic seed germination was standardised for determining seed viability for fresh, desiccated and frozen seeds. MS medium supplemented with 0.6 mg l^{-1} GA_3 + 0.8 mg l^{-1} BAP was optimal. Cryopreservation by air-desiccation and vitrification methods were applied with success achieved in both, however vitrification method yielded much higher recovery (83.4%) after liquid nitrogen exposure than the air desiccation (43.5%). Exposure time to PVS2 of 40 min was optimal amongst the three treatment durations during vitrification. This is the first report on successful cryopreservation of *Coelogyne nitida* seeds.

Key Words: Asymbiotic germination, Cryobanking, Orchid, Protocorms, TTC

Introduction

Orchidaceae is the largest angiosperm family next only to Asteraceae with more than 730 genera and over 26,000 species (Chase *et al*., 2015). All members of Orchidaceae family are listed under the Convention on International Trade in Endangered species of Wild Fauna and Flora (CITES) restricting any trade without permission. Orchids share a large chunk of global floriculture trade and hence are given special attention. Historically they had been a favourite subject of scientific experimentations by Charles Darwin who published books on orchids especially on strategies for fertilisation (Darwin, 1862; 1877). His observations formed the basis for 'Theory of Natural Evolution'. He theorised that orchids and their pollinators had co-evolved. In fact the orchid species *Angraecum sesquipedale* is known as Darwin's orchid. Way back in 19th century, several researchers conducted basic studies on orchids (Gosse, 1863; Moggridge; 1865) and their importance for conservation is still increasingly emphasised (Fay, 2018). Orchids are accepted to be difficult to handle (hence termed non-orthodox) and to store; the seeds have cold sensitivity to freezer storage. Explants like protocorms and protocorm like bodies (PLBs) are desiccation sensitive and there have been very limited studies on pollinia/pollen conservation which is essential for controlled, selected pollinations for hybrid vigour and for conservation of nuclear diversity.

About 186 genera and 1,230 orchid species are found in India which constitute almost 10% of the world's orchid flora. North East India, a mega-biodiversity hotspot, harbours an estimated 151 genera and 876 species of orchids (Medhi *et al*., 2012). Seaton *et al*. (2010) has suggested for *ex situ* conservation of orchids in the warming world. Tissue culture studies and conservation of valuable orchid germplasm have been carried out in various laboratories of the world since long (Seaton and Pritchard, 2008). Gangaprasad *et al*. (1999) attempted restoration of orchids using micropropagation. Cryopreservation is increasingly finding applications for long-term conservation of diverse plant species (Chaudhury and Malik, 2016) and cryoprotocols for different orchids

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are reported (see Vendrame *et al*., 2014, Seaton *et al*., 2015, Popova *et al*., 2016). Cryopreservation in various orchid species has yielded varying degree of success with different desiccation levels (optimal being >14%) and various cryotechniques. The present study was undertaken to develop a cost-effective, long-term conservation strategy for *Coelogyne nitida* (Wall. ex Don) Lindl., a sympodial epiphyte. Investigations were done on handling and transport of fruits and seeds, standardisation of tetrazolium test, asymbiotic *in vitro* seed germination and cryopreservation using air-desiccation and vitrification techniques.

Materials and Methods

Collecting of Material

Coelogyne nitida (Wall. ex Don) Lindl. is a sympodial epiphytic orchid (Nongrum *et al*., 2007), that has pseudobulb, and inflorescence is a raceme which flowers from May to June. It is naturally distributed in Sikkim, West Bengal and Arunachal Pradesh. Mature fruit capsules of *Coelogyne nitida* (IC 617089) were harvested about 12 months after anthesis by the NRC Orchids, Darjeeling, West Bengal from the Institute's greenhouse. Immediately after harvest, the pods of *Coelogyne nitida* were packed in plastic bags and sent to ICAR-NBPGR, New Delhi by speed post air carrier for further studies and cryopreservation, where pods were maintained at 15^{o}C until used in for experimentation (within 15 days).

In vitro asymbiotic germination

Coelogyne nitida capsules were washed with tap water and taken inside laminar flow and sterilized by flaming after dipping in 70% alcohol. This was repeated two times as per the procedure of Nongrun *et al.* (2007). The capsules were then slit open under aseptic conditions and seeds, which were abundant and uncountable being thousands to millions in each of the pods, scooped out carefully. For germination, seeds were distributed in glass culture tubes (15 × 2.5 cm) thinly over the surface of 15 ml of six different Murashige & Skoog (MS) (1962) nutrient media aseptically, viz., basal alone or in combination with various growth regulators namely 0.4 mg l^{-1} BAP, 0.6 mg l^{-1} BAP, 0.1 mg l^{-1} GA_3 + 0.2 mg l^{-1} BAP, 0.6 mg l^{-1} GA_3 + 0.6 mg l^{-1} BAP and 0.6 mg l^{-1} GA_3 + 0.8 mg l^{-1} BAP. Each of the media were supplemented with 3% sucrose, 0.8% agar and pH adjusted to 5.8 prior to autoclaving. Cultures were incubated at 25 $\pm$ 2^{0}C under light intensity of 40 µmol m^{-2} s^{-1} for 16/8 h light/dark cycle. Cultures were observed microscopically at weekly intervals to record growth and development evident earliest by swelling in embryos and appearance of protomeristem. Data on days to initiate germination, protocorm, 1st leaf shoot, root were recorded. Percent seed germination was determined after 60 days of culture and calculated by using the formula: % seed germination = number of germinated seeds/total number of seeds × 100.

Viability test using Tetrazolium

Seed viability was also assessed with a modified Tetrazolium test. Seeds (10 mg) were placed in 1 ml tubes and pre-incubated in a 10% (w/v) sucrose solution. After incubation for 24 h at 25°C, sucrose solution was removed and seeds washed twice with distilled water. Seeds were subsequently distributed in three tubes and individually solutions of 0.1, 0.5 and 1% of 2,3,5-triphenyl tetrazolium chloride (TTC), prepared in distilled water, were added to the seeds and incubated in the dark at 35°C for 24 h. After incubation, seeds with liquid drops of TTC solution were placed on a glass slide and observed under microscope. Red/deep pink seeds were counted as viable, while white seeds were counted as non-viable and percentage viability was calculated. Three replicates each with about 300 seeds per sample were used.

Seed Cryopreservation by Air Desiccation

Freshly extracted seeds were placed on sterile filter paper discs in a laminar flow for 4-5 h. Moisture content (MC) of fresh and desiccated seeds was determined gravimetrically using low constant temperature oven method (ISTA, 1985). Seeds, about 300 in number, were placed in pre-weighed moisture bottles. The samples were weighed again after 17 h drying at 103±2^{o}C. The MC was obtained from 2-4 independent determinations (each with about 300 seeds) and expressed as a mean percentage on fresh weight basis. Seeds were suitably desiccated to 16-18% moisture and transferred to sterile 1 ml polypropylene cryovials. These cryovials were subjected to fast freezing at -196^{o}C by direct plunging in liquid nitrogen (LN) (air desiccation-freezing method). After 24 h cryostorage, seeds were thawed at 37±2 ^{O}C and cultured *in vitro* to study survival and recovery, as done with fresh seeds.

Seed Cryopreservation by Vitrification Technique

Freshly extracted seeds (~300) were transferred to 1.2 ml sterile cryovials and treated with 0.5 ml loading solution (0.4 M sucrose, 2 M glycerol in basal MS medium) for 20 min at 25°C. Loading solution was replaced with 0.5 ml Plant Vitrification Solution 2 [PVS2: 30% (w/v) glycerol, 15% (w/v) ethylene glycol and 15% (w/v) dimethyl sulfoxide and 0.4 M sucrose desolved in MS medium (pH 5.8)] (Sakai *et al*., 1990) for 20, 40 and 60 min at 25°C. Cryovials containing seeds in PVS2 solution were plunged rapidly in LN and held for 1 h. Subsequently, the cryovials were thawed in a water bath at 38±2°C for 1 min with vigorous shaking. The PVS2 was immediately replaced with 0.5 ml unloading solution (1.2 M sucrose in MS basal medium) and incubated at 25°C for 20 min. The solution was removed, seeds blotted dry on sterile filter papers and then cultured *in vitro* to study survival and recovery as done with fresh seeds. In all, growth regulators-supplemented, five MS culture media (detailed in Table 2), were used for recovery, two of them with BAP alone in two concentrations namely 0.4 mg l^{-1} and 0.6 mg l^{-1} and with different concentrations of GA_3 (0.1 mg l^{-1} and 0.6 mg l^{-1}). Experiments were repeated two times.

Transfer of Plantlets to Pots

Seedlings ~4–5 cm tall and with well-formed roots, raised from cryo-retrieved samples, were removed from culture vessels and roots washed thoroughly with tap water to remove adhering medium without damage. Ten of the seedlings were then transplanted in plastic pots (10 × 7 cm size) containing mixture of coco peat, vermicompost and perlite in 1:1:1 ratio. Plantlets were covered with a pierced transparent polythene bag, and allowed to grow in the laboratory under ambient conditions (at 24°C and 80% RH) for 3 weeks with regular watering. Later, pots were shifted outside the lab in partial shade where minimum and maximum temperatures were 18 and 25°C, respectively, and RH was 70–80 %. Plantlets were watered on alternate days and fed with 1/10 strength MS nutrient salt solutions every two weeks. Establishment of plants was assessed after 90 days.

Data Analysis

Data generated in *in vitro* asymbiotic seed germination and cryopreservation experiments were statistically analysed using CRD by Panse and Sukhatme (1985). SPSS software was used to generate ANOVA analyses at the 0.1% level of probability. In the Tables, values followed by the same letter in a column are not significantly different using DMRT.

Results

Healthy undamaged mature pods of *Coelogyne nitida* were successfully transported after harvesting from North East India to ICAR-NBPGR, New Delhi, a distance of about 1500 km. The average weight of pods was 1.71 g and average size was 6.03 cm in length and 1.03 cm in breadth (Fig 1a). The colour of extracted seeds (Fig 1b) ranged from white, yellow and green in different pods. Immature seeds were invariably white and pale. About 15% of seeds from the total pods received were empty, as they lacked the embryos. The MC of fresh seeds averaged 45.9%.

Using TTC test, seed viability of 18.0%, 46.2% and 82.5% was recorded for fresh seeds incubated in 0.1% (Fig 1c), 0.5% (Fig 1d) and 1% (Fig 1e) solutions. The values achieved by 1% TTC solution test for viability were similar to that achieved by *in vitro* germination (Table 1). Seeds after cryoexposure were also randomly checked for seed quality using 1% TTC solution and viability values were similar to those achieved by *in vitro* germination (data not presented here).

Under *in vitro* conditions, fresh seeds cultured on MS basal medium exhibited swelling by 28 days, protocorms (Fig 1b) appeared by 40 days, shoots (Fig 1d) and roots initiated by 71 and 101 days, respectively (Table 1). Amongst the five growth regulators supplemented MS medium, best response was observed with that supplemented with 0.6 mg l^{-1} GA_3 and 0.8 mg l^{-1} BAP and hence data has been presented for this medium in present studies on asymbiotic seed germination. In comparison to MS basal, the responses were faster by 2-5 days for seeds inoculated on MS medium supplemented with 0.6 mg l^{-1} GA_3 and 0.8 mg l^{-1} BAP. Mean seed germination recorded after 60 days of culture was 82%. Seeds desiccated to 17.93% MC and those desiccated and exposed to LN were similarly tested for asymbiotic germinaton on these two culture media namely MS basal and MS with 0.6 mg l^{-1} GA_3 and 0.8 mg l^{-1} BAP. For desiccated unfrozen seeds, initiation of seed germination was observed, within 20 days, on medium supplemented with growth regulators whereas in MS basal media, it was by 29th day of culture. Protocorms were visible by 42 days on MS basal and few days earlier (by 38 days) on growth regulators-supplemented media. Healthy upright shoots emerged by about 80 days in both the culture

media (Fig 1e). In next 30-32 days, rooting was initiated on both the media. Final seed germination percentage was 45.32% on MS basal medium and 75.5% on growth regulators-supplemented MS media.

Desiccated frozen seeds were thawed and germinability assessed on two media used for fresh seeds (Table 1). Initiation of seed germination was observed earlier, within 25 days, on growth regulator-supplemented medium, whereas, on MS basal medium, it was slower and observed by 31st day of culture which was a delay of 3-5 days than in their respective nonfrozen controls. Protocorms initiation and shoot formation was also delayed by 4-8 days in comparison to their respective nonfrozen controls. Rooting in frozen seeds was initiated on MS basal medium by 128 days, a delay of 18 days compared to unfrozen seeds and on growth regulator-supplemented media by 124 days, a delay of 6 days compared to nonfrozen seeds. After 18 weeks of culture, germination was recorded as 16.36% on MS basal medium and 43.5% on MS media supplemented with growth regulators. Hence, lower germinability values were observed in LN exposed seeds when compared to fresh and nonfrozen seeds. In all cases, however, growth regulator-supplemented media were better for germinability. The plantlets raised from the two media appeared normal and healthy and could be transferred to pots with 100% success for one month in healthy conditions (Fig 1f).

Vitrification method was attempted with freshly extracted seeds to investigate the role of cryoprotectants in improving survivability of seeds after LN exposure. Seeds subjected to freezing preceded by 20 min PVS2 showed germination ranging from 80% on MS medium supplemented with 0.6mg l^{-1} GA_3 + 0.8mg l^{-1} BAP to 55% on MS medium supplemented with 0.1 mg l^{-1} GA3 + 0.2 mg l^{-1} BAP (Table 2). Seeds subjected to freezing preceded by 60 min PVS2 treatment showed germination ranging from 70.5% on MS medium supplemented with 0.6 mg l^{-1} GA_3 + 0.8mg l^{-1} BAP to 55.5% on MS medium supplemented with 0.6 mg l^{-1} BAP. Highest recovery values were recorded for seeds subjected to freezing preceded by 40 min PVS2 treatment. Germination as high as 79 to 83% was recorded on MS media supplemented with different combinations of GA_3 + BAP and 75% on MS media supplemented with BAP alone. Overall MS medium supplemented with 0.6 mg l^{-1} GA_3 + 0.8 mg l^{-1} BAP was optimal for all the treatments.

Discussion

Orchids are priority to be conserved through biotechnological interventions (Pritchard, 2016). Generally seeds are the preferred propagules for propagation and storage. However, orchid seeds are reportedly difficult to handle due to varying proportions of seeds formed without embryos and inherently low germination rates. Responses due to heat and cold sensitivity are other causes misunderstood as mishandling (Pritchard, 1989). Despite these difficulties, orchid seeds with low MC are the much preferred explants compared to protocorms, which suffer cryoinjury due to their inherent high moisture levels. Although several reports exist on success in orchid seed storage in conventional -20^{o}C genebanks (Seaton *et al*., 2010), cryobanking has shown greater potential as the viable method (Merritt *et al*., 2014; Popova *et al*., 2016). Generally laboratories worldwide

Table 1. Asymbiotic *in vitro* seed germination and cryopreservation by desiccation, in *Coelogyne nitida* using 300 seeds in 3 replicates

LN exposure	Media Composition	Days taken for initiation of				Final germination Percentage %
		Seed swelling	Protocorm development	Shoots appearance	Roots appearance	
Fresh Seeds	MS Basal	28	40	71	101	81.5±5.7^{ab}
	MS+0.6mg l^{-1} GA_3+0.8mg l^{-1} BAP	26	36	66	97	82.5±1.1^{a}
Desiccated non-frozen controls	MS Basal	29	42	79	110	45.3±8.9^{d}
	MS+0.6mg l^{-1} GA_3+0.8mg l^{-1} BAP	20	38	81	118	75.5±5.6^{c}
Desiccated frozen	MS Basal	31	47	81	128	16.3±4.1^{e}
	MS+0.6mg l^{-1} GA_3+0.8mg l^{-1} BAP	25	46	85	124	43.5±0.8^{d}

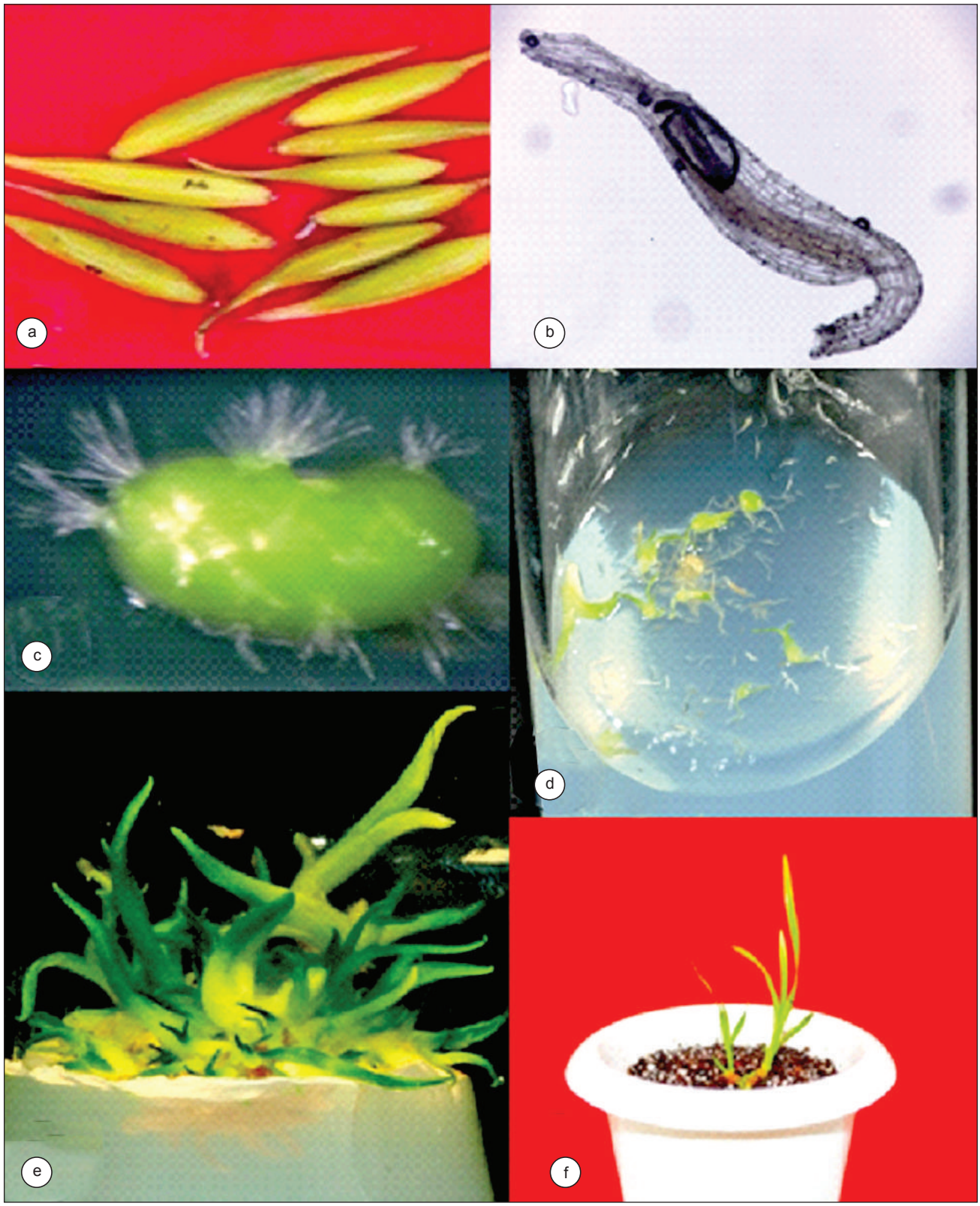

Fig. 1. Asymbiotic *in vitro* seed germination in *Coelogyne nitida*
a. Freshly collected pods; b. Single seed showing well defined embryo; c. *In vitro* A raised protocorm with rhizoids; d. First shoot formations from protocorms; e. Well developed shoots showing root initiation; f. Seedling transferred to pot

do not have the necessity to transport material from one lab to another but use the germplasm locally. This is the first effort to collaborate with Institute situated as far as 1500 km away but needed to exchange *Coelogyne nitida* germplasm in viable condition and fresh pods received through air travel showed reasonably good seed viability, indicating no transportation losses.

Germination tests are the ideal method for correct evaluation of seed quality. However, time taken to evaluate orchid seeds for their full germination potential is several weeks (Merritt *et al.*, 2014). This necessitates use of vital stain 2,3,5-triphenyl tetrazolium chloride for rapid estimation of viability, which is a widely acceptable test for orchid seeds. In the present study, viability determination using 1% TTC preceded by sucrose incubation gave a fair estimate of viability comparable to *in vitro* germination. Hosomi *et al.* (2011) earlier reported an improved colouration and distinction between dead and live seeds of *Cattleya* in TTC test following the pre-conditioning with sucrose. Orchid seeds, due to minute size and oil reserves, often do not have their metabolism activated (Arditti, 1980) and depend on external sucrose for energy source which may be the case for optimal results in TTC test in present studies, using sucrose incubation overnight. For orchid species possessing dark or impermeable seed coats, an improved TTC testing has also been devised that includes a bleaching step (Custodio *et al*., 2016).

For mass propagation of selected orchid species, asymbiotic *in vitro* seed germination and *in vitro* establishment have been standardised by various workers (Arditti, 1984; Popova *et al*., 2016). This helps in conservation of declining orchid population in nature. Role of different culture media for asymbiotic seed germination has been investigated for several orchid species. In our studies, Murashige and Skoog (MS) medium supplemented with GA_3 and BAP was suitable for obtaining survival after various treatments, since fresh seeds exhibited high germinability (82%). This germination value was better than that reported earlier by Nongrum *et al*. (2007) where four culture media viz. Knudson C, Vacin and Went, Mitra and MS were used. MS was also one of the suitable media for further growth of protocorms and plantlets, in their studies. In present work, the microscopic examination showed 10-15% seeds without any embryos corroborating the results of viability achieved with *in vitro* germination and TTC method. Growth regulators reportedly do not have a major role in orchid seed germination, but are essentially needed for subsequent protocorm and seedling formation. Further, long-term conservation of orchid seeds for assured high viability retention for infinite periods needs to be devised for uninvestigated species. Cryopreservation has been increasingly employed with different orchid species with high predictable success in seeds, for both epiphytic and terrestrial species, and for pollen/ pollinia (reviewed by Popova *et al*., 2016). In this context, present study on low temperature tolerance of seeds of *Coloegyne nitida* was carried out using two cryopreservation protocols. There are no reports so far on long-term cryopreservation of seeds of *Coelogyne nitida*.

At ultra-low temperature storage, role of optimal moisture window, achieved by partial drying, for best results in terms of high viability with normal protocorm development has been emphasised (see Popova *et al*, 2016). Most conventional method is to desiccate seeds between 13 to 17% moisture followed by direct plunging in LN. In exceptional cases, seeds with high moisture between 24-25% have been reported to also survive direct LN freezing (Nikishina *et al*., 2001; Wu *et al*., 2013). In the present study, a safe moisture level of 16-18%, reported for most of the orchid species was employed before freezing seeds to avoid any desiccation and also freezing injury. Desiccation of seeds resulted in decline in germinability which was substantial (37% decline) using MS basal medium. Growth regulator supplementation,

Table 2. Vitrification freezing of seeds of orchid species *Coelogyne nitida* using 300 seeds in 3 replicates

Media Composition	% Germination after PVS2 exposure of (min)		
	20 min	40 min	60 min
MS+0.4 mg l^{-1} BAP	67.8±4.0^{c}	75±2.8^{d}	60.5±2.8^{c}
MS+0.6 mg l^{-1} BAP	65.5±5.7^{c}	75±5.7^{d}	55.5±1.4^{d}
MS + 0.1 mg l^{-1} GA_3 + 0.2 mg l^{-1} BAP	55.0±2.3^{d}	78.5±1.1^{cd}	65.7±2.8^{b}
MS + 0.6 mg l^{-1} GA_3 + 0.8 mg l^{-1} BAP	80±3.2^{a}	82.2±1.4^{ab}	70.5±1.4^{a}
MS + 0.6 mg l^{-1} GA_3 + 0.6 mg l^{-1} BAP	71.4±4.9^{b}	83.4±1.7^{a}	59.7±1.4^{d}

however, showed improved response (only 6% decline in germination) for desiccated seeds. In addition, various initiation responses of seeds germination and seedling growth were slower after seed desiccation (Table 1). Seeds after freezing showed a further decline in germinability indicating freezing injury. However, recovery percentage was up to 43% while using growth regulator-supplemented media. Growth regulators were, thus important for optimising recovery of desiccated and cryo-exposed seeds.

Vitrification is a freezing technique which chiefly comprises seven steps namely preculture, osmoprotection, dehydration with highly viscous vitrification solutions like PVS2 (Sakai and Engelmann, 2007), rapid cooling, rapid warming, dilution/exclusion of vitrification solution from the explant and subsequent recovery growth. Vitrification solution is attributed with three functions (Volk and Walters, 2006) i.e. replacing cellular water, altering the freezing behaviour of the cells and impeding water loss on drying. Carefully selected exposure duration to these solutions is the key to success as they themselves can cause stress of osmotic injury and dehydration. PVS2 has proven successful with several orchid species with immature and mature seeds (Hirano *et al*., 2011). Successful reports exist on vitrification of orchid seeds both with or without pre-culture in high sucrose before subjecting to cryopreservation (Merritt *et al*., 2014). In present study, high seed survival (83.4%) was achieved following vitrification (without any preculture treatment) that was as good as in nonfrozen controls (82.5%). Exposure time of 40 min to PVS2 was optimal amongst the three treatment durations and MS media with different concentrations and combinations of GA_3 and BAP were most suitable for recovery. Exposure duration of 20 min was next best followed by 60 min exposure. During vitrification, dehydration of seeds could be achieved using high viscosity cryoprotectants which reportedly leads to glass formation omitting any ice crystallisation (Sakai and Engelmann, 2007). Healthy plantlets were recovered after vitrification defining a successful cryopreservation methodology. Hence, two long-term, economical and safe conservation methods of *Coelogyne nitida* seeds using air desiccation-freezing and vitrification were developed. Lately, cryopreservation has been emphasised as the most suitable alternative bioresources germplasm storage of seeds of short-lived orchid species that cannot be seed-banked at conventional temperatures of -20^0C effectively (Streczynski *et al.*, 2019). Based on this study attempts are being made to cryobank germplasm of *C. nitida* at ICAR-NBPGR, New Delhi, in the form of seeds.

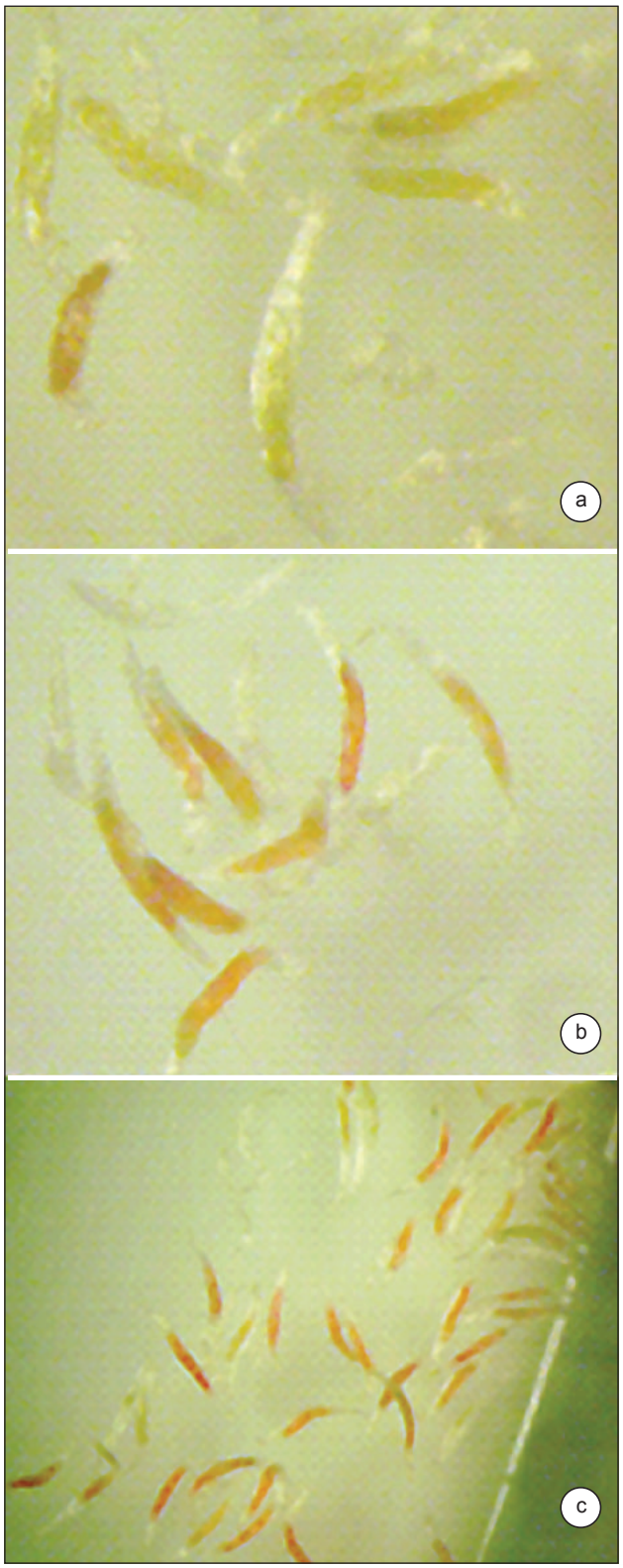

Fig. 2. *Coelogyne nitida* fresh seeds tested for viability using different concentrations of TTC solution
a. 0.1% TTC solution; b. 0.5% TTC solution
c. 1% TTC solution

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RESEARCH ARTICLE

Morpho-Genetic Characterization of Scented Radhunipagal Rice Landrace of South Bengal

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Radhunipagal is a traditional small-grained non-Basmati type scented rice of *rahr* (red and laterite) and *gangetic* plain region of West Bengal. With a view to develop the phenotypic and genetic database of the unique variety, the agro-morphological characterization of Radhunipagal rice was done following DUS test guidelines of PPV&FRA at 'C' Block Farm, B.C.K.V., Kalyani, West Bengal during *kharif* (wet) season of 2012, 2013 and 2014. The variety had long statured plants (scale 7, 135-145 cm height) with distinct anthocyanin colouration on lower nodes and internodes; and it had late heading (120-125 days) and late maturity (scale 7, 150-155 days). The flower was bi-sexual including six yellow coloured anthers, and an ovary with light purple coloured feathery stigma. The colour of lemma and palea of matured grain was straw with purple spot at tip and the grains were awnless, short in length (5.8 mm) with very low test weight (10.03 g). The white-coloured kernels were short-bold (length 3.57 mm and width 1.86 mm) in shape, and the cultivar had low amylose content (17.0%), medium gelatinization temperature (alkali value 3.2) and medium-strong aroma. The DNA-based study using 23 simple sequence repeat (SSR) markers for Radhunipagal rice against non-aromatic international check (IR 36) revealed that two markers (RM 42 and RM 310) recorded near-similar molecular weights for both the varieties, while other two markers (RM 339 and RM 341) made greater genetic distances (184 *vs*. 143 bp and 138 *vs.* 175 bp, respectively) between Radhunipagal and IR 36.

Key Words: Aromatic rice, Morpho-agronomic traits, Grain quality, SSR polymorphism

Introduction

The cultivation and use of small and medium-grained non-Basmati type scented rices in Bengal region of eastern India have been documented in *punthi* (manuscript), district gazetteers, books, folk literatures, doggrels, etc. for a long period. Among such 40 aromatic rice landraces, Radhunipagal is traditionally cultivated in *rahr* (red and laterite) and lower gangetic plains of West Bengal for about 400–500 years. The earliest record of Radhunipagal rice was found in two district gazetteers on 24 Parganas (Hunter, 1875) and Medinipur (Hunter, 1876), and thereafter in two books entitled 'The Dictionary of the Economic Products of India' (Watt, 1891) and 'The Handbook of Agriculture' (Mukherji, 1901). Although it was very popular in Bengali society and culture for preparation of *bhog* (rice intermixed with pulses), *payash* (dessert), *pistak* or *pitha* (home-made cake), etc. during social functions and religious festivals for a long period, but its cultivation has been marginalized to small pockets of a few districts (Birbhum, Burdwan, Hooghly, Bankura, etc.) during last four decades due to large-scale adoption of high-yielding varieties in the region. Farmers in native areas cultivate Radhunipagal rice following traditional practices intermixed with a few modern technologies in recent times during *kharif* (wet) season.

In the present-day agricultural system, it has become a necessity to register important landraces as farmers' varieties under PPV&FRA, 2001 to strengthen the right of the farming community to conserve, cultivate and protect the same against unauthorized utilization by multi-national seed companies at national and global level. Hence, agro-morphological, physico-chemical and molecular characterization of Radhunipagal rice needs to be done as legal evidence to find out phenotypic and genetic distances from other closely related genotypes as well as to avoid duplication in rice germplasm conservation system.

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Materials and Methods

DUS Testing and Determination of Grain Quality

The seeds of Radhunipagal rice were collected from the Regional Research Sub-Station (RRSS), Red and Laterite Zone, Bidhan Chandra Krishi Viswavidyalaya (BCKV), Sekhampur, Birbhum, West Bengal. 25 days old seedlings of Radhunipagal rice @ single / hill were transplanted in an open puddled field with five replications at 'C' Block Farm (22º59′N, 88º27′E and 9.75 m above mean sea level) of BCKV, Kalyani, Nadia, West Bengal, India during *kharif* (wet) season of 2012, 2013 and 2014. Each experimental unit consisted of 6-metre row length comprising 30 rows including row to row distance of 30 cm and plant to plant distance of 20 cm. Standard agronomic practices were adopted in trial plots during the course of investigation. The DUS descriptors following 'DUS Test Guidelines for Rice' of PPV&FRA, Government of India (www.plantauthority.gov.in) were used to define the morphological and related characteristics of Radhunipagal rice. Grain quality parameters like size and shape of grain and kernel, amylose content (Juliano, 1971), gelatinization temperature (Little *et al.*, 1958) and aroma (Nagraju *et al*., 1991) were determined at Aromatic Rice Laboratory, Department of Agronomy, BCKV, Mohanpur, Nadia.

Molecular Characterization by SSR Markers

The molecular characterization of Radhunipagal rice was done at the Division of Plant Biology, Bose Institute, Kolkata, West Bengal during 2006-2008. 3-day old seedlings of Radhunipagal rice along with international non-aromatic check (IR 36) were used for isolation of genomic DNA following the method of Walbot (1988). DNA amplification was carried out by standard PCR method with 23 pairs of simple sequence repeat (SSR) markers in a Peltier Thermal Cycler (MJ Research, USA). Each reaction mixture contained 1 µl of genomic DNA (100 ng), 0.5 µl of each primers (at a concentration of 10 pmole/µl), 2.5 µl of 10× PCR buffer, 0.75 µl of 50 mM MgCl2, 0.25 µl of 2.5 mM dNTP mixture, 0.2 µl (1 unit) of 5 unit/µl Taq DNA polymerase and 19.3 µl of PCR-grade water. The temperature profile of the first PCR cycle was 97°C for 5 minutes, 55-60°C (as necessary in accordance to Table 2) for 2 min; followed by 35 cycles of 1 min at 95°C, 1 min at 55-60°C and 2 min at 72°C. The final extension was at 72°C for 10 min.

The PCR products were resolved by native polyacrylamide gel electrophoresis (PAGE) following the protocol given by Sambrook *et al.* (1989). The size (in nucleotides) of the most intensely amplified band for each microsatellite marker was determined using the Molecular Analyst software (BioRad, USA), based on the migration of the band relative to standard molecular weight size markers (100 bp DNA ladder SibEnzyme Ltd., Russia). IR36 was used as a molecular weight reference in each gel because a sequence-based estimate of allele size in this germplasm was available. The different alleles amplified from the genomic DNA of Radhunipagal rice along with the check were identified on the basis of their length or base pairs (bp) for making genetic characterization of Radhunipagal rice in the study.

Results and Discussion

Agro-morphological Characteristics and Grain Quality

Radhunipagal rice was usually adaptable to rainfed medium land in gangetic alluvium and *rahr* region of West Bengal. The characteristics of Radhunipagal rice following 'DUS Test Guidelines for Rice' of PPV&FRA are described in Table 1.

Plant: Radhunipagal rice belonged to long-duration type with late heading (scale 7, 122 days) and late maturity (scale 7, 151 days) (Fig. 1).

Fig. 1. Radhunipagal rice field at dough stage

Table 1. Plant characteristics of Radhunipagal rice following DUS Test Guidelines.

Sl. No.	Characteristics	Scale	Remarks measured values etc.
1	Coleoptile: colour	2	Green
2	Basal leaf sheath colour	3	Purple lines
3	Leaf : Intensityof green colour	5	Medium
4	Leaf : anthocyanin colouration	1	Absent
5	Leaf : distribution of anthocyanin colouration	–	
6	Leaf sheath : anthocyanin colouratin	9	Present (inner side of lower leaf sheath)
7	Leaf sheath: intensity of anthocyanin colouration	–	
8	Leaf: pubescence of blade surface	5	Medium
9	Leaf : Auricles	9	Present
10	Leaf : anthocynin colorations of auricles	1	Colourless
11	Leaf : collar	9	Present
12	Leaf : anthocyanin colouration of collar	1	Absent
13	Leaf : ligule	9	Present
14	Leaf: shape of ligules	3	Split
15	Leaf: colour of ligule	1	White
16	Leaf : length of blade	7	Long (70.0 cm)
17	Leaf : width of blade	3	Narrow (0.98 mm)
18	Culm : attitude (for floating rice only)	–	
19	Culm : attitude	3	Semi-erect
20	Time of heading (50% of plants with panicles)	7	Late (122 days)
21	Flag leaf attitude of blade (early observation)	3	Semi-erect
22	Spikelet : density of pubescence of lemma	3	Weak
23	Male sterility	1	Absent
24	Lemma: anthocyanin colouration of keel	1	Absent
25	Lemma: anthocynin of area below apex	1	Absent
26	Lemma: anthocynin colouration of apex	7	Strong
27	Spikelet : colour of stigma	4	Light purple
28	Stem: thickness	5	Medium (0.52 cm)
29	Stem: length (excluding panicle)	5	Medium (129.3 cm)
30	Stem: anthocyanin coloration of nodes	1	Absent (Present usually at lower nodes)
31	Stem : intensity of anthocyanin colouration of nodes	3	Weak
32	Stem : anthocyanin colouration of internodes	9	Present
33	Panicle: length of main axis	5	Medium (21.9 cm)
34	Flag leaf: attitude of blade (late observation)	5	Horizontal
35	Panicle: curvature of main axis	7	Drooping
36	Panicle: number per plant	3	Few (9.57)
37	Spikelet: colour of tip of lemma	5	Purple
38	Lemma & Palea : Colour	7	Purple spots / furrows on straw
39	Panicle: awns	1	Absent
40	Panicle: colour of awns (late observation)	–	
41	Panicle: length of largest awn	–	
42	Panicle: distribution of awns	–	
43	Panicle : presence of secondary branching	9	Present

Sl. No.	Characteristics	Scale	Remarks measured values etc.
44	Panicle : secondary branches	2	Strong
45	Panicle : attitude of branches	3	Erect to Semi-erect
46	Panicle: exertion	7	Well exerted
47	Time of Maturity	7	Late (151 days)
48	Leaf : senescence	5	Medium
49	Sterile lemma: colour	4	Purple
50	Grains: weight of 1000 fully developed grains	1	Very low (10.03 g)
51	Grain : length	1	Very short (5.78 mm)
52	Grain : width	2	Narrow (2.35 mm)
53	Grain : phenol reaction of lemma	–	
54	Decorticated grain: length	1	Very short (3.57 mm)
55	Decorticated grain: width	1	Very narrow (1.86 mm)
56	Decorticated grain shape	2	Short bold
57	Decorticated grain: colour	1	White
58	Endosperm: presence of amylose	9	Present
59	Endosperm: content of amylose	3	Low (17.0 %)
60	Varieties with endosperm of amylose absent only-polishedgrain : exertion of white core	–	
61	Gelatinization temperature through alkali spreading value	3	Medium (Alkali score 3.2)
62	Decorticated grain : aroma	9	Present (Medium-strong)

Table 2. Details of SSR markers and base pair length of Radhunipagal rice

SSR Marker	Motif	Rice Chromosome No.	Annealing temperature (^{0}C)	Base pair (bp)	
				Radhunipagal	IR 36 (International check)
RM 42	(GA)6	8	65	155	156
RM 44	(GA)16	8	55	109	113
RM 72	(TAT)5C(ATT)15)	8	55	149	166
RM 80	(CTT)20	8	65	118	122
RM 112	(GAA)5	2	55	130	142
RM 149	(AT)10	8	59	262	247
RM 152	(GGC)10	8	60	142	150
RM 182	(AT)16	7	59	294	296
RM 207	(GA)25	2	65	149	128
RM 210	(GA)23	8	55	136	150
RM 218	(GA)24	3	55	150	155
RM 223	(GA)25	8	55	148	164
RM 250	(CT)17	2	60	156	151
RM 251	(CT)29	3	55	114	120
RM 282	(GA)15	3	59	132	140
RM 284	(GA)8	8	55	147	140
RM 310	(GT)19	8	55	106	108
RM 337	(CTT)4-19(CTT)8	8	59	156	161
RM 339	(CCT)8(CCT9CCT)5	8	59	184	143
RM 341	(CTT)20	2	55	138	175
RM 505	(CT)12	7	55	123	126
RM 530	(GA)23	2	59	157	168
RM 569	(CT)16	3	59	161	168

Stem: Plants were long-statured with average stem length of 129.3 cm excluding panicle. The thickness of stem was medium (scale 5) with mean diameter of 0.52 cm. Anthocyanin colouration was usually present on lower nodes and internodes of Radhunipagal rice (Fig. 2), while the colour of inner side of leaf sheath mainly at plant base of closely related landrace Radhatilak was green. The attitude of the culm could be categorised as erect (scale 1) at booting stage.

Fig. 2. Node and internode

Leaf: Leaves were long, narrow and green. The colour of basal leaf sheath was green (scale 1) with medium intensity (scale 5), but there was anthocyanin colouration on inner side of lower leaf sheath. The average length and width of leaf blade were noted as 70.0 mm and 9.8 mm, respectively. The split-type ligule (scale 3) and sickle-shaped auricle at leaf base were found in the plant. The attitude of the flag leaf was semi-erect (scale 3) at early observation and horizontal (scale 5) at late observation.

Inflorescence: The length of panicle of Radhunipagal rice was categorized as medium (scale 5, 21.9 cm) with the curvature of the main axis as drooping (scale 7). The plant produced very few (scale 3, mean 9.57) well-exerted panicles in the field. The colour of the lemma and palea was green with purple spot at tip at anthesis (Fig. 3), which turned to straw with purple spot at tip at maturity.

Flower: The variety produced bi-sexual flowers including six yellow coloured anthers, and an ovary with light purple coloured feathery stigma (Fig. 4). But the colour of feathery stigma of Radhatilak rice was white.

Grain: The grains of Radhunipagal rice were short in size (mean length 5.8 mm and width 2.4 mm) and awnless. The weight of 1000 fully-developed grains was very low (10.03 g). The colour of lemma and palea was straw with purple spot at tip (scale 7) (Figs. 5 and 6), while that of sterile lemma was purple (scale 4). But the colour of tip of lemma in the spikelet of Radhatilak rice was reddish purple, while that of sterile lemma was red (Rey *et al.,* 2019).

The kernels were short-bold in shape (length 3.57 mm and width 1.86 mm) and white in colour (Fig. 6). The cultivar had low amylose content (17.0%), medium gelatinization temperature (alkali value 3.2) (Fig. 7) and medium-strong aroma.

Fig. 3. Spikelets at anthesis

Fig. 4. Flower

Fig. 5. Grain

Fig. 6. Grains and kernels

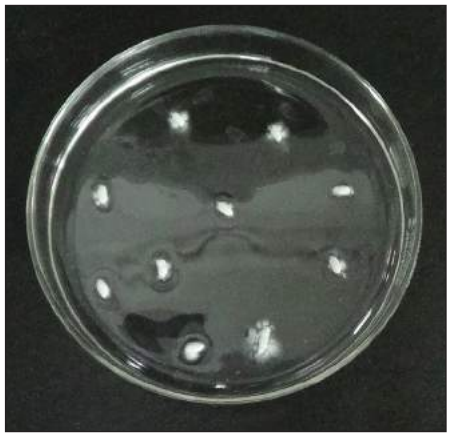

Fig. 7. Alkali digestion test

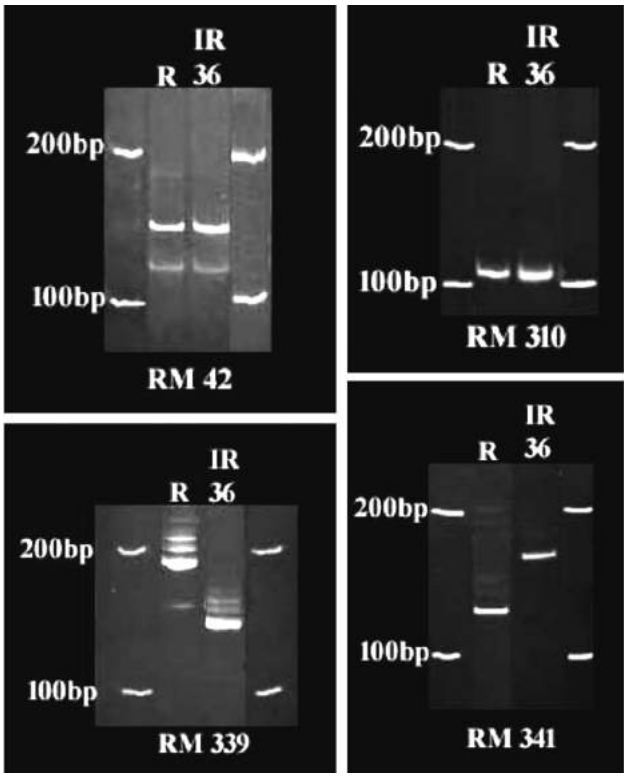

Fig. 8. SSR profile of scented Radhunipagal rice

DNA Amplification Profile

23 SSR markers used in the study were selected from chromosome 2, 3, 7 and 8 because two important traits like aroma (Ahn *et al*., 1992) and cooked kernel elongation ratio (Ahn *et al*., 1993) of scented rice were mapped earlier using RFLP markers. The SSR markers revealed clear and consistent amplification profile in the investigation, which developed the molecular base-pair length database of Radhunipagal rice against the non-aromatic international check variety IR 36 because of availability of sequence-based estimate of allele size of the later reference variety (Table 2). Among the markers used, two markers (RM 42 and RM 310) recorded near-similar molecular weights for both the varieties, while two markers (RM 339 and RM 341) made greater genetic distances (183.95 *vs*. 143.09 bp and 138.38 *vs*. 174.97 bp, respectively) between Radhunipagal and IR 36 varieties in the investigation.

Conclusion

Radhunipagal, an aromatic rice landrace of South Bengal, had late maturity (150-155 days) and the plants were long statured (135-145 cm height) with anthocyanin colouration on lower nodes and internodes. The colour

of lemma and palea of matured grain was straw with purple spot at tip and the grains were short in length (5.8 mm) with very low test weight (10.03 g). The kernels were short-bold in shape (length 3.57 mm and width 1.86 mm) and white in colour, which had low amylose content (17.0%), medium gelatinization temperature (alkali value 3.2) and medium-strong aroma. With these, Radhunipagal could be distinguished from another scented rice (Radhatilak) by the characteristics of anthocyanin colouration on internodes and inner side of leaf sheath, colour of tip of lemma and entire sterile lemma, grain size and weight. Based on molecular base-pair length database developed by 23 SSR markers, two (RM 339 and RM 341) made greater genetic distances between Radhunipagal and IR 36 in the study.

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RESEARCH ARTICLE

Analysis of Genetic Diversity in Pomegranate using SRAP (Sequence-Related Amplified Polymorphism) Markers

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Sequence-related amplified polymorphism (SRAP) was used to assess the genetic diversity of 114 pomegranate accessions sourced from all over the world and maintained in the field gene bank at ICAR-Indian Institute of Horticultural Research, Hesaraghatta, Bangalore. Totally 10 SRAP combinations were used for the study. Genetic parameters such as effective alleles (Ne), Nei genetic diversity (H), Shannon index (I), and polymorphic information content (PIC) were calculated based on molecular data. The number of alleles per locus ranged from 1.66 to 4.00 with an average of 2.03. The average polymorphic information content (PIC) value was 0.24. The expected heterozygosity varied from 0.215 to 0.445 with an average of 0.306. Cluster analysis was performed using R software. All the 114 accessions were grouped into four major clusters. There was no clear grouping based on origin. The analysis of molecular variance (AMOVA) indicated insignificant genetic variation (p=0.31) between pomegranate accessions from different geographical locations. Overall genetic variation among the population groups was low, while 99% of variation was due to within group differences. These results confirmed that SRAP markers could be a powerful and an effective marker system for determining the genetic diversity and population genetic structure of the pomegranate.

Key Words: Pomegranate; Genetic Diversity; SRAP marker

Introduction

Pomegranate (*Punica granatum* L.) is an important ancient fruit crop which belongs to family Punicaeceae (syn. Lythraceae) under order Myrtales. The present scientific name of pomegranate was derived from the Latin name *Malus granatum*, which corresponds to 'seeded apple' (Holland *et al*., 2007). It is one of the favourite fruits of tropical and subtropical regions. Having originated in Iran, it is now widely cultivated all over the world including countries like Spain, Morocco, Egypt, Afghanistan and Baluchistan. In India, pomegranate is commercially cultivated in Maharashtra, followed by Andhra Pradesh, Uttar Pradesh, Tamil Nadu, Karnataka, Gujarat, Rajasthan, Punjab and Haryana (NHB database) http://nhb.gov.in/report_files/pomegranate/POMEGRANATE.htm

The genus, *Punica* is a small genus of fruit-bearing deciduous shrub or small tree, having both cultivated (*Punica granatum* L.) and wild types (*Punica protopunica* Balf.). *Punica protopunica* is endemic to island of Socotra (part of Yemen) and is the only relative of cultivated pomegranate. In almost all the countries where pomegranate is commercially grown, despite the availability of large number of local varieties only few are commercially utilized. The names of the cultivars originate frequently either from the place of cultivation or from colour of the fruit. Varieties are often classified as sweet, sweet-sour and sour, early, mid- season and late, juicy and table fruit, soft seeded and hard seeded. Several cultivars grown today are the result of human selection from naturally occurring variation. There is a great variability in pomegranate regarding its tree habit, mode of pollination, leaf size-shape and various flower characteristics (Mars and Marrakchi, 1999). Pomegranate accessions have been mainly evaluated based on the morphological characters and show a wide range of variation in size of fruits, sweetness, time of ripening, juiciness, and proportion of seeds to flesh. Therefore, breeding for useful traits has gained importance in this crop for which determination of genetic relationships and precise identification of accessions to conserve

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its genetic diversity is required. However, these traits are affected by environment and cultivation conditions and do not result in a clear discrimination among them (Kumar, 1999). The molecular or DNA based markers are more suitable for an accurate discrimination of the accessions and cultivars.

The Sequence Related Amplified Polymorphism (SRAP) technique is a relatively simple and highly reproducible DNA marker technique useful for both mapping and gene tagging in plants (Li and Quiros, 2001). SRAP has been shown to be more informative than other PCR-based techniques in detecting genetic diversity (Budak *et al*. 2004) and has been successfully used to study the genetic diversity of, and relationships among, several species (Castonguay *et al*., 2010; Talebi *et al*., 2011; Abedian *et al*., 2012). The present work was undertaken to demonstrate the usefulness of SRAP markers for diversity studies and for differentiating the accessions in the diverse collection of pomegranates sourced from different regions of the world.

Materials and Methods

The genetic analysis was carried out on 114 pomegranate accessions which included accessions from Turkmenistan, Japan, USA, Albania, former Soviet Union and wild types of unknown origin (Table 1, Fig.1). These accessions were obtained from the USDA National Germplasm Repository in Davis (CA, USA) and maintained at Indian Institute of Horticultural Research, Hesaraghatta, Bengaluru. The plants are in juvenile stage, therefore, the pomological traits recorded for accessions available at the website of USDA National Germplasm Repository was taken into consideration for interpretation of the clustering.

Genomic DNA was extracted from the leaves of each plant by using the CTAB-based method (Doyle and Doyle, 1990). The DNA quality was checked and adjusted to 50ng/µL final concentration for PCR reactions. A total of forty primer combinations (PC) based on literature were selected for the study. These primers were used for screening a panel of ten accessions from diverse origin to identify the polymorphic primers. After the initial screening, ten PCs which gave a reliable amplification product were selected for the molecular characterization of the pomegranate collection (Table 2). The PCR reaction was performed in a total volume of 15µl, containing 50–70ng template DNA, 0.2mM dNTPs, 1.6 mM $MgCl_2$, 0.3µM of each forward and reverse primer, 2.5 µl 10× PCR buffer, 1unit *Taq* DNA polymerase, and sterile double-distilled water. The amplification was conducted in a thermo-cycler programmed with the following thermal condition: initial denaturation at 94°C for 5min followed by 5 cycles of denaturation at 94°C for 1 min, 35°C for 1 min, 72°C for 1 min and annealing for 35 cycles of 94°C for 1min, 57°C for 1 min and extension at 72°C for 1 min, final extension at 72°C for 10 min and final hold at 4°C forever. The PCR product mixed with 2 µl bromophenol blue dye were analysed on 2% agarose gel in 1x TBE (Tris-borate-EDTA) buffer with 100bp DNA ladder as size marker. PCR products were visualized by ethidium bromide staining.

In order to evaluate the effectiveness of the SRAP markers, only the intense, clearly resolved PCR amplified bands were scored manually for their presence (1) and absence (0) in the matrix of SRAP data sheet. The band size was estimated by using medium range DNA ruler (100 bp) which was run along with the amplified products. The number of alleles per locus and the polymorphism information content (PIC) were calculated. The polymorphism information content (PIC) value was calculated by use of the formula: $PIC = 1 - \sum Pi^2$ where Pi is the frequency of the i^{th} allele (Smith *et al*., 1997). The genetic relatedness among the pomegranate accessions was assessed using Jaccard's co-efficient of similarity and by generating dendrogram based on Unweighted Pair Group Method with Arithmetic Mean (UPGMA) using R software (R Core Team 2013). The binary data scored was used to construct a dendrogram. Analysis of molecular variance (AMOVA) was performed to estimate variance components for SRAP data, partitioning the variation into within and among populations, by use of GenAlEx 6.5 - Genetic Analysis in Excel written in Visual Basic for Applications (VBA) within Excel (Peakall and Smouse 2012)

Results and Discussion

Ten SRAP PCs which exhibited the characteristics of good stability and repeatability were selected for this study. These primer pairs produced a total of 64 bands ranging from 80 to 1500bp of which 29 (47.8%) were found to be polymorphic. The number of amplified fragments ranged from 4 (Me3F +Em4R) to 12 (Me5F +Em1R). The polymorphic information content (PIC) value ranged from 0.17 to 0.34 with an average of 0.24. (Table 3). The primer combinations Me1F +Em8R

Table 1. Pomegranate accessions used in this study with their geographical origins and description

Code No.	Accessions	Accession No	Origin	Description
1	110 Podarok	EC798803	Turkmenistan	Soft-seeded, excellent sweet and sharp taste
2	121 Lyubimyi	EC798813	Turkmenistan (Balkan)	cultivated
3	109 Medovyi Vahsha	EC798802	Turkmenistan	Soft-seeded, early, sweet.
4	87 Mae	EC798785	USA (California)	Fruit light red or pink, medium to small. deep red arils, slightly sour
6	29 King	EC798739	Unknown	Tree shrubby, somewhat shy bearer, fruit medium to large, flesh very sweet.
7	16 White Flower	EC798733	Albania	Flowers white, ripens in October.
9	90 Sour	EC798788	USA (California)	Fruit slightly striped red, yellow-green, size medium. arils large, deep red, juice sour, seeds hard, suitable for culinary use.
10	106 Gissarskii Alyi	EC798799	Turkmenistan	Soft-seeded, excellent very sweet
11	10 Nochi-Shibori	EC798728	Japan (Saitama)	
12	81 Wonderful	EC798779	Unknown	Fruit large (up to 5 inches in diameter), bright red, arils red, juice red, sweet sour. Seeds medium soft, very juicy, ripens early, good shipper, large. ripens late September and October.
13	124 Parfyanka	EC798816	Turkmenistan (Balkan)	Seeds medium hard, seed red, flavour sweet-sour
14	42 Pink	EC798744	Unknown	
17	19 Hyrdanar × kirmizy-Akbu	EC798736	Unknown	
18	45 Elf	EC798747	USA	Dwarf, fruit greenish-yellow with orange blush, shape blocky, size small to medium, arils large, translucent pink, slightly tart.
19	38 Balegal	EC798743	USA (California)	Fruit large, round, pale pink, flesh pink, very sweet.
20	61 DPUN 61	EC798761	Unknown	Seeds soft, seed red, skin reddish yellow, flavour sweet
22	139 Myagkosemyannyi Rozovyi	EC798830	Turkmenistan (Balkan)	Seeds soft, seed rose, skin yellowish, flavour sweet-sour
28	112 1/25 Rannii	EC798805	Turkmenistan	Soft-seeded, early, sweet.
30	164 Utah sweet	EC798849	USA (California)	
31	125 Ariana	EC798817	Turkmenistan (Balkan)	Seeds medium hard, seed skin red, flavour sweet-sour
32	107 Gissarskii Rozovyi	EC798800	Turkmenistan	Seeds soft, seed colour rose, skin yellowish
34	9 Ki-zakuro	EC798727	Japan (Saitama)	
37	92 Gold	EC798790	USA (California)	Fruit size medium, vivid gold, arils and juice pink, sweet, seeds medium soft.
39	15 Parfianka	EC798732	Turkmenistan	Sweet-acid, soft seeded type, ripens in October, high juice quality.
40	7 Haku-botan	EC798725	Japan (Saitama)	Ornamental white, fruits large, light yellow, almost white, rind thick, arils medium-sized, white, sour, seeds medium hard.
41	57 Rosamia	EC798757	USA (California)	Produces single crop.
42	134 Myatadzhy	EC798826	Turkmenistan ((Balkan)	Seeds soft, seed colour, skin red, flavour sweet-sour
46	88 ELF	EC798786	USA (California)	Dwarf, fruit greenish-yellow with orange blush, shape blocky, size small to medium, arils large, translucent pink.
47	101 Kukurchiaskii	EC798795	Unknown	
48	108 Desertnyi	EC798801	Turkmenistan	Soft-seeded, excellent sweet and sharp taste
50	111 Shainakskii	EC798804	Turkmenistan	Soft-seeded, excellent sweet and sharp taste
56	75 Surh-anor	EC798773	Soviet Union Former	
58	104 Hotuni Zigar	EC798797	Turkmenistan	
63	13 Sverkhranniy	EC798730	Turkmenistan	Sweet, soft seeded type, ripens in August.

Code No.	Accessions	Accession No	Origin	Description
64	93 Palermo	EC798791	Unknown	Fruit large to extra-large, round, medium, red-yellow rind, flavor rich-tart, slightly acid, cuttings introduced in early 1980's from Italy.
66	2 Double Red	EC798721	USA (California)	Ornamental, some fruiting, rootstocks own, fruit Medium to large, red, rind thin, arils medium sized, pink, juice white, sour, seeds medium hard.
67	149 Gulistan	EC798837	Turkmenistan (Balkan)	
68	17 Dotch legrelley	EC798734	Azerbaijan	Flowers double, red-white.
70	105 Agat	EC798798	Turkmenistan	Soft-seeded, excellent sweet and sharp taste.
71	62 Salavatski	EC798762	Soviet Union Former	
72	167 Ink	EC798850	USA (California)	
73	14 Molla Nepes	EC798731	Turkmenistan	Sweet-sour, soft seeded type. ripens in October
74	4 Orange	EC798723	Unknown	Fruit very large, reddish orange, rind medium thick, arils large, pink, juice pink, seeds medium hard
76	53 Loffani	EC798753	USA (California)	
77	30 How sweet it is	EC798740	USA (California)	
78	117 Vkusnyi	EC798810	Turkmenistan (Balkan)	Seeds soft, seed colour red, skin red, flavour sweet-sour
79	100 Koinekasyrskii Kislosladkii Krasnyi	EC798794	Unknown	
80	5 Small Leaf	EC798724	USA (California)	Fruit small
83	85 Crab	EC798783	USA (California)	Fruit bronze-red, size medium, blocky pleasant appearance, arils deep red, juice red, mild sour, large, red, sour rich red juice, heavy bearing.
84	99 DK from Shevlan	EC798793	Turkmenistan (Balkan)	
85	122 Turan	EC798814	Turkmenistan (Balkan)	
86	59 Sakerdze	EC798759	Soviet Union, Former	High yield, large fruit, thin pericarp of fruit, coarse grain, high taste
89	43 Mae	EC798745	USA (California)	Excellent taste, medium to large fruit with sweet, tangy red flesh, sweet-sour
90	35 Vina	EC798741	USA (California)	Fruit papershell type, rind pale with some pink flush.
91	28 Fleischman's	EC798738	Unknown	
92	44 Eve	EC798746	USA (California)	Fruit red, large, arils deep red, juice red, good sweet/acid balance, seeds hard, productive, rated excellent.
95	50 Eversweet	EC798750	USA (California)	Derived from common home-grown cultivar in Lebanon, plants produce 2-3 crops of mature fruit each year in southern California, beginning in mid-summer and continuing through late fall. Fruit sweet even when small and immature, flesh light pink red, excellent for wine, syrup or fresh.
99	31 Toryu shibori	EC798729	Unknown	
102	54 Loulou	EC798754	USA (California)	Fruits small, sweet.
104	37 Wonderful	EC798742	USA (California)	Seeds medium hard, seed colour very red, skin red, flavour sour, eating quality excellent.
106	48 Ambrosia	EC798749	USA (California)	Fruits large, taste tart and sweet with pleasant aroma, kernels large and thick, good for jelly, syrup, and fresh eating.
108	91 Dewey	EC798789	Unknown	Fruit medium to large, reddish-pink.
109	103 Shirin Zigar	EC798796	Turkmenistan	
113	86 Cranberry	EC798784	USA (California)	Fruit uniformly cranberry red, size medium, suitable for culinary use.
5	127 Kemine	EC798819	Turkmenistan (Balkan)	wild
8	64 DPUN 64	EC798764	Unknown	wild
15	163 Machtumkuli	EC798848	Turkmenistan	wild

Code No.	Accessions	Accession No	Origin	Description
16	130 Messarian	EC798822	Turkmenistan (Balkan)	wild
21	151 Sirenevyi	EC798839	Turkmenistan (Balkan)	wild
23	135 Azadi	EC798827	Turkmenistan (Balkan)	wild
24	60 DPUN 60	EC798760	Unknown	wild
25	47 DPUN 47	EC798748	Unknown	wild
26	157 Chandyr	EC798845	Turkmenistan (Balkan)	wild
27	158 Balkan	EC798846	Unknown	wild
29	131 Dahistan	EC798823	Turkmenistan (Balkan)	wild
33	147 Sumbar	EC798835	Turkmenistan (Balkan)	wild
35	80 DPUN 80	EC798778	Unknown	wild
36	115 11 / 15	EC798808	Unknown	wild
38	123 Saharnyi	EC798815	Turkmenistan (Balkan)	wild
43	56 Purple Heart	EC798756	USA (California)	wild
44	78 DPUN 78	ED798776	Unknown	wild
45	154 Chernaya roza	EC798842	Turkmenistan (Balkan)	wild
49	143 Sogdiana	EC798833	Turkmenistan (Balkan)	wild
51	136 Syunt	EC798828	Turkmenistan (Balkan)	wild
52	137 Andalib	EC798829	Turkmenistan (Balkan)	wild
53	67 DPUN 67	EC798766	Unknown	wild
54	206 WEO 50	EC798851	USA (California)	wild
55	153 Kyz-bibi	EC798841	Turkmenistan (Balkan)	wild
57	74 DPUN 74	EC798772	Unknown	wild
59	113 15/4 Pamyati rozanova	EC798806	Turkmenistan	wild
60	68 DPUN 68	EC798767	Unknown	wild
61	58 DPUN 58	EC798758	Unknown	wild
62	70 DPUN 70	EC798768	Unknown	wild
65	152 Kopetdag	EC798840	Turkmenistan (Balkan)	wild
69	66 DPUN 66	EC798765	Unknown	wild
75	140 Seidi	EC798831	Turkmenistan (Balkan)	wild
81	116 Vishnevyi	EC798809	Turkmenistan (Balkan)	wild
82	142 Anvari	EC798832	Turkmenistan (Balkan)	wild
87	126 Girkanets	EC798818	Turkmenistan (Balkan)	wild
88	133 Hvalynskii	EC798825	Turkmenistan (Balkan)	wild
93	145 Nusai	EC798834	Turkmenistan (Balkan)	wild
94	155 Kara gul	EC798843	Turkmenistan (Balkan)	wild
96	129 Nisa	EC798821	Turkmenistan (Balkan)	wild
97	150 Ovadan	EC798838	Turkmenistan (Balkan)	wild
98	160 Gulyalek	EC798847	Turkmenistan (Balkan)	wild
100	63 DPUN 63	EC798763	Unknown	wild
101	79 DPUN 79	EC798777	Unknown	wild
103	114 31/69	EC798807	Turkmenistan	wild
105	73 DPUN 73	EC798771	Unknown	wild

Code No.	Accessions	Accession No	Origin	Description
107	51 DPUN 51	EC798751	USA (California)	wild
110	120 Kubarchatyi	EC798812	Turkmenistan (Balkan)	wild
111	76 DPUN 76	EC798774	Unknown	wild
112	72 DPUN 72	EC798770	Unknown	wild
114	128 Molla Nepes	EC798820	Turkmenistan	wild

Table 2. List of sequence of forward and reverse SRAP primers used in this study

Sl. No.	Primer name	Sequence (3'-5')
1	Me 1F	TGA GTC CAA ACC GGA TA
2	Me 2F	TGA GTC CAA ACC GGA GC
3	Me 3F	TGA GTC CAA ACC GGA AT
4	Me 4F	TGA GTC CAA ACC GGA CC
5	Me 5F	TGA GTC CAA ACC GGA AG
6	Me 6F	TGA GTC CAA ACC GGA CA
7	Me 7F	TGA GTC CAA ACC GGA CG
8	Me 8F	TGA GTC CAA ACC GGA CT
9	Me 9F	TGA GTC CAA ACC GGA GG
10	Me 10F	TGA GTC CAA ACC GGA AA
11	Me 11F	TGA GTC CAA ACC GGA AC
12	Me 12F	TGA GTC CAA ACC GGA GA
13	Me 13F	TGA GTC CAA ACC GGA AG
14	Em 1R	GAC TGC GTA CGA ATT AAT
15	Em 2R	GAC TGC GTA CGA ATT TGC
16	Em 3R	GAC TGC GTA CGA ATT GAC
17	Em 4R	GAC TGC GTA CGA ATT TGA
18	Em 5R	GAC TGC GTA CGA ATT AAC
19	Em 6R	GAC TGC GTA CGA ATT GCA
20	Em 7R	GAC TGC GTA CGA ATT CAA
21	Em 8R	GAC TGC GTA CGA ATT CAC
22	Em 9R	GAC TGC GTA CGA ATT CAG
23	Em 10R	GAC TGC GTA CGA ATT CAT
24	Em 11R	GAC TGC GTA CGA ATT CTA
25	Em 12R	GAC TGC GTA CGA ATT CTC
26	Em 13R	GAC TGC GTA CGA ATT CTG
27	Em 14R	GAC TGC GTA CGA ATT CTT
28	Em 15R	GAC TGC GTA CGA ATT GAT
29	Em 16R	GAC TGC GTA CGA ATT GTC

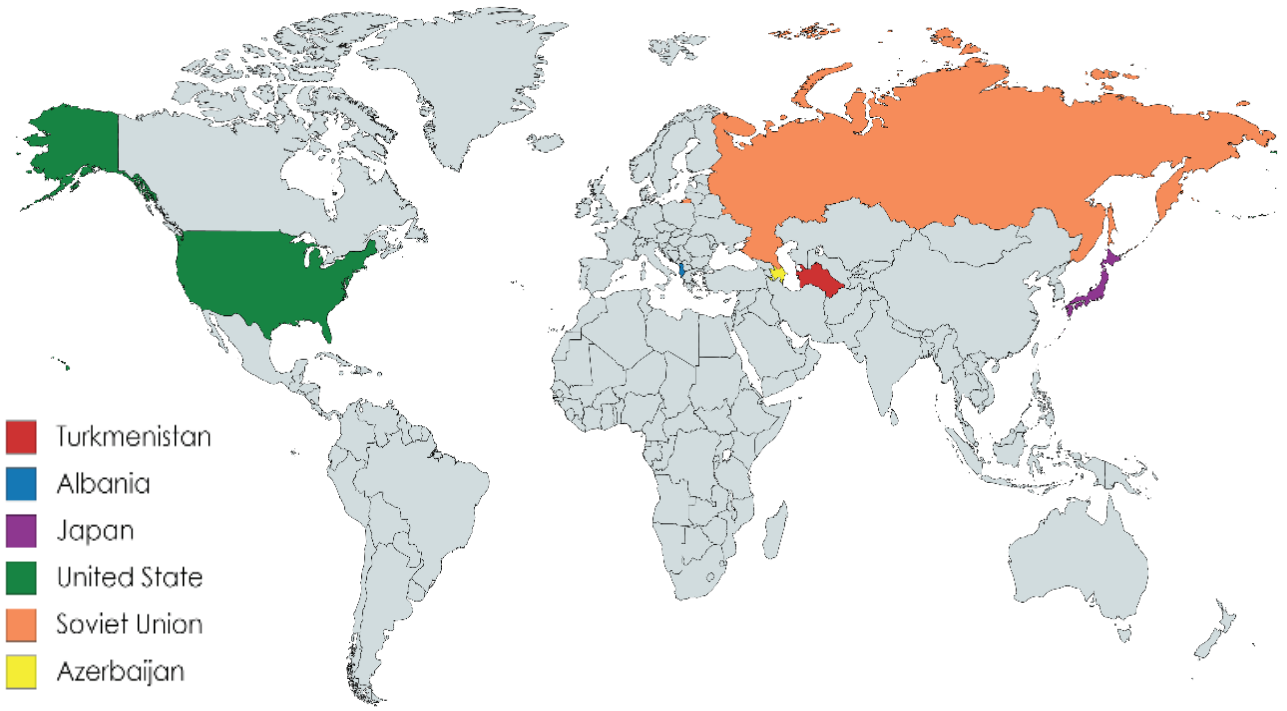

Fig. 1. Pomegranate accessions collected from different regions

and Me5F +Em1R produced maximum number of polymorphic bands (4). These primer combinations were found to be the most informative for genetic diversity studies among pomegranate accessions. The number of alleles per locus ranged from 1.66 to 4.00 with an average of 2.03. The expected heterozygosity varied from 0.215 to 0.445 with an average of 0.306. In previous studies, SRAP markers have been shown to be quite efficient in evaluating the genetic diversity in pomegranate. Substantial polymorphism (53%) was indicated by Soleimani *et al*. (2012) using SRAP marker system on accessions native to different regions of Iran. In another study by Amar and El-Zayat, (2017) this marker system was found to be superior to ISSR and IRAP markers in estimating molecular diversity among the Egyptian pomegranate varieties. Since the accessions in the germplasm have been derived from different geographical regions of the world, this investigation revealed significant polymorphism (47.8%) among the accessions and is in agreement with the result of previous workers. The PIC value of a marker is a useful measure of the efficiency of polymorphic loci in revealing genetic diversity among accessions. Botstein *et al*. (1980) reported that PIC index can be used to evaluate the level of gene variation, when PIC>0.5, the locus was of high diversity; when PIC<0.25, the locus was of low diversity and the locus was of intermediate diversity, when PIC was between 0.25 and 0.5. In this study, five PCs had PIC<0.25 and five had 0.5>PIC>0.25, with an average of 0.24 which is low signifying low variability among the accessions probably due to narrow genetic pool and monogeneric nature of this crop.

The genetic relatedness among the 114 different pomegranate accessions was assessed using Jaccard's co-efficient of similarity and by generating dendrogram based on Unweighted Pair Group Method with Arithmetic Mean (UPGMA). The resulting dendrogram (Fig. 2) was highly fragmented implying that the accessions were genetically distinct and most variation was present within the clusters. All the 114 accessions clustered into 4 major groups, the inter cluster distance lying between 0.3 - 0.4. The I cluster contained 12 accessions out of which 10 (83%) were wild. The II cluster comprised of 5 accessions, all of which belonged to Turkmenistan except number 86 which originated from former Soviet Union. In this cluster there were 2 wild and 2 soft seeded accessions. The III is the largest containing 80% (91) of the accessions. The III cluster was further divided into 3 sub-clusters (III a, III b and III c) comprising of 8, 37 and 46 accessions respectively. The sub-cluster III a, consisted of 8 accessions. Two ornamental accessions available in this germplasm collection came together in this cluster. The sub-cluster III b comprised of 37 accessions out of which 25 (68%) were cultivars. Maximum number of accessions present in this cluster originated from Turkmenistan (38%) followed by USA

Table 3. Polymorphic information content (PIC) value and incidence for 10 SRAP PCs

Sl. No.	Primer combination	No. of total bands	No. PB	PPB (%)	Expected Heterozygosity	PIC
1	Me1F +Em1R	5	3	60	0.3304	0.2476
2	Me1F +Em4R	4	2	50	0.2554	0.1919
3	Me1F +Em8R	6	4	66	0.4459	0.3421
4	Me3F +Em4R	4	2	50	0.3067	0.2401
5	Me6F +Em3R	5	3	60	0.2416	0.1944
6	Me6F +Em4R	5	2	40	0.3329	0.2489
7	Me5F +Em5R	7	3	43	0.2752	0.2193
8	Me2F +Em2R	7	3	43	0.3248	0.2518
9	Me5F +Em1R	12	4	33	0.3359	0.2732
10	Me8F +Em13R	9	3	33	0.2156	0.1709
Total		64	29	-	3.06	2.38
Mean		-	-	45	0.30	0.24

PIC- Polymorphic Information Content.
PPB- Percentage of Polymorphic Bands.

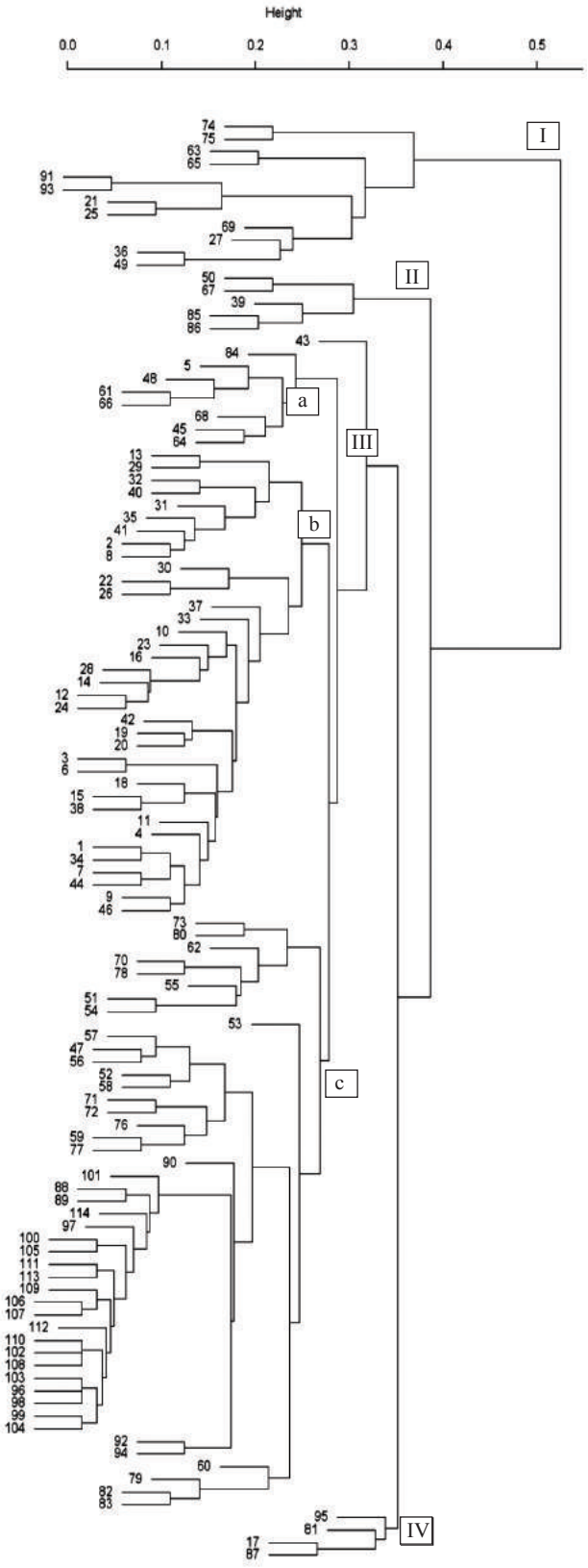

Fig. 2. Neighbour-joining dendrogram of 114 pomegranate accessions based on Unweighted Pair Group Method with Arithmetic Mean (UPGMA) using SRAP markers

(30%) and Japan (8%). This cluster contained maximum number of soft seeded cultivars (16%) and cultivars for which phenotyping data had been generated (numbers 13,22,31, 32 and 42). The sub cluster III c was found to be the largest containing a total of 46 accessions and geographically heterogeneous of all. This sub cluster had high number of accessions from Turkmenistan (43%) and USA (33%) contributing to 27 cultivated accessions. The number of soft seeded cultivars (4%) and accessions from unknown origin (17%) were less in comparison to the previous sub-cluster. The IV cluster was the smallest and consisted of 4 accessions of which, one being a cultivar and one hybrid along with two accessions of wild nature from Turkmenistan. The analysis of genetic distances showed that the distances between the 114 pomegranate accessions ranged between 0.01 and 0.84 with an average of 0.42. The largest distance (0.84) was recorded between ‘‘DPUN121 Lyubimyi’’ (2) and ‘‘DPUN115 32/30’’ (36) which are both wild types; the lowest distance was recorded between several pairs of accessions which were present in sub cluster III c. Analysis of wild pomegranates in the western Himalayas by use of RAPD, DAMD, and ISSR markers (Narzary *et al*., 2009 and 2010), Tunisian pomegranates on the basis of AFLP profiles (Jbir *et al*., 2008), and Iranian pomegranate accessions by use of RAPD and ISSR markers (Talebi *et al*., 2011) has revealed that the geographical diversity of the accessions is not correlated with genetic diversity. Similar result was obtained in the present study which provides evidence that SRAP markers are simple, informative and reproducible approach for evaluation of molecular diversity and phylogenetic relationships in pomegranate germplasm with the added advantage that they target ORFs as functional regions of the pomegranate genome.

The relationship between accessions for genetic, geographical origin and pomological traits was made clear by the clustering pattern. Wild pomegranate is known to occur in the regions from Iran to northern India, as can be observed in the forests of these areas (Stover and Mercure, 2007). These mostly have thicker rind, smaller arils, harder seeds and much higher acidity than the cultivated types. On the basis of SRAP markers, the wild pomegranates from Turkmenistan considered in this study were found to be very similar to each other and they mainly clustered in Group I (83%). The ornamental accessions clustered with cultivars in group III a. These ornamental plants are double-flower forms which, as the name implies, produce double flowers wherein

numerous stamens are modified into petals; they do not usually set fruit but, because of their beautiful flowers, are important as ornamental plants. These sterile flowers can be pollinated manually to yield fruit (Jalikop, 2007). The same result of narrow genetic variation between ornamental and cultivated types has been reported by Jbir *et al*. (2008). Pomegranate accessions can be divided into four groups based on seed hardness: soft, semi-soft, semi-hard and hard seeded (Zamani, 1990). Commercial pomegranate cultivars are often semi-hard or hard-seeded which is a distinct disadvantage, as soft-seeded fruit are the consumer's fruit of choice. According to Levin (1994), completely soft-seeded pomegranates are restricted to a few narrow ecological regions representing an important genetic resource for improving the seed softness trait in commercial pomegranate varieties. There are 8 soft seeded cultivars present in the collection and maximum number (6) clustered together in group III b (13,32,10,12,3,1). This group also comprised of 6 accessions with sweet taste (3, 13,20,28,37,95,102,19), 6 accessions with sweet-sour taste (31,22,12,10,1,42), 3 accessions with tart and watery taste (18,4,46) and 2 sour accessions (9,32). The lowest genetic distance was observed between accessions (110,102,108,103,96,98,99 and 104)) located in group III c. The SRAP data of these accessions are very similar in pattern though they have originated from different geographical areas (Turkmenistan, USA, Japan). Similar observation was made in a recent study by Giancaspro *et al*., 2017 using SSR markers the authors report that pomegranate accessions from different geographical areas appeared more similar with respect to accessions within the same country. They attributed this to the intense flow of genetic materials from Persia to different countries all over the world since ancient times which has led to genetic homogenization among these groups of accessions.

To understand the genetic relationships among accessions that originated from different geographical regions, they were divided into 4 panels (Asia, North America, Europe and Unknown). Four indices of population-level genetic diversity, namely observed number of alleles (Na), expected heterozygosity (He), effective number of alleles (Ne) and Shannon's information index (*I*), were calculated as shown in (Table 4). As a whole among the four populations, the effective number (Ne) of alleles ranged from 1.29 to 1.95 with average of 1.66 the Shannon's information index (*I*) ranged from 0.53 to 0.68 with average of 0.63 and expected heterzygosity (He) ranged from 0.34 to 0.48 with average of 0.44 at the group level. The highest Shannon's information index (*I*), observed and effective number of alleles (Na and Ne), and expected heterozygosity (He) were observed in accessions from the Unknown region which constitute the wild types (Table 4). Analysis of molecular variance (AMOVA) was performed to study genetic differentiation among four population from distinct geographic regions and to estimate the percentage of intra and inter- population genetic variation (Table 5). The results from AMOVA analysis revealed that 99% of total genetic variation occurred within population and only 1% was attributed to among populations. The analysis indicated insignificant genetic variation (p = 0.31) between pomegranate accessions in different populations, low differentiation in allele frequencies (F-statistics = 0.019) and high gene flow (Nm =24.353) among the four populations (Table 5) which indicates that the genetic diversity of pomegranate is independent of their geographical origin. The mean genetic distance among groups ranged from 0.165 to

Table 4. Summary of genetic variation statistics for 10 SRAP primer combinations in four pomegranate population by AMOVA (Analysis of molecular variance)

Population	Sample size	Band frequency	Na	Ne	*I*	He
Asia minor	54	0.611	2.000	1.885	0.662	0.469
North America	25	0.760	2.000	1.296	O.678	0.485
Europe	5	0.400	2.000	1.537	0.534	0.349
Unknown	30	0.683	2.000	1.956	0.682	0.488
Total	114	2.454	8.000	6.674	2.556	1.791
Mean	-	-	2.000	1.668	0.639	0.447

Na - Observed number of alleles,
Ne - Effective number of alleles,
I - Shannon's information index,
He -Expected heterozygosity,

Table 5. Analysis of molecular variance (AMOVA) among and within the pomegranate accessions for four populations using SRAP markers

Source of variation	Degrees of freedom	Sum of Squares	Mean Square	Estimated Variance	Percent variance	*P* value	Fst	Nm
Among Pops	3	1.712	0.571	0.005	1%	0.31	0.019	24.353
Within Pops	110	49.700	0.452	0.452	99%			
Total	113	51.412		0.457	100%			

Fst- F statistics
Nm- Gene flow

0.011. The highest genetic distance (0.165) was observed between the North America and Europe population and lowest (0.011) was detected among the Asia and the Unknown population (Table 6). The result obtained from analysis of molecular variance and pair wise F-statistics test showed insignificant differences among the populations. The result of AMOVA is in agreement with genetic relationship between accessions by cluster analysis, where accessions belonging to different origins have grouped together. The low differentiation in allele frequencies among populations (Fst = 0.019) and high gene flow (Nm = 24.35) observed in this study indicated that the genetic diversity of pomegranates is independent of their geographical origin. Similar result has been reported by Giancaspro *et al*. (2017); Soleimani *et al*. (2012); Jbir *et al*. (2008) and Talebi *et al*. (2011) using different molecular markers which explains that the geographical diversity of the accessions is not correlated with genetic diversity. Pomegranate is an ancient fruit crop which has been propagated clonally by cuttings. It is adaptable to various soil and climate conditions resulting in its spread to different parts of the world. It can therefore be concluded that the higher level of genetic diversity within populations and the lower level among geographical groups is a result of movement of genetic material from the centre of origin (Central Asia) to other countries such as Japan, USA, European countries leading to high level of migration and gene flow among regions. The present investigation clearly demonstrated the efficiency of SRAP markers in detecting sufficient polymorphism to establish genetic relationships and population genetic structure of a diverse collection of cultivars and wild pomegranates sourced from different geographical regions of the world. These markers have a great potential since they target the ORFs as functional regions of the pomegranate genome which could be useful in identifying trait-marker association of interest in the marker-assisted breeding programs. Close relationship between wild and cultivated pomegranates indicates that the cultivated pomegranates have descended from wild plants and they are genetically not very diverse. Low genetic differentiation between accessions from different geographical regions of the world points to narrow genetic base, migration and gene flow in this crop since ancient times.

Table 6. Matrix of genetic distances among pomegranate populations

	Asia minor	North America	Europe	Unknown
Asia minor	0.000			
North America	0.052	0.000		
Europe	0.034	0.165	0.000	
Unknown	0.011	0.015	0.078	0.000

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RESEARCH ARTICLE

Characterization of Sorghum [*Sorghum bicolor* (L.) Moench] Germplasm for Agro-Morphological Traits

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The experiment on characterization of 2500 accessions of sorghum germplasm including indigenous and exotic collections representing 42 countries was conducted at Indian Council of Agriculture Research-Indian Institute of Millets Research (ICAR-IIMR), Hyderabad, India. The experiment was laid in Augment Block Design with two checks. These accessions were characterized for yield and yield attributing traits. The analysis of descriptive statistics showed that wide range of variability for all quantitative traits. There was significant positive association between yield and yield attributing traits except for ear head length which had negative significant association with grain yield and 100-seed weight. Sorghum accessions were grouped into 10 clusters as per Ward's method and found that there was 13.65% and 86.35% of variability was found within and between clusters. This variability in the germplasm can be utilized for further sorghum improvement programme. The potential germplasm identified for different agro-morphological traits may be used to develop parents and varieties in sorghum improvement programmes.

Key Words: Sorghum, Principle Component Analysis, Genetic Diversity, Association and Clusters

Introduction

Sorghum is an important cereal crop of the semi-arid tropics, which is being grown in *Kharif* and *Rabi* season. it is fifth most important cereal crop grown in more than 100 countries worldwide. India, Nigeria, Mexico, USA, Argentina and Ethiopia are the major sorghum producing countries. The sorghum production is 63.93 mt over 44.77 mha area worldwide with the productivity of 14279 kg ha^{-1} (FAO 2016). India is the leading sorghum producing county in South Asia which covers 5.65 mha area with the production of 44 mt and productivity of 7805 kg ha^{-1} (FAO 2016). Sorghum crop is grown mainly for food and fodder in Africa, Asia and Central America. The high brix content sorghum (Sweet sorghum) is used as an alternative source for ethanol production, beer, alcohol, syrup, bakery items, industrial starch, etc. (Umakanth 2012).

It is drought hardy plant which withstands extreme environmental changes. Sorghum has diverse populations and species rich ecosystem and have greater potential to adapt to climate change. The improvement of any crop depends on the availability of diversity of the crop. Wide genetic diversity is the key for present and future. The genetic resources (germplasms and landraces) are the best sources to maintain the wide genetic diversity and sustainable crop improvement. Hence there is urgent need to conserve the genetic resources of all crops as much as possible, before we lose them permanently due to replacement of landraces by improved cultivars besides several other factors (Upadhyaya and Gowda, 2009a).

Many international and national genebanks had large collections and conservations of germplasm as well as represents the wide genetic diversity in different crops including sorghum. In recent years, decline curve observed for international efforts to collect and conserve the plant genetic resources (FAO 2009). There is need of critical assessment of collection of germplasm, identifying gaps and launching germplasm collection mission in unexplored and under explored areas. This is important to enrich the genetic variability further and enhance the efficiency of crop improvement program.

There are several genebanks in the world conserve germplasm of many crops including sorghum.

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International Crops Research Institute for Semi-Arid Tropics (ICRISAT) Hyderabad, India, conserves more than 39000 sorghum accessions from 90 plus countries including > 6200 countries from South Asia (Upadhyaya *et al*., 2016) at international level. The National Genebank at ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR) New Delhi, India conserves 4.3 lakh germplasm of all crop plants and horticulture crops as well. ICAR-Indian Institute of Millets Research (IIMR) Hyderabad India is also involved in collection and conservation of millets germplasms including sorghum, pearl millet and other six small millets, which maintain > 6000 sorghum germplasms (Elangovan *et al*., 2009). Maharashtra, Karnataka, Tamil Nadu, Andhra Pradesh, Telangana, Madhya Pradesh and Uttar Pradesh are major sorghum growing states. There is ample of scope to enrich the existing sorghum genetic resources and filling gap of collection, conservation and characterization. The sorghum germplasm is collected/augmented and conserved at the National Genebank (NGB), ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR), New Delhi; ICRISAT, Patancheru, Hyderabad; and ICAR-Indian Institute of Millets Research (ICAR-IIMR), Hyderabad. ICAR-IIMR-Hyderabad is one of the National Active Germplasm Sites (NAGS) with the collection and augmentation of sorghum, pearl millet and small millets, which includes 32000 acc. of sorghum and 15000 acc. of other millets.

Materials and Methods

A total of 2500 accessions of sorghum germplasm representing 42 countries obtained under the Consortium Research Platform on Agrobiodiversity on Sorghum were used for agro-morphological characterization. In which, data of 2460 accessions were used for analysis and the rest of them did not germinate or could not collect the complete phenotypic data. Among the 2460 accessions represents, 842 accessions of indigenous collections and 1616 accessions of exotic collections. The characterization was done at ICAR-Indian Institute of Millets Research (IIMR) Hyderabad with the geographical coordinate of 17.3207°N latitude, 78.3959°E longitude and 476.5 meters above M.S.L. The experimental was conducted in augmented block design along with two checks CSV 29R and M35-1 which were repeated in every 100 accessions. The plot was with 1 m long row with 60 cm distance between each row. The distance between the plants was maintained at 10 cm after two weeks of thinning. Fertilizers were applied at the rate of 80 kg/ha N and 40 kg/ha P_2O_5 in during crop growth and all necessary package of practice were followed for good crop stand. Regular irrigation was given to maintain sufficient moisture and the crop was protected from weeds, pest and diseases.

Observations recorded

The accessions were characterized for 9 quantitative traits during crop growth durations. The days to 50% flowering was calculated plot based in which 50% plants completes its 50% of the panicle flowers from days after sowing. The other traits were total number of leaves, leaf length (cm), leaf width (cm), ear head length (cm), ear head width (cm), plant height (cm), grain yield (g/plant) and 100-seed weight. The observations were collected from 3 representative plants.

Data analysis

The data was analysed for calculating the mean, range and variance for all the quantitative traits using GENSTAT, association analysis using SPSS software, Principle Component Analysis (PCA) and clustering Analysis using Gen Alex software.

Results

Range: The wide range of variation has been observed for quantitative traits under the study (Table 1) for both Exotic and Indigenous collections for days to 50% flowering, total number of leaves, leaf length, leaf width, ear head length, ear head width, plant height, grain weight and seed weight. Variation was found in the indigenous collection for days to 50% flowering (47 to 96 days), total number of leaves (3.8 to 13.6), leaf length (36.46 to 91.18 cm), leaf width (2.5 to 10.36 cm), ear head length (8.1 to 48.94 cm), ear head width (3.3 to 14.2 cm), plant height (64 to 296 cm), grain yield (3.25 to 144.5 g/plant) and 100-seed weight (1.00 to 6.23 g). Whereas wide range in the exotic collections was observed for days to 50% flowering (44 to 97 days), total number of leaves (2.2 to 14.5), leaf length (23.2 to 88.5 cm), leaf width (2.46 to 10.85 cm), ear head length (7.82 to 73.2 cm), ear head width (2.2 to 19.2 cm), plant height (66 to 348 cm), grain yield (2.5 to 149 g/plant) and 100-seed weight (1.00 to 6.39 g).

Mean: The mean was calculated to compare the indigenous and exotic germplasm (Table 1). The exotic collection germplasm were significantly higher for all the quantitative traits except ear head length. The indigenous germplasm were significantly early flowering (62.28

days) type compare to exotic germplasm (66.62 days). The exotic germplasm had higher total number of leaves (9.17) when compare to indigenous (8.03). Leaf length and leaf width will determine the canopy coverage and photo synthetic area in which exotic germplasms are having longer leaf (68.84 cm) and wider leaf (7.29 cm) than indigenous germplasms (62.64 cm and 6.68 cm respectively). The yield of the plant is decided by the length and width of the ear head, the exotic germplasm had wider ear head (7.13 cm) compare to indigenous (6.15 cm), However, indigenous germplasm reported for longer ear head (25.47 cm) than exotic (18.28 cm).

Association studies: Grain yield is positively associated with all the traits except ear head length. Days to 50% flowering had positive significant association with total number of leaf, leaf length, leaf width, plant height, grain yield and 100-seed weight. However, it had negative correlation with ear head width. Total number of leaves had significant positive correlation with all quantitative traits under the investigation except for ear head length which showed negative correlation. Leaf length had positive significant association with all traits. Leaf width had positive association with remaining quantitative traits, whereas, it had negative correlation with ear head length. Ear head length had positive association with leaf length and plant height; whereas, it associated significant negatively with total number of leaves, leaf width, ear head width, grain yield and 100-seed weight. Plant height, grain yield and 100-seed weight had positive significant association with each other as well as with other quantitative traits, simultaneously grain yield and 100-seed weight had negative significant association with ear head length (Table 2).

Phenotypic diversity using Principle Component Analysis (PCA): The first three principle components considered for the variability because of having more than one as Eigen value. These principal components had contributed 61.51%, 65.38% and 64.38% variability of exotic, indigenous and in total respectively. The first principle component was most important due to its highest contribution for phenotypic variation in the germplasm.

Table 1. Descriptive statistics of quantitative traits of sorghum accessions

	Range				
Traits	Mean	Minimum	Maximum	Standard deviation	SE
Days to 50 % flowering	63.77	44	97	8.323	0.168
Ear head length (cm)	23.01	7.82	73.2	8.324	0.168
Ear head width (cm)	6.493	2.2	19.2	1.442	0.0291
Grain yield (g/plant)	39.35	2.5	149	16.8	0.339
leaf length (cm)	64.77	23.2	91.18	9.258	0.187
Leaf width (cm)	6.895	2.46	10.85	1.258	0.0254
Plant height (cm)	188	64	348	52.43	1.058
100-Seed weight (g)	2.85	1	6.39	0.927	0.0187
Total number of leaves per plant	8.425	2.2	14.5	1.644	0.0331

Table 2. Association of quantitative traits of Sorghum accessions

	DTF	TNL	LL	LW	EHL	EHW	PH	GW	SW
DTF	1	0.539**	0.475**	0.296**	-0.03	-0.071**	0.460**	0.223**	0.014
TNL		1	0.520**	0.485**	-0.10**	0.114**	0.515**	0.399**	0.058**
LL			1	0.516**	0.092**	0.191**	0.596**	0.425**	0.015
LW				1	-0.09**	0.288**	0.281**	0.506**	0.073**
EHL					1	-0.033	0.068**	-0.12**	-0.182**
EHW						1	0.152**	0.377**	0.110**
PH							1	0.334**	0.063**
GW								1	0.110**
SW									1

DTF= Days to 50 % flowering, EHL= Ear Head Length (cm), EHW= Ear Head Width (cm), GW= Grain Yield (g/plant), LL= Leaf Length (cm), LW= Leaf Width (cm), PH= Plant Height (cm), SW= 100-Seed weight and TNL= Total Number of Leaves per plant

The first principal component had contributed more variability through days to 50% flowering (0.41), total number of leaves (0.63), leaf length (0.65), plant height (0.52) and grain yield (0.45). Second, third and fourth PCs contributes largest cosine for the characters viz., ear head width (0.35), ear head length (0.42) and seed weight (0.51) respectively (Table 3).

Clustering: The Wards method of clustering followed to group the germplasm accessions. There were 10 clusters formed. The cluster mean were calculated and presented in the Table 4. The cluster I was best performed for total number of leaves, leaf width, ear head width, grain yield and 100-seed weight, whereas, cluster V was superior for late flowering, leaf length, ear head length and plant height. All clusters include both indigenous and exotic germplasm and there was 86.35% variability between the clusters and 13.65 % within clusters. Indigenous germplasm belongs to cluster I, II, IV and V were majorly from Karnataka, Maharashtra, Andhra Pradesh, whereas most of the exotic germplasm belongs to African countries viz., Mali, Sudan, Uganda, Chad, Ethiopia and Burkina Faso as well as United States of America. The clusters III, VI, VII and VIII consists of indigenous germplasm from Madhya Pradesh, Gujarat, Orissa, Bihar and Uttar Pradesh, whereas most of exotic germplasm were from United States of America, South Africa, Mali, Ethiopia, Burkina Faso and Uganda. Clusters IX and X consists of exotic germplasm from United States of America, Mexico, Zimbabwe and with mixture from African countries.

Discussion: The germplasm is the basic source of all characters and base for all breeding programmes. Plant genetic resources are important component of biodiversity. They include diverse genetic material in terms of traditional varieties, modern cultivars, crop wild relatives, germplasm and landrace etc. They are the source material of all traits including morphological, physiological, biochemical, biotic and abiotic stress

Table 3. Percentage of variation explained by the first seven principal components (PCs) in sorghum accessions evaluation at ICAR-IIMR, Hyderabad

PC	Eigenvalue	Variability (%)	Cumulative %	DTF	TNL	LL	LW	EHL	EHW	PH	GW	SW
F1	3.32	36.84	36.84	0.4134	0.6279	0.6531	0.5112	0.0049	0.1159	0.5195	0.4544	0.0155
F2	1.37	15.19	52.03	0.1745	0.0172	0.0383	0.0519	0.2616	0.3544	0.07	0.1459	0.2533
F3	1.11	12.35	64.38	0.1216	0.0426	0.0169	0.0136	0.4292	0.2691	0	0.0328	0.1856
F4	0.87	9.69	74.07	0.0001	0.011	0.0071	0.0546	0.1903	0.0054	0.0765	0.0196	0.5074
F5	0.64	7.07	81.14	0.001	0	0.0012	0.225	0.0763	0.1229	0.1657	0.0068	0.0373
F6	0.50	5.61	86.75	0.071	0.024	0.0058	0.0229	0.0014	0.1253	0.0449	0.2091	0.0003
F7	0.47	5.24	91.99	0.1285	0.0043	0.0915	0.0509	0.0268	0.0049	0.0344	0.1302	0.0001

DTF= Days to 50 % flowering, EHL= Ear Head Length (cm), EHW= Ear Head Width (cm), GW= Grain Yield (g/plant), LL= Leaf Length (cm), LW= Leaf Width (cm), PH= Plant Height (cm), SW= 100-Seed weight and TNL= Total Number of Leaves per plant.

Table 4. Cluster mean for quantitative traits of sorghum accessions evaluated at ICRA-IIMR Hyderabad

Cluster	DTF	TNL	LL	LW	EHL	EHW	PH	GW	SW
I	69.15	10.16	73.09	8.14	19.19	7.57	256.18	74.52	3.13
II	66.08	9.34	71.17	7.74	20.28	7.30	206.64	64.74	2.90
III	65.06	8.50	66.87	6.83	23.27	6.39	210.13	34.88	2.72
IV	68.69	9.46	69.65	7.37	21.33	6.71	242.47	42.38	3.10
IX	59.16	7.59	57.04	6.52	24.00	6.17	125.22	34.60	2.87
V	70.74	9.85	73.83	7.27	30.94	6.48	275.39	39.50	2.83
VI	60.75	7.48	59.10	5.84	23.11	5.97	173.35	24.58	2.73
VII	61.41	8.10	65.69	7.18	20.78	6.77	167.87	46.68	2.84
VIII	59.98	7.49	58.52	6.30	23.13	6.14	145.96	27.39	2.92
X	58.87	7.32	56.67	6.59	23.62	6.18	99.67	30.77	2.66

DTF= Days to 50 % flowering, EHL= Ear Head Length (cm), EHW= Ear Head Width (cm), GW= Grain Yield (g/plant), LL= Leaf Length (cm), LW= Leaf Width (cm), PH= Plant Height (cm), SW= 100-Seed weight and TNL= Total Number of Leaves per plant.

resistances and necessary to improve the status and lively hood of the mankind.

Conservation of these germplasm sources are very important to meet the requirement of exploding population and rapid climate changes which leads the man to seek resilient and nutritious food crops. The wild relatives, landraces and germplasm are necessary to bring under domestication to make desirable changes to meet breeder's objectives and to enhance the variability.

In India, ICAR-National Bureau of Plant Genetic Resources (NBPGR), New Delhi; International Crops Research Institute for Semi-Arid Tropics (ICRISAT), Patancheru, Hyderabad and ICAR-Indian Institute of Millets Research (IIMR), Hyderabad are the nodal institutes which are having the mandate of collection and conservation of sorghum and other millets. In collaboration with the Consortium Research Platform on Agro-Biodiversity (CRP-AB) on Sorghum, ICAR-NBPGR-New Delhi, ICAR-IIMR had conducted an experiment on characterization of 2500 sorghum germplasm accessions during *Rabi* (post-rainy) 2015-16. These accessions include indigenous and exotic collections of sorghum germplasm. They were the representative sample accessions from 42 countries worldwide and all the accessions with FAO in trust.

The variability can also estimate by range. Sufficient genetic variability has been observed (Elangovan *et al*., 2009) which will helps breeders to select diverse genotypes for the breeding programme. There is differential expression of indigenous and exotic germplasms, the exotic germplasms shows higher variability than indigenous accessions.

Representation of the sorghum germplasm from 42 countries indicates that wide genetic diversity (Upadhyaya *et al*., 2016). In India, the accessions were representative of 17 states which covers almost 60% of Indian states. In which, Maharashtra (127 acc.) had represented with highest number of accessions, followed by Andhra Pradesh (75 acc.), Madhya Pradesh (65 acc.), Gujarat (46 acc.) and Karnataka (41 acc.). The list of representative countries of exotic collections and states of India is presented in Table 5. Elangovan *et al*. (2007, 2012) collected and characterized 157 and 107 sorghum accessions from Karnataka and Gujarat respectively and identified suitable accessions for yield and yield related traits. Ganesamurthy *et al*. (2010) evaluated 130 genotypes for genetic diversity and identified different clusters for different traits as well as identified suitable genotypes for grain yield and fodder yield.

Table 5. representative countries of exotic collections and states of India

Country	Number of germplasms	Country	**Number of germplasms**
United States of America	714	Central African Republic	2
India	495	Japan	2
Sudan	238	Kenya	2
South Africa	77	Argentina	1
Ethiopia	73	Cameroon	1
Mali	62	Greece	1
Nigeria	57	Iraq	1
Uganda	57	Myanmar	1
Burkina Faso	53	Pakistan	1
China	36	Sierra Leone	1
Mexico	32	Thailand	1
Zimbabwe	26	**India (States)**	
Chad	17	Maharashtra	127
Zaire	15	Andhra Pradesh	75
Tanzania	12	Madhya Pradesh	65
Egypt	10	Gujarat	46
Korea	8	Karnataka	41
Senegal	8	Rajasthan	28
Botswana	7	Uttar Pradesh	26
Ghana	6	Orissa	14
Zambia	6	Tamil Nadu	13
Indonesia	5	Haryana	11
Italy	5	Punjab	9
USSR	5	Bihar	8
Iran	4	Bihar (Jharkhand)	6
Malawi	4	Kerala	5
Taiwan, Province of China	4	Jammu & Kashmir	3
Algeria	3	West Bengal	2
Afghanistan	2	Bihar (Chhattisgarh)	1
Australia	2		

The association of traits are helpful for breeders to select traits which contributes to grain yield and yield attributing traits (Fig. 1). In the present study, grain yield traits under the study were associated with each other, where grain yield had positive significant association with all traits except ear head length with which negative association was observed (Elangovan

et al., 2009). The days to 50% flowering, total number of leaves per plant, leaf length, leaf width, plant height, grain weight and seed weight had positive significant association among themselves, which indicates that all the traits are contributing positively to yield and improvement of one traits influences the improvement of other traits as well (Elangovan *et al.,* 2007, 2009, 2013, Jain *et al*., 2009, 2011). Ear head length had negative association with total number of leaves per plant, leaf width grain weight and seed weight, which indicates that improvement in ear head length will reduce the leaf width and total number of leaves, which may be due to more photosynthets required for the ear head length. Hence, the total energy will be utilized for the elongation of ear head length and may fail to accumulate in to grain.

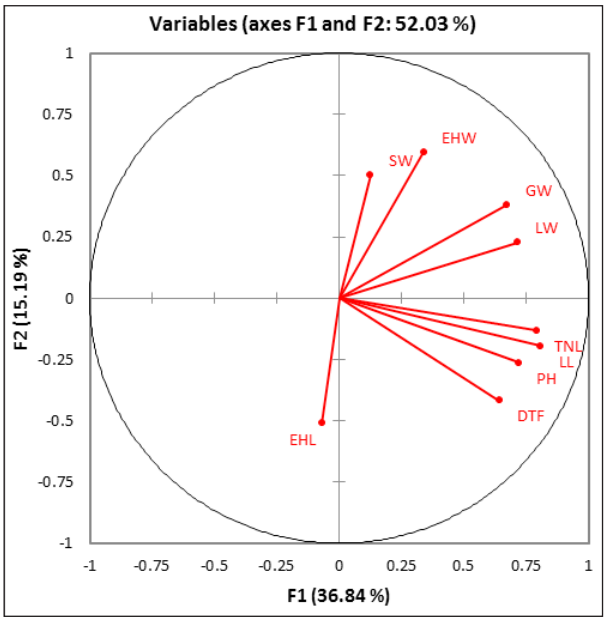

Fig. 1. Sorghum quantitative trait association evaluated at ICAR-IIMR Hyderabad

Clustering of all accession were done according with ward's method to group the genotypes with minimum variance. According to ward's method all accessions grouped in to 10 different clusters with maximum of 451 genotypes in cluster III and minimum of 89 accessions in cluster I (Elangovan *et al.,* 2007, 2009, 2013, Jain *et al.,* 2009, 2011). Within clusters there was minimum of 13.65% variance observed and 86.35% between clusters (Table 6). Cluster mean will help the breeder to select the diverse genotypes from different clusters. The cluster I showed better mean performance for total number of leaf, leaf width, ear head width, grain yield and 100-seed weight. The breeders can select their genotypes from the cluster I for grain yield. Similarly cluster V had performed better mean for leaf length, ear head length, plant height and longer days to 50% flowering, which are suitable for the high biomass fodder yield. The list of better performing top 20 accessions was presented in Table 7. The accession EC 483357 was better performed for grain yield and ear head length and EC 483872 was good for plant height and 100-seed weight, hence these two accessions can serve as parent for the grain yield improvement breeding programmes. EC 484133 was better for total number of leaves, leaf length and late flowering, EC 484182 and IC 289370 were good for grain yield and leaf length and width hence these accessions can be useful for the improvement of high biomass as well as grain yield. Breeding for high bio mass is gaining importance because it is mainly used as animal feed and now a days it is using for the extraction of ethanol which is utilizing as bio fuel to blend with petrol to reduce the use of petroleum products (Umakanth *et al*., 2012).

Table 6. the variation found within and between clusters of sorghum accessions

	Absolute	Percent
Within-class	445.2904	13.65%
Between-classes	2816.9717	86.35%
Total	3262.2620	100.00%

Conclusions

The experiment on characterization of sorghum germplasm was conducted at ICAR-IIMR-Hyderabad for 2500 indigenous and exotic germplasm collection representing 42 countries. Wide genetic diversity was observed within and between clusters identified and the superior accessions identified for all quantitative traits. These accessions can be used by the breeders as parents in the breeding programmes. Still there is need to utilize the remaining germplasm identified instead of using only few accessions for breeding programmes. There is a scope for collection and conservation of germplasm across the country and need to introduce these germplasm to new areas to increase variability and to meet nutritious food requirement of growing population.

Table 7. Best performing top 20 accessions identified for quantitative traits of sorghum evaluated at ICAR-IIMR Hyderabad

Days to 50% Flowering (early)	EC 483629, EC 483759, IC 289886, IC 289923, EC 483128, EC 483154, EC 483429, EC 483484, EC 483533, EC 483578, EC 483655, EC 483941, IC 289835, IC 289848, IC 289849, IC 289850, IC 289924, IC 289963, IC 289966 and IC 289967
Days to 50% Flowering (Late)	EC 484149, EC 484544, EC 484130, IC 289194, EC 483963, EC 483840, IC 289183, EC 483833, EC 483832, IC 289184, EC 484155, EC 484133, EC 483859, EC 484126, IC 289433, IC 289327, IC 289170, EC 484270, EC 484061 and EC 483818
Total number of leaves	EC 484133, IC 289462, IC 289412, IC 289364, IC 289251, IC 289375, IC 289371, IC 289861, IC 289857, IC 289479, IC 289425, IC 289433, IC 289582, IC 289585, IC 289280, IC 289281, IC 289450, IC 289423, EC 484037 and IC 289169
Leaf length (cm)	IC 289431, IC 289919, EC 484133, IC 289898, EC 484182, IC 289675, IC 289273, EC 484628, EC 484178, EC 483088, IC 289800, IC 289465, EC 484245, IC 289893, EC 483404, EC 483087, IC 289423, EC 483339, IC 289954 and EC 484569
Leaf width (cm)	EC 484152, EC 484129, EC 484037, IC 289613, IC 289594, IC 289284, EC 484589, IC 289273, IC 289169, IC 289370, EC 483821, EC 484146, IC 289364, EC 483615, EC 484039, EC 484157, EC 483666, IC 290005, EC 483908 and IC 289281
Ear head length (cm)	EC 483900, EC 483542, EC 483093, EC 483091, EC 483092, EC 483095, EC 483088, EC 483089, EC 483085, EC 483094, EC 483084, EC 484509, EC 483096, EC 483083, EC 483815, EC 484180, EC 483787, EC 483090, EC 483087 and EC 483086
Ear head width (cm)	EC 484053, EC 484520, EC 484747, IC 289850, IC 289834, IC 289845, IC 289918, IC 289511, EC 483357, IC 289851, IC 289813, IC 289835, EC 484494, IC 289842, IC 289859, EC 484572, IC 289848, EC 484293, EC 484180 and IC 289831
Plant height (cm)	EC 484491, EC 484106, EC 483502, EC 483509, EC 484108, EC 483599, EC 484109, EC 484107, EC 483088, EC 484672, EC 484670, EC 483503, EC 484066, EC 484114, EC 483872, EC 484111, EC 484115, EC 483087, EC 484151 and EC 483093,
Grain yield (g/plant)	EC 483357, IC 289667, EC 483915, EC 483959, EC 484182, IC 289770, EC 484482, IC 289668, IC 289953, EC 483785, IC 289370, EC 484405, EC 483961, IC 289875, IC 289286, IC 289872, IC 289352, IC 289576, IC 289867 and EC 483441
100-Seed weight (g)	EC 483541, IC 289347, IC 289959, EC 483258, EC 484171, IC 289786, EC 483300, EC 484416, EC 484434, EC 483261, EC 483592, EC 483254, EC 483263, IC 289797, EC 483872, EC 483328, EC 484486, EC 484433, EC 483252 and EC 483267

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RESEARCH ARTICLE

Genetic Diversity, Variability and Correlation Studies in Bitter Gourd (*Momordica charantia* L.)

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Thirty one genotypes of bitter gourd (*Momordica charantia* L.) were evaluated for 10 quantitative traits during August 2016. Significant differences among bitter gourd genotypes indicated the presence of wide variation for all the traits. All the 10 traits showed significant and positive association with yield. Genotype IIHR-147-4 took the shortest time to first female flower appearance (26.57 days after planting). A close proximity in the phenotypic and genotypic coefficients of variability was observed for peduncle length, fruit length, fruit girth, number of fruits, fruit weight and fruit yield indicating little influence of environment for the expression of these traits. High heritability was recorded for peduncle length, fruit length, fruit girth and fruit weight. Number of fruits followed by fruit weight, fruit length and fruit girth exhibited maximum positive direct effect on fruit yield. Principal component analysis revealed that first three principal components (PC1, PC2 and PC3) accounted for 72.28% of the total variation with the proportionate contribution values of 40.83, 17.43, and 14.01 respectively. The genotypes were grouped in to four different clusters based on the genetic distance. The divergence value for cluster analysis indicated that the genotypes from clusters I and II had the highest inter-cluster distance and were expected to provide high heterosis if crossed and to show wide variability in genetic architecture.

Key Words: Bitter gourd, Variability, Heritability, Genetic advance, Principle component, D^2 analysis

Introduction

Bitter gourd (*Momordica charantia* L.; 2n= 2x=22.), also known as bitter melon, bitter cucumber, bitter squash, balsam pear, karela, cassilla and maiden apple (Morton, 1967), is an important fast growing warm seasonal climbing annual cucurbit vegetable grown in tropics and subtropics. It is widely cultivated in India, China, Malaysia, Africa, and South America (Minraj *et al*., 1993; Singh, 1990). It is mainly valued for its nutritional and medicinal properties. It has been used for centuries in the ancient traditional medicine of India, China, Africa, and Latin America. Bitter gourd fruits also possess anti-oxidant, anti-microbial, anti-viral, anti-diabetic activities (Welihinda *et al*., 1986; Raman and Lau, 1996). The immature fruits are used as fried, stuffed, dried and pickled (Morton, 1967). Among the cucurbits, bitter gourd is considered a prized vegetable because of its high nutritive values in respect of ascorbic acid and iron (Behera, 2004) besides, its immense medicinal values, mainly, for its hypoglycemic properties. The ripe fruits are rich in vitamin A. Fruit also contains two major alkaloids viz., momordicin and cucurbitacin; momordicin is the momordicoside glycoside of tetracyclic triterpinoides with cucurbitane skeleton (Vandana and Chandra, 1990). Lee *et al*. (1995) has identified a protein MAP-30, in both seeds and fruits of bitter gourd, which inhibits human immunodeficiency virus (HIV).

In India, the genetic analysis based on quantitative traits has been made in this crop by Mishra *et al*. (1998), Ram *et al*. (2000), Dalamu *et al*. (2012) and Resmi and Sreelathakumary (2012). Success in any plant breeding programme solely depends upon the existence of genetic variability present in the population. It is proved that larger the variability, greater is the scope for selection and improvement. It is the genotypic variability and more specifically the additive variances, which is most important for a plant breeder as, it determines the genetic gain through selection. Yield is a complex entity which is associated with a number of component characters. Before aiming at an improvement in yield, it is necessary

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to have information on genetic variability and heritability, in respect of important characters associated with yield. Genotypic and phenotypic coefficients of variation are useful in detecting the amount of variability present in the available genotypes. The main purpose of estimating heritability and the genetic parameters that compose the heritability estimate is to compare the expected gains from selection based on alternative selection strategies (Holland *et al*., 2003). The efficiency of selection depends on the direction and magnitude of association between yield and its component characters. The correlation coefficients indicate association between two characters and form a basis for selecting desirable plant type and path coefficient analysis splits the correlation coefficients into direct and indirect effects to measure the relative importance of each character. Information on character association and direct and indirect effects of component traits on yield would greatly help in formulating the selection criteria and using them effectively in crop improvement programme (Sharma and Bhutani 2001, Bhave 2003, Singh *et al*., 2008, Islam 2009).

Improvement in yield is normally attained through exploitation of the genetically diverse parents in hybrid breeding programmes. Since, the crossing programme involving genetically diverse parents is likely to produce high heterotic effects and also more variability could be expected in the segregating generations. Genetic diversity between genotypes indicates the differences in gene frequencies. For identifying such diverse parents for crossing, multivariate analysis using Mahalanobis D^2 statistic (1936) has been used in several crops. This is a valuable tool to study genetic divergence at inter varietal and sub-species level in classifying the crop plants.

Considering the availability of genetic variability, its scope of yield improvement, the present investigation was undertaken to study the character association and direct and indirect effects of component characters on yield and genetic divergence among 31 genotypes of bitter gourd to locate suitable parental groups likely to provide superior segregants on hybridization.

Materials and Methods

The experimental materials comprised of 31 genotypes of bitter gourd and advanced breeding lines maintained at Division of Vegetable Crops, ICAR- IIHR, Bengaluru. The experiment was laid out in a randomized complete block design (RCBD) with three replications at the experimental farm, Division of Vegetable Crops, ICAR- IIHR, Bengaluru, during August 2016. Seeds were sown in 98-cell plug-trays, using cocopeat as a growing medium. The seedlings were ready for transplanting 15 days after germination (two true- leaf stage) and transplanted in the main field in raised beds, covered by white polythene mulch, at spacing of 150 cm between beds and 50 cm between plants. Treatments were allotted at random in rows of each replication. All the recommended cultural practices were followed to raise a healthy crop.

Thus ten plants per genotype were maintained in each row. The fruits were harvested at marketable stage. Five plants were selected at random from each experimental plot (5 m X 1.5 m) for recording observations on 10 quantitative traits viz., days to first male flower, node to first male flower, days to first female flower, node to first female flower, peduncle length (cm), fruit length (cm), fruit girth (cm), number of fruits per plot, individual fruit weight (g) and total fruit yield per hectare (kg). Means across three replications were calculated for each character.

The data were subjected to analysis of variance according to Panse and Sukhatme (1967). The genotypic and phenotypic coefficients of variation were computed according to Burton and Devane (1953). The broad sense heritability was computed according to Falconer and Mackay (1996). Genetic advance over mean was worked out according to Johnson *et al*. (1955). Path coefficient analysis provides an effective means of partitioning correlation coefficients into unidirectional and alternative pathways thus permitting a critical examination of specific factors that produce a given correlation, which can be successfully employed in formulating an effective selection programme (Salahuddin *et al*. 2010). The genetic diversity was analyzed by Mahalanobis D^2 analysis (Mahalanobis 1936) and clustering of genotypes was done according to Tocher's method as described by Rao (1952). The programming codes were developed using statistical analysis system (SAS) package available at ICAR- IIHR, Bangalore, India (SAS V 9.3).

Results and Discussion

The analysis of variance for 10 quantitative characters exhibited significant differences for all the characters under study which indicated considerable amount of variation among the genotypes. Mean performance of the 31 genotypes for different traits studied was presented

in Table 1. Maximum variability was recorded for fruit yield per hectare followed by fruit weight, number of fruits per plot and fruit length (Table 2).

Table 1. Analysis of variance for different quantitative traits in bitter gourd

Character	Source of variation		
	Replication	Treatment	Error
Df	2	30	60
Days to first male flower	3.00	13.21**	6.93
Node to first male flower	0.72	7.61**	3.88
Days to first female flower	4.74	10.26**	7.67
Node to first female flower	0.20	10.35**	9.85
Peduncle length	19.02	29.60**	2.99
Fruit length	0.59	61.23**	4.09
Fruit girth	1.53	10.31**	1.24
Number of fruits / plot	8782.26	3365.22**	836.42
Fruit weight	461.54	2845.48**	377.41
Fruit yield	99734160	30789504**	7055594

** Significant at P<0.01 level

The higher degree of variation was observed in phenotypic and genotypic variance among the characters studied. Maximum variation was observed by fruit weight followed by fruit yield per hectare. Low variance was observed for days to first male flower and days to first female flower. Characters such as peduncle length, fruit length, fruit girth showed narrow differences between the values of GCV and PCV (Table 2) indicating variability due to genetic constitution indicating better scope of selection through these traits for improvement. High heritability was recorded for peduncle length, fruit length, fruit girth and fruit weight indicating low environmental influence for the expression of these traits (Table 2). The rest of the traits showed low to moderate level of heritability. The higher estimates of heritability coupled with higher genetic advance observed for fruit weight, whereas moderate heritability with high genetic advance for number of fruits per plot indicated that heritability of these traits is mainly owing to additive effects.

The genotypic correlation were higher than their corresponding phenotypic correlation for all the traits studied suggesting strong inherent association between these traits at genotypic level (Table 3), which was in agreement with the results obtained by Srivastva and Srivastva (1976), Singh *et al*. (1977), Indresh (1982), Lawande and Patil (1989), Panthi *et al*. (1995) and Singh *et al*. (2013). Yield per hectare showed significant and positive association with all the 10 traits studied. Fruit weight has significant and positive correlation with days to first male flower, node to first male flower, days to first female flower, node to first female flower, peduncle length, fruit length and fruit girth and significant negative correlation with number of fruits per plot. Fruit girth had a positive significant correlation with days to first female flower, node to first female flower, peduncle length. Fruit length showed significant positive association with node to first male flower, days to first female flower, node to first female flower, peduncle length.

It was observed from path analysis that number of fruit followed by fruit weight, fruit length and fruit girth exhibited maximum positive direct effect (Table 4). Node to first female flower exhibited negative direct effect on fruit yield and its positive and significant association with fruit yield was due to its high indirect positive effect through days to first female flowering. Islam *et al*. (2009), Sundaram (2010), Sharma and Bhutani (2001) Dalamu and Behera (2013) also reported maximum direct contribution of number of fruits per plant in bitter gourd. Through present path analysis study, it may be concluded that improvement in yield could be bought by selection for component character like number of fruits followed by fruit weight, fruit length and fruit girth.

The residual factor determines how best the causal factors account for the variability of the dependent factor. The residue obtained was 0.214 indicating that selected characters contributed the remaining 79 percent. Similar observations were made by earlier workers (Mahesh *et al*., 2014).

Principle component analysis and clustering of genotypes help in selection of suitable genotypes to be used in breeding for improvement in desired characters (Cheema *et al*., 2011). Eigen values of 10 principal component axes and percentage of variation accounting for them obtained from the principal component analysis revealed that the first axis largely accounted for the variation among the genotypes (40.83 %) (Table 5). Two dimensional ordinations of 31 bitter gourd genotypes on PC axis 1 and 2 revealed scattered diagram of genotypic distribution pattern on axis. Three principle components had eigen values > 1 and explained most of the total variation with PC1, PC2 and PC3 contributions and accounts for 72.28 per cent of total variation amongst bitter gourd genotypes. This finding was in agreement with that of Kundu *et al*. (2012) and Singh *et al*. (2014) in bitter gourd. Moreover, the first

6 principal components contributed 90.57 per cent of the total variation with proportionate contribution values of 40.83, 17.43, 14.01, 7.71, 5.56 and 5.00 per cent respectively. The first PC has positive association with all the 10 traits. The second PC has positive association with days to first male flower, node to first male flower, number of fruits and fruit yield per plot, while negative association with days to first female flower, node to first female flower, peduncle length, fruit length, fruit girth and fruit weight. The third PC has positive association with days to first male flower, node to first male flower, days to first female flower, node to first female flower, while negative association with peduncle length, fruit length, fruit girth, number of fruits per plot and yield

Table. 2. Mean performance of 31 bitter gourd genotypes for yield and yield attributes

Sl. No.	Genotype	Days to first male flower	Node to first male flower	Days to first female flower	Node to first female flower	Peduncle length (cm)	Fruit length (cm)	Fruit girth (cm)	Number of fruits / plot	Individ-ual fruit weight (g)	Fruit yield (Kg/ Ha)
1	Arka Harit	29.71	6.43	29.86	10.75	7.25	8.19	9.99	37.00	58.08	2040
2	IIHR-144-1	32.58	9.31	30.25	11.2	3.56	5.93	7.64	137.33	11.56	5560
3	IIHR-80-1-2	33.00	8.00	33.00	10.00	1.12	4.64	7.78	79.33	6.48	2453.33
4	Pusa Vishesh	25.68	5.35	29.67	12.00	7.50	15.67	12.08	51.33	67.78	5453.33
5	Pusa Do Mausami -5	28.67	7.13	30.85	12.83	9.44	18.30	11.46	104.00	69.60	9533.33
6	Phule Ujwala	29.32	8.78	31.58	12.73	11.61	15.50	12.40	91.67	95.98	8546.66
7	IIHR -30-4	30.81	10.56	31.85	12.83	8.63	11.40	14.60	105.67	97.00	11160
8	IIHR -49-34-5	28.26	7.53	30.64	12.36	11.50	13.80	12.25	169.67	47.40	8613.33
9	IIHR -46-1	31.11	9.50	30.4	13.00	10.40	16.20	12.74	75.67	89.34	10106.67
10	NDBI-09-7	31.29	9.57	31.00	13.00	10.00	16.10	12.7	60.00	73.575	6480
11	NDBT-7-9	26.67	6.78	29.63	17.00	4.58	16.58	11.02	87.33	67.92	8853.33
12	CO-1-2	31.25	7.00	32.4	14.25	9.56	17.20	12.34	59.67	132.00	7266.66
13	IIHR -38-2	31.10	9.15	30.11	12.22	13.64	19.50	14.66	90.00	62.76	9960
14	IIHR -151-2	29.52	6.94	30.54	11.36	7.75	10.37	11.45	65.00	36.60	4053.33
15	IIHR -40-1	31.70	10.50	35.29	14.00	11.20	16.20	12.46	112.33	83.22	13786.67
16	IIHR -148-7	37.33	12.00	31.65	15.08	12.58	16.28	13.78	153.33	80.48	13826.67
17	IIHR -101-1-7	28.67	8.84	33.14	14.62	11.90	9.10	11.30	61.33	34.62	3373.33
18	IIHR -17-1	29.8	10.50	30	15.8	10.53	12.00	12.56	56.00	91.30	5760
19	IIHR -8-2	31.46	10.15	30.67	15.75	11.92	12.10	11.32	116.33	108.48	10786.67
20	IIHR -86-11-6	29.35	8.59	30.62	14.14	11.40	10.30	12.96	107.67	30.375	6066.66
21	IIHR -147-4	29.89	8.11	26.57	9.89	8.85	8.24	12.84	172.33	27.28	9093.33
22	IIHR -12-6	29.61	9.33	29.4	12.60	13.14	20.14	10.79	132.67	44.16	10760
23	IIHR -80-1-3	27.33	9.50	26.33	11.00	1.60	4.57	8.30	83.00	5.22	1306.66
24	Preethi-1	29.53	8.83	28.91	11.4	8.83	17.50	11.68	122.67	81.00	10360
25	IIHR- 44-2	31.44	8.41	33.25	14.71	9.06	15.89	14.55	118.00	89.82	12093.33
26	NABG-1-5	30.78	9.00	32.00	14.14	8.50	14.14	12.28	85.00	40.86	7293.33
27	IIHR- Sel-5-8	28.62	6.26	28.79	11.33	8.31	11.67	13.09	88.67	61.26	6200
28	IIHR- 145	30.90	10.53	33.20	13.20	11.38	23.50	11.62	74.33	104.58	7093.33
29	Hirkani- 6	28.85	6.06	30.6	10.64	6.80	11.10	13.80	80.67	65.48	6213.33
30	Meghana- 1	31.29	8.12	31.00	11.29	11.2	14.40	14.30	107.00	55.48	9106.66
31	IIHR -14-4	29.89	7.88	28.94	9.86	5.75	12.50	14.38	89.33	70.12	7720
	Mean	30.13	8.49	30.74	12.72	9.01	13.55	12.11	95.94	64.17	7771.71
	CD 5 %	4.21	3.15	4.43	5.02	2.76	3.23	1.78	46.28	31.08	4250.86
	SEm ±	0.07	0.05	0.07	0.09	0.04	0.05	0.03	0.83	0.56	76.67

Table 3. Estimate of genetic parameters for morphological traits in bitter gourd

Character	Range	CV	PVAR	GVAR	PCV	GCV	h^2	GA	GAM
Days to first male flower	25.68 - 37.33	8.739	9.027	2.093	9.972	4.803	23.195	1.437	4.771
Node to first male flower	5.35 – 12.00	23.209	5.131	1.244	26.666	13.130	24.246	1.131	13.338
Days to first female flower	26.33 - 35.29	9.011	8.536	0.861	9.504	3.019	10.093	0.608	1.979
Node to first female flower	9.86 – 17.00	24.685	10.025	0.165	24.892	3.202	1.655	0.108	0.849
Peduncle length (cm)	1.12 - 13.64	19.203	11.863	8.870	38.230	33.057	74.768	5.312	58.968
Fruit length (cm)	4.57 - 23.50	14.932	23.143	19.047	35.494	32.200	82.302	8.168	60.264
fruit girth (cm)	7.64 - 14.66	9.201	4.268	3.025	17.049	14.353	70.874	3.020	24.927
Number of fruits	37 - 172.33	30.142	1679.358	842.933	42.711	30.260	50.194	42.373	44.227
Fruit weight (g)	5.22 – 132.00	30.271	1200.103	822.688	53.979	44.693	68.552	48.921	76.338
Fruit yield (Kg/ Ha)	1306.667 - 13826.67	34.178	14966897	7911303	49.779	36.192	52.859	4218.72	54.283

CV: coefficient of variation, PVR: phenotypic variance, GVR: genotypic variance, PCV: Phenotypic coefficient of variation, GCV: genotypic coefficient of variation, h^2 heritability (broad sense), GA: genetic advance, GAM: genetic advance over mean

Table 4. Correlation coefficient at genotypic (above diagonal) and phenotypic (below diagonal) level in bitter gourd genotypes

	Days to first male flower	Node to first male flower	Days to first female flower	Node to first female flower	Peduncle length (cm)	Fruit length (cm)	Fruit girth (cm)	Number of fruits / plot	Individual fruit weight (g)	Fruit yield (Kg/ Ha)
Days to first male flower	0.000	1.162^{**}	1.107^{**}	0.741^{**}	0.246^{*}	0.115^{NS}	0.050^{NS}	0.530^{**}	0.136^{NS}	0.782^{**}
Node to first male flower	0.398^{**}	0.000	1.038^{**}	2.483^{**}	0.464^{**}	0.227^{*}	-0.003^{NS}	0.447^{**}	0.253^{*}	0.668^{**}
Days to first female flower	0.250^{*}	0.086^{NS}	0.000	2.403^{**}	0.736^{**}	0.682^{**}	0.332^{**}	-0.411^{**}	0.788^{**}	0.475^{**}
Node to first female flower	0.062^{NS}	0.171^{NS}	0.279^{**}	0.000	1.810^{**}	1.927^{**}	0.830^{**}	-0.265^{*}	2.831^{**}	2.046^{**}
Peduncle length (cm)	0.179^{NS}	0.276^{**}	0.156^{NS}	0.249^{*}	0.000	0.696^{**}	0.661^{**}	0.210^{*}	0.522^{**}	0.652^{**}
Fruit length (cm)	0.017^{NS}	0.147^{NS}	0.158^{NS}	0.191^{NS}	0.556^{**}	0.000	0.515^{**}	0.050^{NS}	0.693^{**}	0.729^{**}
fruit girth (cm)	0.096^{NS}	0.074^{NS}	0.097^{NS}	0.050^{NS}	0.463^{**}	0.371^{**}	0.000	0.095^{NS}	0.580^{**}	0.642^{**}
Number of fruits	0.151^{NS}	0.165^{NS}	0.003^{NS}	-0.018^{NS}	0.190^{NS}	0.030^{NS}	0.104^{NS}	0.000	-0.228^{*}	0.529^{**}
Fruit weight (g)	0.167^{NS}	0.165^{NS}	0.193^{NS}	0.127^{NS}	0.425^{**}	0.566^{**}	0.416^{**}	-0.108^{NS}	0.000	0.638^{**}
Fruit yield (Kg/ Ha)	0.136^{NS}	0.259^{*}	0.239^{*}	0.157^{NS}	0.470^{**}	0.496^{**}	0.453^{**}	0.656^{**}	0.410^{**}	0.000

Table 5. Path coefficient analysis of yield contributing characters in bitter gourd genotypes

	Days to first male flower	Node to first male flower	Days to first female flower	Node to first female flower	Peduncle length (cm)	Fruit length (cm)	Fruit girth (cm)	Number of fruits / plot	Individ-ual fruit weight (g)	Fruit yield (Kg/ Ha)
Days to first male flower	**0.109**	0.0265	0.2820	-0.0241	-0.076	0.0390	0.016	0.354	0.056	0.782^{**}
Node to first male flower	0.126	**0.023**	0.265	-0.081	-0.143	0.077	-0.009	0.298	0.103	0.668^{**}
Days to first female flower	0.120	0.024	**0.255**	-0.079	-0.227	0.232	0.102	-0.274	0.321	0.475^{**}
Node to first female flower	0.081	0.057	0.613	**-0.033**	-0.557	0.655	0.256	-0.177	1.152	2.046^{**}
Peduncle length (cm)	0.028	0.011	0.188	-0.059	**-0.307**	0.237	0.204	0.140	0.213	0.652^{**}
Fruit length (cm)	0.013	0.005	0.174	-0.063	-0.214	**0.340**	0.159	0.034	0.282	0.729^{**}
fruit girth (cm)	0.005	-0.001	0.085	-0.028	-0.204	0.176	**0.308**	0.063	0.236	0.642^{**}
Number of fruits	0.058	0.010	-0.105	0.009	-0.065	0.018	0.029	**0.668**	-0.097	0.529^{**}
Fruit weight (g)	0.015	0.006	0.201	-0.092	-0.065	0.236	0.179	-0.152	**0.407**	0.638^{**}

per hectare. Similar findings were also reported by Singh *et al.* (2014) in bitter gourd. The fourth PC had positive association with days to first male flower, days to first female flower, fruit girth and fruit weight, while negative association with node to first male flower, node to first female flower, peduncle length, fruit length, number of fruits per plot and fruit yield. The fifth component has positive association with days to first female flower, node to first female flower, fruit length, number of fruits per plot and fruit yield, while negative association with days to first male flower, node to first male flower, peduncle length, fruit girth and fruit weight. The sixth PC has positive association with days to first female flower, node to first female flower, peduncle length, fruit girth and number of fruits per plot, while negative association with days to first male flower, node to first male flower, fruit length, fruit weight and fruit yield. The traits having positive association with PCs have major role in genetic diversity analysis and explaining total genetic variation in bitter gourd and which is in agreement with findings of Kundu *et al.* (2012) and Singh *et al.* (2014).

Cluster analysis helps group individuals with the same description (Hair *et al.* (1995). Genotypes in a cluster exhibit a high degree of homogeneity (Cheema *et al.,* 2011). Cluster analysis grouped 31 genotypes into four clusters (Table 6). Cluster 1 had six genotypes, cluster 2 had five genotypes, cluster 3 had three genotypes, and cluster 4 had 17 genotypes accommodating more than 50 per cent of total genotypes. Average inter-cluster distance was found maximum (10590.56) between cluster I and cluster II followed by cluster I and cluster III (7094.97) (Table 7). Therefore, hybridization between the genotypes from cluster I and cluster II and cluster I and cluster III are likely to be fruitful for developing divergent heterotic cross combinations which may be potentially exploited in bitter gourd breeding programmes. Cluster IV exhibited highest cluster mean values for most of the traits viz., days to first male flower, node to first male flower, days to first female flower, node to first female flower, peduncle length, fruit girth, fruit weight and fruit yield per hectare (Table 8). Cluster II represented highest cluster mean value for fruit length and number of fruits per plot. Lowest mean value for days to first female flower was observed in cluster II whereas the least cluster mean for node to first female flower was recorded in cluster III.

Table 6. Principle component analysis of various traits in bitter gourd

Parameter	PC 1	PC 2	PC 3	PC 4
Eigen value (Root)	4.08	1.74	1.40	0.77
Percent	40.83	17.43	14.01	7.71
Cumulative percent	40.83	58.27	72.28	80.00
Traits		Eigen vectors		
Days to first male flower	0.23	0.49	0.27	0.43
Node to first male flower	0.27	0.44	0.24	-0.22
Days to first female flower	0.25	-0.01	0.53	0.31
Node to first female flower	0.29	-0.12	0.32	-0.65
Peduncle length (cm)	0.39	-0.08	-0.14	-0.07
Fruit length (cm)	0.36	-0.28	-0.12	-0.08
Fruit girth (cm)	0.31	-0.21	-0.34	0.41
Number of fruits	0.13	0.52	-0.47	-0.18
Fruit weight (g)	0.36	-0.33	0.08	0.11
Fruit yield (Kg/ Ha)	0.42	0.15	-0.28	-0.02

Table 7. Clustering pattern of bitter gourd genotypes

Cluster	Number of genotypes	Genotypes
Cluster I	6	Arka Harit, IIHR- 151-2, Pusa Vishesh, IIHR Sel 5-8, Hirkani- 6, IIHR- 14-4
Cluster II	5	PDM- 5, Preeti- 1, IIHR- 12-6, IIHR- 49-34-5, IIHR- 147-4
Cluster III	3	IIHR- 144-1, IIHR-80-1-2, IIHR-80-1-3
Cluster IV	17	Phule Ujala, IIHR- 46-1, NDBI- 9-7, IIHR-38-2, Megana-1, Co-1-2, IIHR-145, IIHR- 17-1, IIHR-8, NDBT- 7-9, IIHR- 101- 1- 7, IIHR- 86- 11-6, NABG-1-5, IIHR- MC- 84-4, Preeti (rkvy), IIHR- 40-1, IIHR- 148-7

Table 8. Average Intra- (diagonal) and inter-cluster distances (D^2) for studied traits in bitter gourd genotypes

Cluster	1	2	3	4
1	0.00			
2	10590.56	0.00		
3	7094.97	3495.61	0.00	
4	3819.15	6771.49	3275.93	0.00

The knowledge of the characters contributing to divergence is an important factor and this study helps in identifying the diversity in different proportion which ultimately helps in deciding the utilization of genetic material for the improvement of specific character (Table

Table 9. Cluster means for different characters in bitter gourd

Cluster	Days to first male flower	Node to first male flower	Days to first female flower	Node to first female flower	Peduncle length (cm)	Fruit length (cm)	Fruit girth (cm)	Number of fruits / plot	Individual fruit weight (g)	Fruit yield (Kg/ Ha)
Cluster I	28.71	6.48	29.73	10.99	7.22	11.58	12.46	68.66	59.88	5280
Cluster II	29.19	8.18	29.27	11.81	10.35	15.59	11.80	140.26	53.88	9672
Cluster III	30.97	8.93	29.86	10.73	2.09	5.04	7.90	99.88	7.75	3106.66
Cluster IV	31.21	9.38	31.54	14.09	10.54	14.65	12.93	96.49	77.27	9311.37

Table 10. Relative contribution of various traits towards genetic divergence in bitter gourd genotypes

Sl. No.	Character	Percent contribution
1	Days to first male flower	10 %
2	Node to first male flower	9 %
3	Days to first female flower	18 %
4	Node to first female flower	12%
5	Peduncle length	9 %
6	Fruit length	6 %
7	fruit girth	12 %
8	Number of fruits	10 %
9	Fruit weight	10 %
10	Fruit yield	4 %

9). The maximum contribution to genetic divergence was by days to first female flower followed by node to first female flower, fruit girth, fruit weight and days to first male flower. Therefore necessary attention is required to be focused on these characters. In contrary to this result, Singh *et al.* (2014) has reported maximum contribution of fruit weight and fruit length to the diversity of bitter gourd. There is always difference of opinion in specifying the trait that is contributing high or low towards the genetic diversity. The contribution mainly depends upon the genotypes included in the study and the environmental influence over the character.

For future experiment, traits contributing maximum to genetic diversity should be given priority as selection parameters and the diverse genetic material of the present study may be utilized for attempting heterotic cross combination and developing hybrid varieties for Comment [DRK13]: Not required. May be deleted. improvement of bitter gourd yield. Based on the findings of present study, it is concluded that for selection of superior genotypes, primary emphasis should be given on fruit length, fruit width, fruit weight and number of fruits. Hybridization between the genotypes from cluster I and cluster II and cluster I and cluster III and selection in segregating population, based on fruit length, fruit width and fruit weight for yield components, should result in improvement of fruit yield and quality of bitter gourd.

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RESEARCH ARTICLE

African Bitter Leaf [*Vernonia amygdalina* Delile]: Study on Seasonal Variations in Total Phenols and Seed Germination in India

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Owing to reported medicinal value and seasonal variations in leaf bitterness, African bitter leaf (*Vernonia amygdalena* Delile) was studied for total phenol contents, as an indicator for accumulation of secondary metabolites. The total phenol content in the leaves were found variable [range from $0.158^{a}\pm0.01$ to $0.675^{f}\pm0.02$] in different months. Organoleptic assessment was done to correlate the results to assess leaf bitterness during different months of the consucutive year. Besides, seed germination study was also undertaken to record data on the viability of seeds for conservation in NGB.

Key Words: African bitter leaf, India, Leafy vegetable, Medicinal plant, Seed germination, *Vernonia amygdalina* Delile

Introduction

African bitter leaf or bitter leaf plant [*Vernonia amygdalina* Delile, *Gymnanthemum amygdalinum* (Delile) Sch. Bip. ex Walp.], a native to tropical Africa is a perennial plant shrub/tree of height between 1-6 m with dark green leaves and rough bark, this species has been cultivated in many parts of West Africa (Igile *et al*., 1994). *Vernonia amygdalina* Delile is a species under the genus *Vernonia* Schreb (Family Asteraceae) which has over 1,000 species (Swee Keong Yeap *et al*., 2010). Plant grows near water bodies, in forests margins, woodland and grassland up to 2800m altitude, in areas with mean annual rainfall 750-2000mm (Nwosu *et al*., 2013). Humid environment is more suitable for its growth (Ndaeyo, 2007). Ability of plant to thrive on wide ecological range and all types of soil has led it to perform better in adverse climate.

African bitter leaf plant produces large mass of forage for medicinal and vegetable use (Bonsi *et al*., 1995; Smith and Eyzaguirre, 2007). The species is widely cultivated in Yemen and Ethiopia, South Uganda, Kenya, Tanzania and Brazil (Robinson, 1999). Variations in plant growth, leaf size with degree of bitterness (less or more bitter types) are reported in areas of native regions. Leaves from less watered plant are bitter as compared to those under irrigated condition.

Literature review facilitated in identification of the neutraceutical potential. In view of its hardy nature and success of planting in extreme hot and cold condition in Delhi, it deserved to be assessed for health value.

It is a proven herbal medicine used in polyherbal therapy (combination of herbs or phytochemicals from several sources) to cure or manage many diseases (Ebong *et al*., 2008; Atangwho *et al*., 2011). The leaves are commonly used as a treatment against nematodes in humans and chimpanzees as well as for other intestinal worms (Huffman and Seifu, 1989). It is a well known traditional anti-diabetic plant known in Africa (Akah and Okafor, 1992; Atangwho *et al*., 2011).

In India this species may be a recent introduction from its native region. It is reported under sporadic cultivation in Bihar, Madhya Pradesh, Odisha and West Bengal for medicinal uses (Kumar and Verma, 2012; Bhattacharjee *et al*., 2013). This species has little known medicinal value and popularity in India (Pandey *et al*., 2014). Earlier publications have no mention of the species cultivation or its reported use from India (Ambasta *et al*., 1986; Rao *et al*., 1988; Uniyal, 1995; Karthikeyan *et al*., 2009). In view of its multiple uses as vegetable and in disease management, study was carried out to evaluate total phenols with respect to seasonal variation. Organoleptic assessment was also carried out to correlate

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the phenol contents in different seasons. Besides one of the thrust issues on propagation by seed was studied using seeds set under Delhi conditions with the objective to find out multiplication by seeds to facilitate conservation in National Gene Bank at ICAR-NBPGR, New Delhi.

Materials and Methods

Study was carried out during 2014-18 at ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR), New Delhi. Ten mature leaf samples of African bitter leaf tree (third leaf from fifth node at tip) of 0-5 year's age, planted in the experimental garden, were harvested in the first week of each month. The leaves were cleaned using distilled water, surface water was removed by gently tapping on filter paper sheet and the samples were immediately extracted in 80% ethanol for estimating total phenols using folin ciocalteu reagent as mentioned in Arivalagan *et al.* (2018). For data on organoleptic use and medicinal value of the species was gathered from 10 respondents from Delhi and Bihar who were using the leaves for control of diabetes. Leaves of the plant were provides to 10 persons of different age in different months and intensity of bitterness was recorded in numeric scale.

For seed germination study, fully matured flowers were harvested during February-March from three year old plants. The filled seeds were separated using floatation test and were subjected to germination studies. Two replications of 50 seeds each were plated in petriplates with Whatman filter paper soaked in 5 ml of distilled water. The petriplates were kept in germinator at 25°C±2 in dark. In control after 21 days, no germination was observed and therefore seeds were subjected to the following treatments: 1) Co-application of Ethrel (100%); and 2) Presoaking in GA_3 (1000 ppm, 500 ppm, 250 ppm and 100 ppm) for 24 hours, then seeds were washed. After the treatment, 50 seeds in two replications were placed in petriplates at 25°C under dark conditions. Observations were taken on every alternate day for a period of 30 days. Statistical analysis for total phenol content and organoleptic value was done using *Duncan's multiple range Test* (DMRT) to ascertaining significant differences during study period.

Results and Discussion

Evaluation of total phenols

Leaves of *Vernonia amygdalina* have very bitter and astringent taste, which affects its intake when used as medicine or vegetable, etc. (Bonsi *et al*., 1995a). The phytochemical studies revealed the presence of saponins, flavonoids, alkaloids, terpenes, steroids, coumarins, phenolic acids, lignins, xanthones, anthraquinones, edotides and sesquiterpenes (Owoeye *et al*., 2010).

In this study plants of age above 2-3 years showed best phenol contents and there was no significant variation observed in leaves harvested from plants of different age groups. Total phenol content increased with high temperature and low relative humidity (RH) was evident from highly significant negative correlation (-0.716) at error confidence of 1%. Negative correlation was observed between bitters and high temperature with low RH. The phenol contents were assessed using organoleptic test involving 10 respondents in different seasons. Organoleptic value was minimum level in the month of November (2.875^{a}±0.83) and was maximum in the month of April (8.125^{e}±0.83) which is also associated to total phenol contents.

Table 1. Variation in organoleptic value and total phenol contents in different months

Month	Total Phenol (%)	Organoleptic value
January	0.289^{bc}±0.04	4.00^{b} ±0.75
February	0.253^{ab}±0.01	4.00^{b}±0.75
March	0.378^{cd}±0.03	5.00^{c}±0.75
April	0.675^{f}±0.02	8.125^{e}±0.83
May	0.564^{e}±0.05	7.75^{e}±1.03
June	0.384^{cd}±0.10	5.5^{cd}±0.92
July	0.300^{bc}±0.06	5.5^{cd}±0.92
August	0.475^{de}±0.11	6.375^{d}±0.74
September	0.278^{bc}±0.02	4.00^{b}±0.75
October	0.264^{b}±0.06	4.125^{b}±0.83
November	0.158^{a}±0.01	2.875^{a}±0.83
December	0.358^{bc}±0.03	3.625^{ab}±1.06

Correlation is significant at the 0.01 level (2-tailed)

A periodic cyclic increase and decrease in phenol content was observed. There was high phenol content in April (0.675^{f}±0.02) and May (0.564^{e}±0.05) when temperature was high, and rises in August, thereafter it showed continuous decline up to November and then the increased in December and January when temperatures are very low with corresponding values of August 2016-17. Leaves from 0.5-1 year old plants had high phenol contents during the month of May to August in comparison to other age group plants. Similarly 4-5 years old plants had high phenol contents from September to December. Similarly 2-3 years old plants had high phenol contents in the month of January, March and April. Overall results revealed that total phenol contents

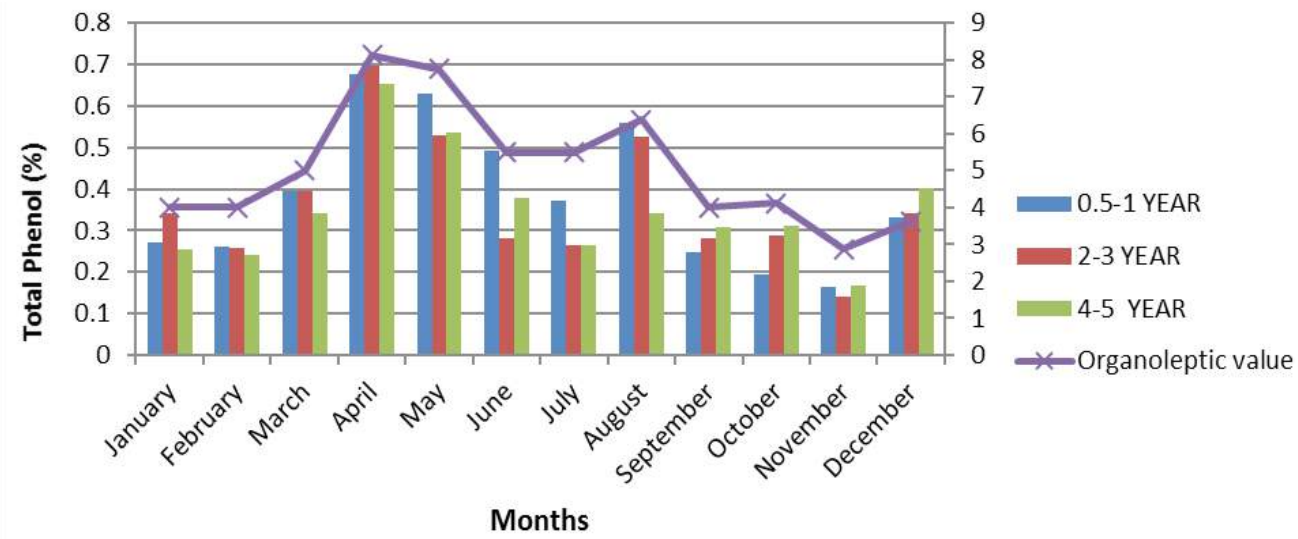

Fig. 1. Data on periodic variation in total phenol contents in plants of different age (2016 and 2017) on primary vertical axis and trends of bitterness in leaf of African bitter leaf based on organoleptic assessment (2016 and 2017) on secondary vertical axis

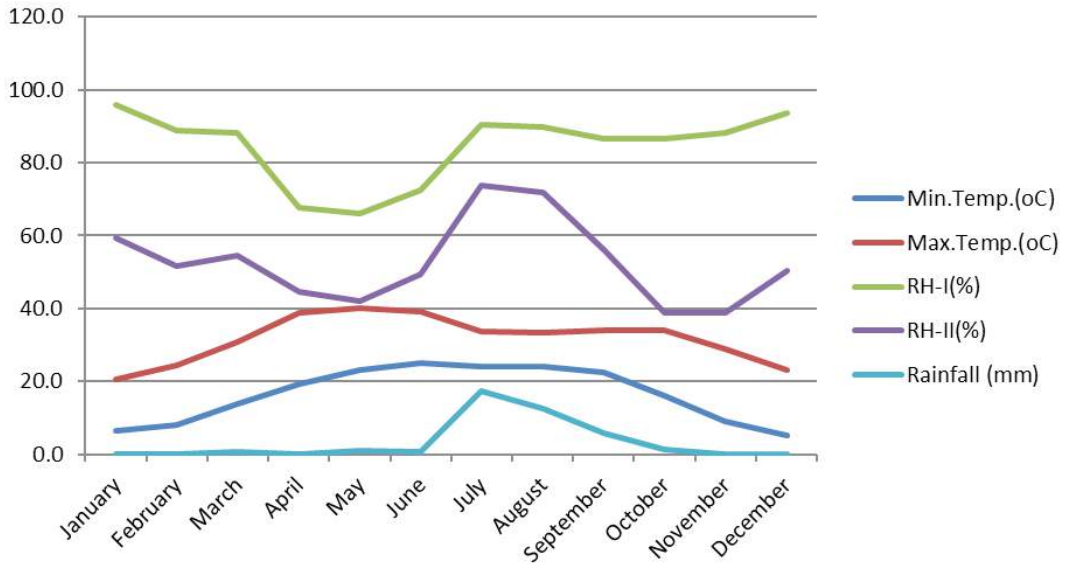

Fig. 2. Variation in climatic variables (rainfall, relative humidity and temperature) recorded during experimentation in Delhi (2016 and 2017)

were highest up to age of 2-3 years, later the phenol contents were decreased, indicating the efficacy of the leaves from plants under cultivation will be only up to three years.

Seed Germination

In planted cuttings in the experimental plots, flowering was reported after three years of age in Delhi. Seeds were harvested for seed germination study to check viability through seed floatation test. Two third of the seeds were found unfilled that was also confirmed through examining under the microscope and found poor or no germination. More routinely *Vernonia amygdalina* in its native area is propagated through stem cuttings although seed propagation is poorly known (Pandey *et al*., 2014). (Anonymous, 2000; http://www.fao.org/livestock/agap/frg/Visit/Ida/Vernonia%20amygdalina.htm accessed on 28-06-18). Pandey *et al*. (2014) have also confirmed that mature seed from dry flower heads raised in nursery in Delhi conditions showed poor seedling emergence.

Fig. 3. Propagation by vegetative cuttings at ICAR-NBPGR, New Delhi; flowering twig; seeds harvested from mature flowers

Its use as vegetable can be explored by selecting and introducing suitable types from native area. In dry months, 2-3 years old plants are best for medicinal use due to high phenol contents; for vegetable use high irrigation is needed to make it suitable for consumption as leafy vegetable. In Africa in highly irrigated conditions the bitters were found low which is in concurrence with our study undertaken in Delhi (Fig. 1).

Conclusions

Vernonia amygdalina has multiple economic uses, mainly as vegetable and for medicinal value (especially for anti-diabetic property) and therefore, deserves for further research in exploring its potential in Indian condition. Study highlights the preliminary work on analysis of total phenols during growth period. Maximum total phenols and organoleptic value was high in the month of April which is suitable for medicinal purpose. Similarly minimum value was recorded in the month of November which indicates lower bitterness and suitable for fresh leafy vegetable or dried powder for future use.

The following thrust areas were identified:

- Selection of types having suitability as vegetable or nutraceutical value.
- Developing agri-horticultural practices for best growth.
- Study on detailed phytochemical profile under Indian conditions and standardization of protocol for different use.
- Develop linkages for medicinal and health use with clinical trials.

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RESEARCH ARTICLE

Evaluation of Selected Indian Wheat Cultivars for Identification of Tolerant Lines under Terminal Heat Stress

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In the present study, 102 Indian wheat cultivars were evaluated against terminal heat tolerance under normal and late sowing conditions at the ICAR-NBPGR Farm, New Delhi for two consecutive years in augmented block design. Observations were recorded for ten characters including phenological, yield and yield component traits. Genotype, season, and genotype by season interaction on grain yield and other traits were highly significant. Six genotypes, IC75240(C306); IC75215(PBW34); IC252632(HD2687); IC303070(HD2781); IC75226(WG377); IC75191(DL153-2) were common among the 30 top yielding genotypes across the environments. The Relative Heat Tolerance (RHT) ranged from 0.09% in genotype RAJ3765 (38) to -57.19% in genotype RAJ2184(70). Grain yield was positively correlated with days to spike emergence, days to physiological maturity, biological yield, tillers per plant, grains per spike and harvest index under normal sown condition, whereas it was associated additionally with thousand grain weight under late sown condition. The sufficient genetic variability recorded in the cultivars warrants for improving heat tolerance using combination breeding among these lines and further search for novel variation in untapped genepool.

Key Words: Cultivar, Grain yield, Heat tolerance, Terminal heat, Wheat

Introduction

Wheat (*Triticum aestivum* L.) is one of the most important staple food crops of the world, occupying 17% of crop acreage worldwide, feeding about 40% of the world population and providing 20% of total food calories and protein in human nutrition (Gupta *et al.,* 2008). India has witnessed a significant increase in total food grain production to the tune of 277.49 m tones with a major contribution of wheat 98.61 MT and productivity of 3172 kg/ha (2017). On the other hand, India is also the second largest wheat consumer after China. Global reports indicated a loss of 10-40% in crop production by 21st century because of the impact of climate change (Dutta *et al.,* 2013). Continual heat stress is a problem in about 7 m ha while terminal heat stress is a problem in about 40% of the irrigated wheat growing areas of the world (Joshi *et al.,* 2007). High temperature during grain filling stage in wheat is a major constraint and reduces the number of grains per ear, grain weight and subsequently the harvest index, resulting in reduced grain yield (Bansal *et al.,* 2013). The wheat crop in the northern plains are exposed to higher ambient temperatures at the time of grain filling, which significantly reduces the productivity. Modern wheat varieties are well adapted to controlled cultural practices, but they are generally not highly tolerant to extreme environmental stresses such as high temperature (Morgunov,1994). Heat tolerance thus should be essential characteristic of wheat cultivars to be developed. In general, photosynthetic rate is maximum at 20-22^{o}C and decrease abruptly at 30-32^{o}C. Heat stress injuries of the photosynthetic apparatus during reproductive growth of wheat diminish source activity and sink capacity which results in reduced productivity.

Late planting of wheat in India is common due to the intensive cropping system, which often delays the sowing of the wheat crop up to the middle of January, particularly in North East India where it is generally sown after harvest of paddy. As a result, a portion of maturity period of the crop is pushed forward and thus has to face higher temperature of the summer with hot spells often occurring at the time of maturity (Abrol *et al.,*1991). Wheat is a sink limited crop and high temperature during grain filling causes the production

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of shrivelled grains due to forced maturity (Savin and Slafer, 1991). Late sown crop gets exposed to mean maximum temperature of about 35°C during grain growth and causes yield reduction of 270 kg ha^{-1} per degree rise in temperature (Rane *et al.,* 2000). Higher temperatures affect all phases of crop growth, accelerate floral initiation, reduce the period of spike development, resulting in shorter spike with lower number of spikelet and adversely affecting pollen development. The duration of grain growth in the post-anthesis period is considered the most significant determinant of yield in wheat (Evans *et al.,* 1975). Both the day and night temperatures have a pronounced effect on the duration of grain filling. It could extend to over 80 days at 15 to 10 °C day/night temperature and is reduced to less than 40 days at 30/25°C. Higher temperatures further associated with limitation of water cause rapid shrinkage of grain volume. The knowledge from the germplasm evaluation will be of great significance for selection of heat tolerant genotypes and also for improving grain yield under high temperature. Keeping in view the above points an experiment was conducted to evaluate the yield potential of wheat cultivars under terminal heat stress.

Materials and Methods

The experiment was conducted at ICAR-NBPGR, Farm IARI, New Delhi during 2010-2011 and 2011-2012 with 102 wheat accessions (Indian cultivars) alongwith three checks (C306, HDR77 and WR544) in augmented block design under natural field conditions. The detail of the material is given in Table 1. The crop was sown on 20th November (normal sown) and 20th December (late sown) in both the years. Each accession was sown in three rows of 2 m row length and row to row distance was 25 cm. Recommended fertilizer dose (120 kg N: 60 kg P_2O_5: 40 kg K_2O in per hector area) were applied in field. Standard agronomic practices were followed for raising the crop. Observations were recorded for different phenophases, yield and yield attributes under normal and late sown conditions. The relative heat tolerance was calculated by the formula $(Yh - Yc)/Yc) \times 100\%$ as described by Haque *et al.* (2009), where *Yh* is grain yield in the heat stressed condition (late season), *Yc* is grain yield in the control (normal season). Augmented design analysis was performed using SAS 9.3 software and Pearson's correlation coefficients of the traits were obtained using SPSS version 16 software (SPSS, Chicago, Illinois, USA).

Results and Discussion

Effects of genotype, stress condition, year and their interaction

The effect of genotype was highly significant for all the traits except for biomass, spike length and grains per spike (Table 2). The effect of sowing condition was consistently highly significant for all the traits, whereas year was not significant for plant height. The effects (mean squares) of genotype and stress condition interaction and genotype × year × sowing condition were significant for days to physiological maturity and 1000 grain weight whereas genotype and year interaction was significant for biomass, tillers per plant and 1000 grain weight. Significant effects of genotype, environment and genotype × environment obtained are similar to the findings of Tadesse *et al.* (2012), Degewione *et al.* (2013) and Mohamed (2013). The effect of sowing condition (SC) was consistently much greater than its corresponding effects due to genotype, year and other interaction on each of the traits which revealed that most of the observed variations are mainly due to environmental effects, in this case high temperature during grain filling. Moreover, the effect of G was constantly greater than its corresponding effect of G×S except in biomass, spike length, grains per spike and 1000 grain weight (Table 2). Furthermore, the significant genotype by year interaction showed that each genotype responded differently to the two seasons in respect to biomass, tillers per plant and thousand grain weight. Sharma *et al.* (2010) also explained that wheat grain yield (GY) is highly influenced by production environments. More importantly, the significant genotypic effect obtained on GY and other traits revealed the existence of sufficient genetic variability among the wheat germplasm that can be exploited in the heat tolerance breeding programs.

Grain yield and other traits' performance of the wheat genotypes under normal and late sown condition

The mean performance of wheat genotypes for all the traits was significantly ($p \leq 0.05$) higher in the normal season than in the late season (Table 3) which is similar to numerous reports on impact of heat stress on wheat (Singh *et al.,* 2007; Rahman *et al.,* 2009). Exposure to higher temperatures can significantly reduce grain yield (Tewolde *et al.,* 2006). Kushwaha *et al.* (2006) recorded significantly less tillers/m^2, spike length and spikelets/ear under late planting in wheat. Singh

Table 1. List of wheat varieties studied for terminal heat tolerance

S.N.	Acc. ID	Variety	S.N.	Acc. ID	Variety	S.N.	Acc. ID	Variety
1	IC75240	C306 C	37	IC128152	CPAN1676	73	IC252927	RW346
2	IC128184	HDR77 C	38	IC443766	RAJ3765	74	IC75219	SKML1
3	IC296383	WR544 C	39	IC443767	RS31-1	75	IC393885	SONAK
4	IC536265	UP262	40	IC443769	UP115	76	IC145735	SONORA64
5	IC536269	UP368	41	IC443768	Utkalika	77	IC145753	UP2121
6	IC535622	WL1562	42	IC528119	DBW16	78	IC252954	UP2425
7	IC112258	VL401	43	IC116274	AJANTA	79	IC445595	UP2338
8	IC128150	BW11	44	IC144904	AKW381	80	IC75223	WL2265
9	IC128157	DL784-3	45	IC128155	SANGAM	81	IC113722	MOTIA
10	IC128175	HD2327	46	IC145280	HD1925	82	IC547563	DBW17
11	IC128180	HD2428	47	IC145283	HD1941	83	IC128167	HD2189
12	IC128195	HS240	48	IC145971	HD1949	84	IC443725	HD2285
13	IC128206	J24	49	IC145286	HD1981	85	IC75242	HD2329
14	IC128210	JU12	50	IC128209	HD1982	86	IC252612	HD2402
15	IC128213	K7410	51	IC252611	HD2177	87	IC443727	HD2643
16	IC128228	HI385	52	IC128168	HD2204	88	IC252632	HD2687
17	IC128261	VL404	53	IC128169	HD2236	89	IC290174	HD2733
18	IC128266	WH157	54	IC128170	HD2270	90	IC303070	HD2781
19	IC128267	WH283	55	IC128171	HD2278	91	IC443728	HD2824
20	IC128268	WH291	56	IC128172	HD2281	92	IC540910	HD2894
21	IC128270	WH416	57	IC128174	HD2307	93	IC574476	HD2967
22	IC128272	WH410	58	IC128178	HD2385	94	IC574388	HD2987
23	IC128273	WL711	59	IC128181	HD2501	95	IC296299	DBW14
24	IC252742	HW2004	60	IC122726	HUW468	96	IC75191	DL 153-2
25	IC252818	Lal Bahadur	61	IC252732	HUW510	97	IC138631	DL788-2
26	IC252951	UP2382	62	IC128211	K65	98	IC443722	DL803-3
27	IC290230	K9644	63	IC128212	K68	99	IC402064	HD2851
28	IC335540	Kharchia 65	64	IC128235	Narmada4	100	IC437081	HD2864
29	IC75234	HD2009	65	IC128244	PBW154	101	IC528118	HD2888
30	IC75212	PBW12	66	IC128666	PBW222	102	IC519900	HD2932
31	IC75226	WG377	67	IC420923	PBW226	103	IC309874	PBW343
32	IC144903	AKW1071	68	IC420925	PBW299	104	IC240798	PBW373
33	IC128177	HD2380	69	IC75215	PBW34	105	IC565811	PBW590
34	IC145420	HUW213	70	IC75389	RAJ2184			
35	IC443729	HD2833	71	IC410028	RAJ4037			
36	IC282300	K7903	72	IC252929	RW3016			

c- Check

Table 2. Combined analysis of variance of agronomical traits of 105 wheat varieties as influenced by date of sowing and year

Source	DSE	DPM	PH	BM	TP	SL	GS	GYP	TGW	**HI**
Gen	54.41*	60.61**	976.29**	6.30	25.49**	2.97	73.44	40.93**	529.80**	1470.38**
Yr	2460.56**	1541.33**	18.75	4318.27**	271.54**	33.78**	7458.39**	853.50**	579.61**	383.08**
SC	8225.06**	33849.48**	2862.37**	2255.71**	176.97**	184.61**	1764.19**	456.53**	18207.96**	212.02**
Gen*Yr	42.76	0.03	2.75	146.36**	6.21**	4.16	32.71	5.92	996.67**	28.17
Gen*SC	0.61	16.87*	73.73	1.38	4.54	1.79	83.25	0.02	456.47**	30.38
Gen*Yr*SC	34.98	40.35**	59.04	10.46	2.58	0.92	62.04	0.11	44.05*	11.06

Days to spike emergence (DSE); days to physiological maturity (DPM); plant height (PH); biological yield per plant (BYP); tillers per plant(TP); spike length(SL); grains per spike (GS); grain yield (GYP) 1000-grain weight (TGW); and harvest index (HI).

Table 3. Mean performance of wheat cultivars for agronomical traits under timely and late sown conditions averaged across years

Traits	Mean performance		CV(%)		% Reduction
	Timely sown	Late Sown	Timely sown	Late Sown	
DSE	91.48±0.31	82.74±0.27	5.11	4.83	9.56
DPM	136.65±0.23	118.95±0.14	2.47	1.82	12.95
PH	96.57±0.80	91.59±0.81	12.23	13.06	5.16
BYP	21.87±0.39	17.41±0.42	26.26	35.31	20.40
TP	6.35±0.10	5.09±0.09	23.29	27.88	20.00
SL	12.92±0.11	11.61±0.09	12.61	12.15	10.14
GS	56.18±0.81	52.20±0.69	21.40	19.63	7.09
GYP	8.74±0.17	6.71±0.17	29.76	39.01	23.20
TGW	43.33±0.47	30.53±0.49	15.97	23.85	29.54
HI	39.84±0.38	38.43±0.42	14.33	16.16	3.55

Days to spike emergence (DSE); days to physiological maturity (DPM); plant height (PH); biological yield per plant; g(BYP); tillers per plant (TP); spike length, cm (SL); grains per spike (GS); grain yield; g (GYP) 1000-grain weight; g (TGW); and harvest index % (HI).

et al. (2016) reported general reduction in the values of all traits under late planting condition as compared to normal planting due to temperature stress. The higher temperature associated with the late season as evidently reported in the meteorological data (Fig. 2) impacted heat stress on the crop leading to the greater significant reduction in all the traits especially on thousand grain weight (29.54%) and grain yield (23.50%). In late season 1, grain yield ranged from 2.1 in genotype Lal Bahadur and PBW12 to 10.3 g in genotype HD2687, while in late season 2 it extended from 4.3 in genotype Lal Bahadur to 15.3 g in genotype HD2687. It ranged from 2.8 g in genotype Lal Bahadur to 13.2 g in genotype HD2189 in the normal season 1, whereas it stretched from 5.0 g in genotype Lal Bahadur to17.9 g in genotype HD2687 in the normal season 2. Prasad *et al*. (1999) confirmed that heat stress also imparts to the decreased seed filling duration thus resulting in production of smaller seed size. Mitchell and Jones (2005) found five climatic variables available which included cloud cover, diurnal temperature range, precipitation, temperature and vapour pressure. Janjua *et al.* (2010) analyzed the impact of climate change on wheat production and concluded that higher temperature affected the growth process of wheat negatively thus resulting in severely hampered productivity. A considerable decrease in the number of grains was observed on exposure of floral initiation stage and spikelet development to high temperature conditions thus adversely impacting the maximum yield potential. Sink strength and source capacity are considered two vital factors in modifying the grain yield and quality of wheat genotypes exposed to chronic heat as well as a heat shock reported by Yang *et al.* (1996). Kumar *et al.* (2013) recorded reduction in biomass, grain growth and grain yield are the consequence of impaired biological processes at cellular, molecular and organ level under thermal stress.

Table 4. Thirty top yielding genotypes in each of the environment (L1: late sown-first year; N1: normal sown-first year; L2: late sown-second year; N2: normal sown-second year). Genotypes in Bold indicates stable genotypes across the environments

Sl. No.	Gen (L1)	GYP (g)	Sl. No.	Gen (N1)	GYP (g)	Sl. No.	Gen (L2)	GYP (g)	Sl. No.	Gen (N2)	GYP (g)
88	**IC252632, HD2687**	10.30	83	IC128167, HD2189	13.20	**88**	**IC252632, HD2687**	15.33	**88**	**IC252632, HD2687**	17.93
84	IC443725, HD2285	9.90	**88**	**IC252632, HD2687**	13.10	**69**	**IC75215, PBW34**	15.14	**69**	**IC75215, PBW34**	15.97
54	IC128170, HD2270	9.50	70	IC75389, RAJ2184	12.70	64	IC128235, Narmada4	13.16	64	IC128235, Narmada4	15.44
69	**IC75215, PBW34**	9.50	103	IC309874, PBW343	12.50	102	IC51990, HD2932	11.89	10	IC128175, HD2327	15.24
83	IC128167, HD2189	9.30	64	IC128235, Narmada4	11.75	2	IC128184, HDR77	11.87	92	IC540910, HD2894	14.52
85	IC75242, HD2329	9.20	101	IC528118, HD2888	11.30	10	IC128175, HD2327	11.45	70	IC75389, RAJ2184	14.33
74	IC75219, SKML1	9.00	86	IC252612, HD2402	11.25	**96**	**IC75191, DL153-2**	11.43	9	IC128157, DL784-3	14.04
90	**IC303070, HD2781**	8.60	**31**	**IC75226, WG377**	11.00	104	IC240798, PBW373	11.27	103	IC309874, PBW343	13.49
1	**IC75240, C306**	8.20	92	IC540910, HD2894	10.90	92	IC540910, HD2894	11.14	91	IC443728, HD2824	13.39
17	IC128261, VL404	7.55	**69**	**IC752215, PBW34**	10.83	36	IC282300, K7903	11.08	**90**	**IC303070, HD2781**	13.21
27	IC290230, K9644	7.00	2	IC128184, HDR77	10.75	53	IC128169, HD2236	11.06	36	IC282300, K7903	12.99
31	**IC75226, WG377**	7.00	9	IC128157, DL784-3	10.65	17	IC128261, VL404	11.01	17	IC128261, VL404	12.37
46	IC145280, HD1925	6.85	15	IC128123, K7410	10.50	87	IC443727, HD2643	10.90	11	IC128180, HD2428	12.21
96	**IC75191, DL153-2**	6.85	**96**	**IC75191, DL153-2**	10.50	97	IC138631, DL788-2	10.80	101	IC528118, HD2888	12.20
53	IC128169, HD2236	6.80	36	IC282300, K7903	10.30	5	IC536269, UP368	10.74	53	IC128169, HD2236	12.07
35	IC443729, HD2833	6.65	102	IC519900, HD2932	10.30	89	IC290174, HD2733	10.69	102	IC519900, HD2932	11.99
77	IC145753, UP2121	6.50	**90**	**IC303070, HD2781**	10.25	**31**	**IC75226, WG377**	10.65	78	IC252954, UP2425	11.83
82	IC547563, DBW17	6.50	54	IC128170, HD2270	10.20	**90**	**IC303070, HD2781**	10.64	104	IC240798, PBW373	11.82
5	IC536269, UP368	6.45	74	IC75219, SKML1	10.20	15	IC128213, K7410	10.61	**31**	**IC75226, WG377**	11.81
68	IC420925, PBW299	6.40	24	IC252742, HW2004	10.05	**1**	**IC75240, C306**	10.42	89	IC290174, HD2733	11.81

Sl. No.	Gen (L1)	GYP (g)	Sl. No.	Gen (N1)	GYP (g)	Sl. No.	Gen (L2)	GYP (g)	Sl. No.	Gen (N2)	GYP (g)
75	IC393885, SONAK	6.25	87	IC443727, HD2643	9.90	54	IC128170, HD2270	10.33	**1**	**IC75240, C306**	11.61
60	IC122726, HUW468	6.05	85	IC75242, HD2329	9.85	78	IC252954, UP2425	10.21	2	IC128184, HDR77	11.60
104	IC240798, PBW373	6.00	93	IC574476, HD2967	9.70	39	IC443767, RS311	10.10	63	IC128212, K68	11.51
95	IC296299, DBW14	5.95	45	IC128155, SANGAM	9.50	65	IC128244, PBW154	10.00	20	IC128268, WH291	11.48
50	IC128209, HD1982	5.90	63	IC128212, K68	9.40	103	IC309874, PBW343	9.83	**96**	**IC75191, DL153-2**	11.46
43	IC116274, AJANTA	5.80	84	IC443735, HD2285	9.25	83	IC128167, HD2189	9.76	19	IC128267, WH283	11.43
52	IC128168, HD2204	5.75	10	IC128175, HD2327	8.95	20	IC128268, WH291	9.51	87	IC443727, HD2643	11.37
89	IC290174, HD2733	5.75	11	IC128180, HD2428	8.95	27	IC290230, K9644	9.46	8	IC128150, BW11	11.34
67	IC420923, PBW226	5.70	**1**	IC75240, C306	8.90	19	IC128267, WH283	9.41	15	IC128213, K7410	11.31
39	IC443767, RS311	5.65	82	IC547563, DBW17	8.80	46	IC145280, HD1925	9.24	24	IC252742, HW2004	11.26

Table 4 showed the top thirty high yielding genotypes in each of the four environments (late season 1, late season 2, normal season 1, and normal season 2) with mean grain yield of 5.1, 8.4, 7.7, and 9.9 g in the four environments, respectively. These genotypes yielded above 5.65 g per plant under late planted condition and above 9.24 g under non stress condition. Among them, six genotypes viz.,1-IC75240 (C306), 69- IC75215(PBW34), 88-IC 252632(HD2687), 90-IC303070 (HD2781), 31-IC75226(WG377), 96-IC75191(DL153-2) were stable for grain yield in all the environments, hence recommended for both heat favourable and heat stressed environments. The stability of these six genotypes were due to stability in different yield components. For example, genotype 1-IC75240 (C306) was stable in terms of plant height, tillers per plant, spike length, grains per spike and harvest index as evident by % reduction less than 10% for these traits (Table 5). Genotype 31-IC75226(WG377) had stable spike length and thousand grain weight and harvest index, whereas genotypes 69-IC75215(PBW34) had moderate reduction for all the traits except thousand grain weight. Genotypes 88-IC252632(HD2687), 90-IC303070 (HD2781) and 96-IC75191(DL153-2) had less reduction for plant height, spike length and grains per spike. The combination breeding can be opted for suitably combining the component traits. Gavhane *et al.* (2016) identified genotypes NIAW2972, NIAW34 and HI977 as terminal heat tolerant to be used in the crossing programme for developing terminal heat tolerant variety/line. Similarly, tolerant genotypes like DBW14, RAJ3765, HD2643 and HALNA performed physiologically better in terms of higher membrane stability index (MSI), chlorophyll content, photosynthesis rate (Pn), harvest index under heat stress conditions by Nagar *et al.,* 2015. Al-Otayk (2010) argued that a genotype with stable and high yield across different environments would be a more suitable cultivar and perhaps a donor parent for further breeding for heat tolerance. Specifically, genotypes 69- IC75215(PBW34); 88-IC252632(HD2687) were consistently among the top five yielding genotypes in the two late seasons will be ideal for heat stressed environments, while genotypes 88-IC252632(HD2687) and 64-IC128235(NARMADA4) that were consistent in the two normal seasons are recommended for heat favourable (non-stressed) condition only. The genotype

Table 5. Percent reduction of stable wheat cultivars under terminal heat stress condition over the years for agronomical traits

Acc No	1	31	69	88	90	96
Acc ID/Cultivar	IC75240 (C-306)	IC75226 (WG377)	(IC75215) PBW34	IC252632 (HD2687)	IC303070 (HD2781)	IC75191 (DL1532)
Source/ Pedigree	UP, RGN/CSK3//2*C591/3/ C217/N14//C281	UP (WG 143 x USA-255) x PV 18	PAU, Anhinga S x Flamingo S	Delhi CPAN 2009/HD-2329 (CPAN 2009 =KVZ/TO-RIM//POTAM/ANA)	Delhi BOW/C 306//C 591/HW 2004	UP TANORI 71/NP 890
Traits	**Percent reduction**					
DSE	10	7.1	11.68	9.28	11.83	12.17
DPM	12.83	14.65	14.64	10.7	12.22	14.03
PH	6.6	-3.65	8.65	2.34	1.08	-1.68
BYP	15.74	17.87	9.19	20.84	17.35	29.05
TP	25.37	34.48	5.56	4.35	8.33	22.22
SL	8.66	9.71	10.66	7.66	9.81	5.68
GS	2.49	11.04	-3.23	6.8	3.33	-5.38
GYP	10.09	22.62	8.06	17.4	17.99	16.76
TGW	26.4	9.93	21.11	35.96	30.5	50
HI	-7.12	6.39	-1.14	11.09	0.73	-16.45

Days to spike emergence (DSE); days to physiological maturity(DPM); plant height(PH); biological yield per plant; g(BYP); tillers per plant(TP); spike length; cm(SL); grains per spike (GS); grain yield; g (GYP) 1000-grain weight; g (TGW); and harvest index,% (HI).

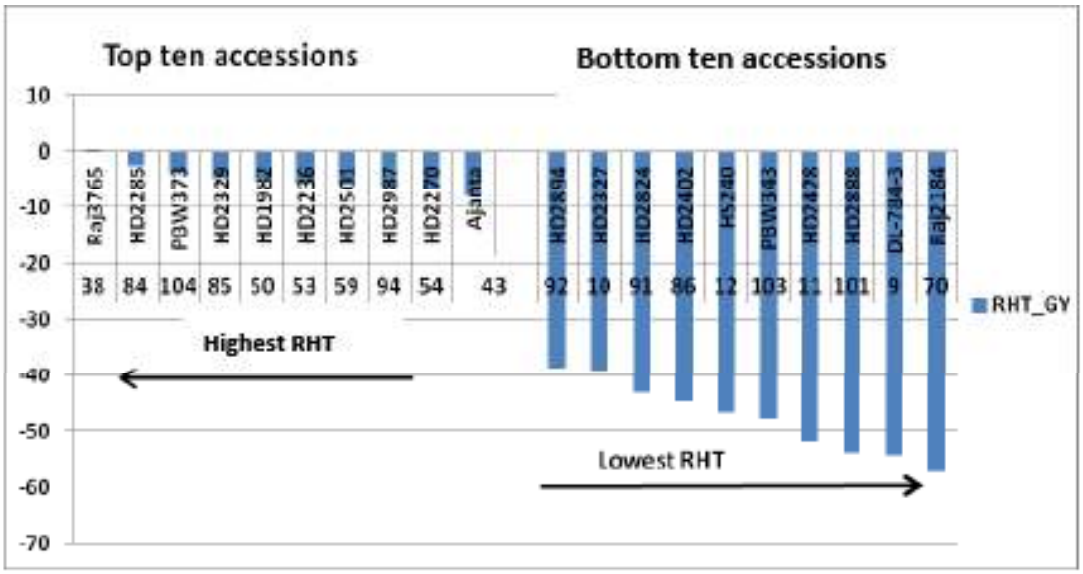

Fig. 1. Relative heat tolerance for grain yield (%) of the wheat germplasm under late sown condition with respect to normal sown

88-IC252632(HD2687) can be recommended for both heat stressed environments and heat favourable (non-stressed) conditions.

Relative heat tolerance (RHT) of wheat germplasm

Figure 1 showed the grain yield plasticity/stability of the genotypes in the late seasons (heat stressed environments) with respect to their corresponding normal seasons (control/heat favourable environments). The RHT ranged from 0.09% in genotype RAJ3765(38) to -57.19% in genotype RAJ2184(70). None of the top ten and bottom genotypes were found among six stable and common genotypes high yielding genotypes under timely and late sown conditions. However, genotype no. 104-IC240798(PBW373), 53-IC128169(HD2236) and 54-IC128170(HD2270) were among top thirty

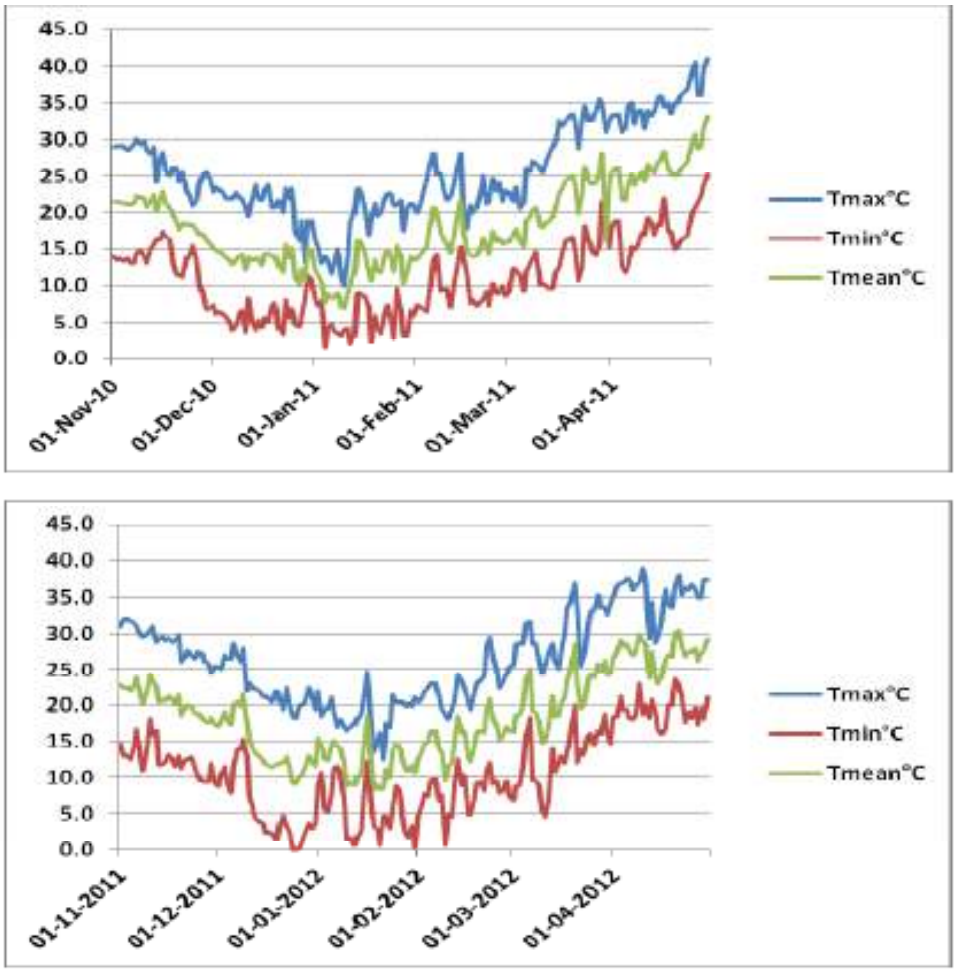

Fig. 2. Minimum, maximum and mean temperature(°C) during 1st and 2nd years of screenin

under three environments. 104-IC240798(PBW373) and 53-IC128169(HD2236) were superior under all environments except non-stress condition during first year. 54-IC128170(HD2270) was superior under all environments except non-stress condition during second year. 84-IC443725 (HD2285) and 85-IC75242(HD2329) were superior during first year evaluation only. Rehman *et al.* (2009) previously reported that wheat planted in the late season suffered drastic yield loss which exceeded 50%. Top ten tolerant genotypes could be genetic sources for heat tolerance breeding program. Wheat has the tendency to adopt diverse types of responses to temperature stress as well as a heat shock by developing thermo-tolerance for the enhancement of the grain quality and yield (Iqbal *et al.,* 2017). End-of-season or 'terminal' heat stress is also likely to increase for wheat in the near future (Semenov, 2009) due to increase in global warming. Therefore, breeding for heat tolerance in wheat is a major global concern (Paliwal *et al.,* 2012).

Association of the grain yield with other traits

Days to spike emergence (DSE) was significantly positively correlated with days to physiological maturity (DM), biological yield per plant, tillers per plant, spike length, grains per spike, grain yield per plant under normal sown condition, whereas under late sown condition, association was found with days to physiological maturity, biological yield per plant, tillers per plant, grains per spike, grain yield per plant and harvest index. Plant height was correlated with biomass under normal sown condition. Grain yield was positively correlated with days to spike emergence, days to physiological maturity, biological yield, tillers per plant, grains per spike and harvest index under normal sown condition, whereas it was associated additionally with thousand grain weight under late sown condition (Table 6). Thousand grain weight was positively correlated with grain yield under late sown condition. Grain yield

Table 6. Pearson correlation of grain yield and other studied traits of wheat cultivars under normal (below diagonal) and late sown conditions (above diagonal)

	DSE	DPM	PH	BYP	TP	SL	GS	GYP	TGW	HI
DSE	1	0.67**	0.18	0.30**	0.26**	-0.01	0.32**	0.37**	0.09	0.25*
DPM	0.59**	1	0.31**	0.27**	0.38**	0.04	0.38**	0.33**	0.08	0.21*
PH	-0.02	0.10	1	0.26**	0.24*	-0.16	-0.02	0.19	0.06	-0.03
BYP	0.39**	0.43**	0.05	1	0.54**	0.00	0.36**	0.89**	0.34**	0.10
TP	0.32**	0.38**	-0.05	0.66**	1	0.04	0.20*	0.48**	0.17	0.08
SL	0.21*	0.09	-0.14	0.17	0.08	1	0.43**	-0.02	-0.10	-0.06
GS	0.31**	0.18	-0.10	0.31**	0.07	0.41**	1	0.41**	-0.05	0.24*
GYP	0.36**	0.35**	0.00	0.91**	0.56**	0.07	0.29**	1	0.37**	0.53**
TGW	-0.25	-0.11	0.11	-0.23	-0.24	-0.35	-0.29	-0.14	1	0.19
HI	0.00	-0.06	-0.10	0.05	-0.06	-0.19	0.04	0.44**	0.20*	1

Days to spike emergence (DSE); days to physiological maturity (DPM); plant height (PH); biological yield per plant; g(BYP); tillers per plant (TP); spike length; cm(SL); grains per spike (GS); grain yield; g (GYP) 1000-grain weight; g (TGW); and harvest index % (HI).

(GY) significantly correlated positively with DSE, and DM indicating that longer the maturation of the crop especially if the grain filling duration is prolonged, the more the increase in the GY. Although early maturing germplasm escape heat stress and ideally suitable for heat stressed conditions (Mondal *et al.,* 2015), but based on this study these genotypes may not produce more seed yield in comparison to medium to late maturing germplasm. This suggests that days to maturity could be favourably selected for enhanced grain yield, except under intense and protracted heat stress especially during the late growing season. Menshawy (2007) also indicated that early maturing cultivars are preferable to escape heat stress injury that occurs at the end of the growing season. The correlation showed that medium maturing genotypes are ideal for heat stressed as well as heat favourable environments (non-stressed condition). These early maturing genotypes possess earliness *per se* genes that hasten the developmental and flowering time under heat stressed environments as soon as vernalization and photoperiod requirements have been fulfilled. Gavhane *et al.* (2016) reported positive correlation of yield under stress environment (<0.05) (r=0.95) association with that of non stress environment.

The significant impact of the heat stress on yield performance of the wheat genotypes underlines the urgent need for breeding for heat tolerance. The improved and released cultivars certainly have the good amount of genetic variability for heat tolerance that can be harnessed for yield improvement of wheat. Further significant genotypic variation present in cultivars will also guide for search of novel genotypes in unexplored germplasm that can be exploited in the heat tolerance breeding programs. Based on this study, genotypes with stable yield performance under stressed as well as non-stressed environments; 1- IC75240(C306); 69-IC75215(PBW34); 88-IC252632(HD2687); 90-IC303070(HD2781); 31-IC75226(WG377); 96-IC75191(DL153-2) are recommended for both heat favourable (non-stressed condition) /normal sown) and heat stressed (late sown) environments. Also, genotypes 104- IC40798, (PBW373); 53-IC128169(HD2236) and 54-IC128170(HD2270) that were among the 10 heat tolerant genotypes based on relative heat tolerance were among top thirty high yielders under three environments, therefore could be suggested as donor lines for heat tolerance breeding program.

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RESEARCH ARTICLE

Evaluation of Mango Germplasms for Resistance against Mango Shoot Gall Psylla, *Apsylla Cistellata* Ruckton (Homoptera: Psyllidae)

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Mango shoot gall psylla, *Apsylla cistellata* Buckton (Psyllidae: Homoptera) is one of the region specific serious pest of mango in India. The pest forms hard conical shaped green shoot galls in place of axillary and apical buds which hampers the initiation of inflorescences and retard the growth. A total of 100 mango genotypes were screened and evaluated against *A. cistellata* infestation during years 2011-12 to 2014-15. Genotype, Himayuddin, Lal Sinduria, Mulgoa Hill and Hybrid- 11/4 were found resistant against *A. cistellata* in field conditions. These genotypes can further be utilized for shoot gall psylla resistant breeding programmes.

Key Words: Mango, Shoot gall psylla, Resistance, Screening

Introduction

Mango, *Mangifera indica* L. the "king of fruits" is one of the main commercial tropical and sub-tropical grown fruit species in the world (Vasugi *et al*., 2012). Mango is believed to be originated from India and this country is considered as the home of several mango germplasms (Butani, 1979). More than 188 insect-pest species have been reported to attack on mango from India (Tandon and Vergheses, 1985). Among them, mango shoot gall psylla, *Apsylla cistellata* Buckton (Psyllidae: Homoptera) is one of the important pest of mango in various part of northern India, Nepal and Bangladesh (Singh, 2000). Its distribution is restricted to districts of Punjab, Tarai regions of Uttar Pradesh and Uttarakhand, Himachal Pradesh, parts of Bihar and entire Jharkhand of India (Rahman *et al*., 2016; Raina and Srivastava, 2018). The pest directly affects the mango production through formation of inflorescences and further fruit setting. The pest forms hard conical shaped green shoot galls in place of axillary and apical buds due to sucking of nymphs at particular place. Meanwhile nymphs feed inside the fully formed galls and grow up to adults. The infested twig shows the die back symptoms in due course of time. Ultimately an infested mango tree produces only 4-5 per cent fruits when compared to healthy trees (Singh, 2000; Raina and Srivastava, 2018). Mango shoot gall psylla is considered as specific and univoltine pest of mango. Adults usually emerged from galls in month of February-March and after that start lying eggs inside the marginal side of midrib of younger leaves. Hatching of eggs observed in the month of August-September and completes the life cycle in a year (Monobrullah *et al*., 1998; Kumar *et al*., 2007).

Absence of effective biocontrol agents, use of recommended insecticides such as profenophos, thiomethoxam, dimethoate, quinalphos etc. is only viable reported control strategy against mango shoot gall psylla (Singh *et al*., 2015; Kadam, *et al*., 2017). Spray in the month of August-September at the time of egg hatching is also usually not effective due to heavy rains in a particular period. Once the insect makes the galls, spraying is not found too effective because the protective gall gives protection to gall insect against insecticidal sprays. Host Plant Resistance (HPR) is an eco-friendly and cost effective viable management strategy against such insect pest and is also recommended as a component of integrated pest management of *A. cistellata* in mango. The identified resistance sources can be used directly or indirectly in breeding programmes for development of *A. cistellata* resistant mango. Hence, in the present study, mango germplasm were screened in field conditions to find out the sources of resistance against *A. cistellata*.

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Materials and Methods

The experiment was conducted in National Germplasm Repository of Sub-tropical fruit crops at ICAR Research Complex for Eastern Region, Research Centre, Ranchi (23°45' N latitude, 85°30' E longitude, elevation 620 m AMSL) Jharkhand, India. The germplasms in orchard are uniformly 25 years old. A total of 100 mango genotypes were selected for screening against mango shoot gall psylla over four consecutive years i.e. 2011-12 to 2014-15 under ICAR-National Innovations on Climate Resilient Agriculture (ICAR-NICRA) project. Each genotype has three replications at spacing of 10m x 10m. The agronomic practices for germplasm maintenance were same for all the assessed genotypes. The germplasm blocks were kept free from application of any insecticide and fungicides during the study period. Five shoots were selected randomly from each of four quadrants and therefore a total of 20 shoots were chosen from a tree. If psylla infested shoots were observed than number of galls on infested shoots were counted one shoot from each quadrant. For each genotype, three plants were selected for observations. Finally data were changed in per cent shoot infested and number of gall/s recorded per infested shoot for interpretation as a source of resistance. The data were observed at weekly intervals (NICRA team of mango pest surveillance, 2011). Genotypes were categorised in to four groups based on level of *A. cistellata* infestation and flower panicle initiation viz., genotype recorded no/less than 20 per cent shoots infestation with no/less than 3 number of galls per shoot were grouped as resistant (category I), genotype recorded 20 to 60 per cent shoots infestation with less than 3 number of galls per shoot or less than 20 per cent shoots infestation but more than 3 and up to 15 galls per shoots were grouped as moderately resistant (category II), genotype recorded 20 to 60 per cent shoots infestation with more than 3 and up to 15 galls per shoot were grouped in to susceptible (category III) and genotypes which recorded more than 60 per cent shoots infestation by shoot gall psylla along with more than 15 number of galls per shoot were grouped in to highly susceptible genotypes (category IV). The per cent shoot gall psylla infestation and number of gall per shoot were analysed through one way ANOVA and means were compared using Tukey's honestly significant difference (HSD) tests for comparison at probability level of 5%. All statistical analyses were performed using SPSS 21.0.

Results and Discussion

Germplasm of mango showed the statistically significant difference in terms of shoot gall psylla infestation (Table 1). Eleven genotypes were categorized as resistant source which have recorded less than 20 per cent shoots infested with less than three numbers of galls on infested shoots. The genotype, Himayuddin was found to consistently resistant where no galls were observed on shoots during observation period. The other three genotypes, Lal Sinduria, Mulgoa Hill and Hybrid- 11/4 also had high level of resistant against shoot gall psylla infestation (1.50, 2.92 & 0.75 per cent shoots infestation and 0.13, 0.48 & 0.06 galls per shoot, respectively). The other mango genotypes for resistant against shoot gall psylla infestation were observed as Hyder Saheb, Lucknow selection, Arka Neelkiran, Swarnajahangir, Hamlet, Hur and Benisan. These genotypes can be serving as potential resistance source for *A. cistellata* resistant breeding programmes. The genotypes, Kohitur (14.25 % shoot infested & 3.43 galls), Bhadaiya Sukul (15.00 % shoot infested & 4.23 galls), Arka Anmol (19.25 % shoot infested & 5.77 galls), and Kesar (11.83 % shoot infested & 3.11 galls) were found moderately resistant consistently during observed years (2011-12 to 2014-15). Although, Dilsad, Hybrid-51, Neelgoa, Kalipari, Sammar bahist chausa, Sahable, Surkhavarna, Khiros Patti and Amir Pasand were categorized under II (moderately resistant) based on mean of observed data but based on individual year data, some of observed year particularly 2013 data theses genotypes regularly did not fall under moderately resistant criterion. Vastara (76.83 % shoot infested & 20.63 galls) followed Mithua Bihar (69.73 % shoot infested & 19.86 galls), Hathi Jhool (60.33 % shoot infested & 16.43 galls), Jhapatta (60.42 % shoot infested & 15.64 galls), Bhatuhi (60.33 % shoot infested & 15.01 galls) and Gulabi (60.75 % shoot infested & 18.56 galls) were found to highly susceptible. Total 70 mango genotypes were categorized as susceptible against *A. cistellata*. Therefore, on the basis of susceptible/ resistant group index, 11 genotypes were found resistant, 13 were categorized as moderately resistant, 70 were susceptible and remaining 6 genotypes were found to be in group of highly susceptible to *A. cistellata* in present study (Table 2). Infestation and formation of galls on shoots are very critical especially gall formation in place of apical buds, where directly interfere the initiation of inflorescence (Raina and Srivastava, 2018). The number of galls and per cent shoot infestation is also directly

Table 1. Mango genotypes evaluated in the field conditions against mango shoot gall psylla, *Apsylla cistellata* infestation during years 2011-12 to 2014-15

Genotype	2012		2013		2014		2015		Mean	
	Per cent infested shoots*	No. of galls/ infested shoot	Per cent infested shoots	No. of galls/ infested shoot	Per cent infested shoots	No. of galls/ infested shoot	Per cent infested shoots	No. of galls/ infested shoot	Per cent infested shoots	No. of galls/ infested shoot
Vastara	51.33^{de}	9.68^{cd}	91.67^{e}	20.92^{z}	72.67^{kl}	29.08^{p}	91.67^{l}	22.83^{jk}	76.83^{j}	20.63^{m}
Jahangir-I	30.01^{cd}	6.33^{bcd}	71.67^{de}	17.12^{xy}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	25.42^{cd}	5.86^{c}
Kala Pahar	12.33^{bc}	2.33^{ab}	86.67^{e}	17.85^{z}	51.67^{hi}	20.77^{lm}	85.67^{k}	22.77^{jk}	59.08^{hi}	15.93^{kl}
Piyara Phulo	20.00^{b}	4.58^{bc}	70.33^{de}	14.68^{v}	19.33^{cd}	9.00^{f}	94.00^{l}	22.50	50.92^{g}	12.69^{ghi}
Hathi Jhool	16.67^{bc}	3.90^{bc}	71.67^{de}	13.75^{u}	66.00^{ik}	25.75^{o}	83.00^{k}	22.32^{jk}	60.33^{hi}	16.43^{k}
Kohitur	1.33^{ab}	0.15^{a}	49.00^{c}	10.03^{op}	6.67^{ab}	2.55^{c}	0.00^{a}	0.00^{a}	14.25^{b}	3.18^{bc}
Jhapatta	37.33^{cd}	5.32^{bcd}	75.00^{de}	13.93^{u}	50.67^{hi}	21.45^{m}	78.67^{i}	21.87	60.42^{hi}	15.64^{kl}
Bhadaiya Sukul	0.00^{a}	0.00^{a}	11.67^{ab}	1.62^{ab}	20.00^{bc}	8.75^{f}	28.33^{c}	6.53^{de}	15.00^{b}	4.23^{ef}
Bag-e-bahar	11.00^{ab}	1.70^{ab}	45.00^{c}	8.43^{mn}	32.00^{ef}	14.37^{ij}	58.33^{g}	13.85^{gh}	36.58^{ef}	9.59^{fg}
Sari Khas	1.67^{ab}	0.43^{ab}	66.67^{de}	13.00^{s}	50.67^{hi}	22.75^{mn}	78.33^{i}	20.73^{jk}	49.33^{g}	14.23^{i}
Jarda	17.33^{bc}	2.58^{ab}	58.67^{d}	14.80^{v}	22.33^{de}	9.92^{g}	20.00^{b}	5.92^{cd}	29.58^{de}	8.30^{ef}
Hyder Sahab	0.00^{a}	0.00^{a}	43.33^{c}	2.80^{cd}	0.00^{a}	2.50^{c}	0.00^{a}	0.00^{a}	10.83^{b}	1.33^{b}
Mundappa Black	7.67^{ab}	0.78^{ab}	10.00^{ab}	1.33^{ab}	64.00	26.52^{o}	81.33^{k}	20.70^{jk}	40.75^{ef}	12.33^{h}
Black Andrew	0.00^{a}	0.00^{a}	74.67^{de}	6.03^{ij}	21.67	10.07^{fg}	0.00^{a}	0.00^{a}	24.08^{cd}	4.03^{c}
Anfas	3.00^{ab}	0.35^{a}	65.00^{d}	13.12^{t}	76.33	30.53^{p}	78.67^{i}	20.05^{kj}	55.75^{gh}	16.01
Kalapaddy	0.00^{a}	0.00^{a}	38.33^{bc}	8.08^{m}	60.00^{ij}	26.33^{o}	78.00^{i}	19.28^{j}	44.08^{fg}	13.43^{ik}
H/51/1	0.00^{a}	0.00^{a}	30.00^{bc}	4.67^{fg}	32.33^{ef}	14.62^{ij}	55.67^{fg}	13.92^{gh}	29.50^{d}	8.30^{ef}
Lucknow Selection	0.00^{a}	0.00^{a}	8.33^{ab}	1.25^{ab}	6.67^{ab}	3.00^{c}	0.00^{a}	0.00^{a}	3.75^{a}	1.06^{a}
Ituraba	0.00^{a}	0.00^{a}	13.33^{ab}	1.33^{bc}	65.00^{ik}	27.82	82.67^{jk}	21.68^{jk}	40.25^{e}	12.71^{h}
Mulgoa hill	0.00^{a}	0.00^{a}	11.67^{ab}	1.92^{bc}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	2.92^{a}	0.48^{ab}
Swarnrekha-1	5.00^{ab}	1.15^{ab}	65.00^{d}	11.95^{qr}	41.33^{gh}	16.28^{jk}	89.33^{k}	20.13^{j}	50.17^{g}	12.38^{h}
Dilsad	20.67^{bc}	3.47^{bc}	30.00^{b}	5.58^{hi}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	12.67^{bc}	2.26^{b}
Ratna	0.00^{a}	0.00^{a}	76.00^{de}	16.35^{x}	3.33^{ab}	1.87^{c}	71.67^{i}	20.18^{jk}	37.75^{ef}	9.60^{fg}
Neelphanso	29.00^{bc}	4.65^{bc}	72.00^{de}	14.03^{u}	40.33^{fg}	19.70^{l}	84.67^{jk}	21.68^{jk}	56.50^{h}	15.02^{k}
Nileshwari	7.33^{a}	1.45^{ab}	71.67^{de}	12.58^{s}	36.00^{fg}	15.40^{j}	74.33^{i}	20.65^{jk}	47.33^{fg}	12.52^{h}
Barbelia	46.00^{de}	7.82^{cd}	71.67^{de}	14.08^{u}	54.67^{hi}	25.60^{no}	51.67^{f}	14.32^{h}	56.00^{h}	15.45^{k}
Dashehari Mahmooda	18.00^{bc}	4.05^{bc}	38.33^{bc}	7.42^{kl}	35.00^{fg}	16.28^{jk}	46.00^{e}	12.55^{g}	34.33^{e}	10.08^{fg}
Arka Neelkiran	5.67^{ab}	0.80^{ab}	33.33^{bc}	2.85^{cd}	5.00^{ab}	2.52^{c}	0.00^{a}	0.00^{a}	11.00^{b}	1.54^{b}
Hybrid/51	0.00^{a}	0.00^{a}	30.00^{bc}	4.33^{fg}	40.00^{fg}	17.73^{k}	62.33^{h}	18.97^{i}	33.08^{e}	10.26^{g}
Arka Anmol	2.00^{ab}	0.33^{a}	46.67^{cd}	11.08^{q}	28.33^{ef}	15.63^{j}	0.00^{a}	0.00^{a}	19.25^{c}	6.76^{de}
Bennet Alphanso	4.00^{ab}	0.65^{ab}	30.00^{bc}	5.75^{ij}	33.67^{fg}	16.07^{j}	21.67^{bc}	2.83^{b}	22.33^{cd}	6.33^{de}
Ratnagiri Alphanso	7.33^{ab}	1.55^{ab}	81.67^{e}	18.23^{z}	24.33^{de}	11.32^{h}	73.00^{i}	17.38^{ij}	46.58^{fg}	12.12^{h}
Amini	0.00^{a}	0.00^{a}	23.33^{b}	4.08^{ef}	0.00^{a}	0.00^{a}	75.33^{i}	18.18^{ij}	25.42^{cd}	5.57^{d}
Sabari	5.00^{ab}	0.70^{ab}	61.67^{d}	11.08^{q}	47.00^{gh}	15.77^{j}	74.67^{i}	17.67^{ij}	47.08^{fg}	11.30^{gh}
Bara Sinduria	37.00^{cd}	6.33^{bc}	78.33^{e}	16.00^{wx}	6.00^{ab}	2.43^{bc}	56.67^{g}	10.17^{fg}	44.50^{fg}	8.73^{ef}
Jawahar	24.00^{c}	3.93^{bc}	51.67^{cd}	10.42^{pq}	45.67^{g}	18.37^{k}	47.67^{ef}	8.92^{f}	42.25^{f}	10.41^{gh}
Sindhu	12.00^{bc}	1.62^{ab}	45.00^{cd}	8.50^{mn}	13.00^{abc}	4.18^{d}	45.00^{e}	10.08^{f}	28.75^{d}	6.10^{de}

Genotype	2012		2013		2014		2015		Mean	
	Per cent infested shoots*	No. of galls/ infested shoot	Per cent infested shoots	No. of galls/ infested shoot	Per cent infested shoots	No. of galls/ infested shoot	Per cent infested shoots	No. of galls/ infested shoot	Per cent infested shoots	No. of galls/ infested shoot
Lal Sinduria	0.00^{a}	0.00^{a}	5.00^{b}	0.50^{b}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	1.25^{a}	0.13^{a}
Hybrid/27	4.33^{ab}	0.62^{ab}	30.00^{bc}	0.65^{b}	13.33^{abc}	6.53^{e}	40.00^{d}	7.08^{e}	21.92^{cd}	3.72^{c}
Sabari-1	0.00^{a}	0.00^{a}	94.00^{e}	7.72^{l}	0.00^{a}	0.00^{a}	81.67^{j}	18.17^{ij}	43.92^{fg}	6.47^{de}
Chinnarasam	0.00^{a}	0.00^{a}	80.33^{e}	15.82^{vw}	2.67^{ab}	1.05^{ab}	68.33^{h}	16.98^{hi}	37.83^{ef}	8.46^{e}
Swarnajahangir	3.33^{ab}	0.28^{a}	12.67^{a}	3.00^{de}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	4.00^{b}	0.82^{b}
Swarnaguddi	27.67^{c}	5.98	63.33^{d}	13.10st	0.00^{a}	0.00^{a}	23.00^{bc}	5.32^{c}	28.50^{d}	6.10^{de}
Pedarasam	1.67^{ab}	0.18^{a}	49.33^{cd}	10.17^{op}	0.00^{a}	0.00^{a}	50.00^{f}	10.88^{f}	25.25^{c}	5.31^{c}
Totapuri	21.67^{b}	3.30^{bc}	71.33^{d}	14.98^{v}	28.33^{ef}	10.60^{g}	29.67^{cd}	6.17^{d}	37.75^{f}	8.76^{ef}
Nileshan	5.33^{ab}	0.80^{ab}	21.67^{b}	4.97^{gh}	38.33^{f}	14.78^{j}	25.67^{b}	5.98^{c}	22.75^{cd}	6.63^{de}
Hamlet	0.00^{a}	0.00^{a}	33.00^{bc}	2.50^{cd}	0.00^{a}	1.80^{bc}	0.00^{a}	0.00^{a}	8.25^{b}	1.08^{ab}
Neelam	38.67^{cd}	5.95^{cd}	27.33^{bc}	6.33^{jk}	42.33^{gh}	17.15^{k}	50.00^{f}	12.00^{g}	39.58^{e}	10.36^{gh}
Chandrakiran	16.67^{bc}	1.67^{ab}	79.33^{e}	17.48^{z}	0.00^{a}	0.00^{a}	73.33^{i}	15.57^{h}	42.33^{f}	8.68^{ef}
Alampur Benisan	22.67^{cd}	7.65^{cd}	78.00^{e}	17.27^{yz}	79.00^{kl}	19.47	28.33^{cd}	6.65^{de}	52.00^{gh}	12.76^{hi}
Dudhia Malgoa	14.33^{bc}	2.87^{bc}	81.33^{e}	15.93^{w}	78.00^{kl}	18.82^{kl}	67.33^{h}	16.08^{i}	60.25^{i}	13.43^{hi}
Jahangir-II	48.33^{de}	12.25	69.67^{de}	13.43^{tu}	28.67^{ef}	11.62^{h}	61.00^{g}	12.27^{g}	51.92^{gh}	12.39^{hi}
Kesar	4.67^{ab}	0.78^{ab}	21.67^{b}	6.62^{k}	0.00^{a}	0.00^{a}	21.00^{b}	4.03^{c}	11.83^{b}	2.86^{bc}
Champa	52.91^{de}	15.39	53.67^{cd}	11.25^{qr}	26.67^{de}	11.20^{h}	45.33^{e}	12.15^{g}	44.64^{fg}	12.50^{hi}
Kesington	11.67^{bc}	2.95^{bc}	47.33^{cd}	9.62^{no}	6.67^{ab}	2.55^{c}	0.00^{a}	0.00^{a}	16.42^{c}	3.78^{c}
Intemax	11.33^{b}	2.08^{ab}	43.33^{cd}	7.98^{m}	21.67^{de}	8.88^{f}	44.33^{e}	13.08^{gh}	30.17^{de}	8.01^{ef}
Illaichi	0.00^{a}	0.00^{a}	26.67^{bc}	5.93^{ij}	0.00^{a}	0.00^{a}	26.67^{cd}	5.08^{c}	13.33^{bc}	2.75^{c}
Lahutia	45.25^{d}	10.47^{ef}	45.00^{cd}	10.08^{op}	24.33^{de}	10.18^{g}	22.67^{cd}	6.37^{d}	34.31^{e}	9.28^{f}
Neeluddin	22.00^{bc}	3.07^{bc}	58.33^{cd}	12.87st	13.33^{abc}	6.35^{e}	0.00	0.00^{a}	23.42^{c}	5.57^{d}
Bhatuhi	60.67^{ef}	10.32^{e}	72.67^{de}	17.27^{xy}	3.33^{ab}	13.10^{i}	95.67	17.55^{ij}	60.33^{i}	15.01^{k}
Hybrid-51	7.00^{ab}	1.42^{ab}	35.33^{bc}	6.67^{k}	22.33^{de}	8.27^{f}	0.00^{a}	0.00^{a}	16.17^{bc}	4.09^{c}
Neelgoa	5.00^{ab}	0.48^{ab}	29.33^{bc}	4.45^{fg}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	8.58^{b}	1.23^{ab}
Jarda II	8.00^{ab}	1.05^{ab}	31.33^{bc}	4.80^{gh}	14.33^{abc}	6.20^{e}	27.67^{cd}	5.95^{cd}	20.33^{cd}	4.50^{c}
Mohan Thakur	9.67^{ab}	1.43^{ab}	26.33^{bc}	5.03^{gh}	3.33^{ab}	1.42^{b}	26.67^{c}	5.98^{cd}	36.50^{ef}	3.47^{c}
Swarnarekha - 2	5.00^{ab}	0.58^{ab}	42.33^{c}	8.17^{mn}	38.00^{fg}	17.15^{k}	75.67^{i}	15.45^{h}	40.25^{ef}	10.34^{gh}
Hybrid-13	26.33^{c}	3.75^{bc}	35.00^{bc}	7.57^{lm}	56.67^{ij}	23.10^{n}	0.00^{a}	0.00^{a}	29.50^{de}	8.60^{ef}
Hybrid-14	18.67^{bc}	4.30^{bc}	71.33^{de}	12.67st	25.00^{de}	9.30^{f}	53.33^{f}	12.12^{g}	42.08^{f}	9.60^{fg}
Hybrid-165	17.33^{bc}	4.08^{bc}	61.67^{d}	15.33	15.33^{abc}	0.28^{a}	51.00^{f}	11.92^{g}	36.33^{f}	7.90^{ef}
Hybrid-20	1.67^{a}	0.28^{a}	20.00^{b}	4.90^{gh}	17.00^{abc}	8.38^{f}	24.00^{bc}	5.43^{c}	15.67^{b}	4.75^{c}
Pulihara	38.67^{cd}	5.43^{bc}	53.33^{cd}	10.95^{q}	62.67^{ij}	27.23^{op}	66.67^{h}	17.27^{ij}	55.33^{gh}	15.22^{k}
Peter	20.33^{bc}	4.02^{bc}	62.00^{d}	12.52st	48.00^{gh}	24.65^{no}	78.33^{ij}	19.83^{j}	52.17^{gh}	15.25^{k}
Mithua Bihar	53.91^{de}	13.07^{f}	86.67^{e}	18.08	59.00^{ij}	28.95^{p}	79.33^{ij}	19.32^{j}	69.73^{i}	19.86^{m}
Hybrid-11/4	3.00^{ab}	0.00^{a}	0.00^{a}	0.25^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.75^{a}	0.06^{a}
Himayuddin	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
Gulabi	31.00^{bc}	8.12^{cd}	55.00^{cd}	14.78^{v}	76.67^{kl}	30.80^{pq}	76.33	20.55^{jk}	60.75^{hi}	18.56^{lm}
Rajapuri	19.00^{bc}	3.25^{bc}	20.00^{b}	4.00^{ef}	26.67^{de}	11.78^{h}	27.00^{c}	6.92^{de}	23.17^{cd}	6.49^{de}
Kalipari	20.33^{bc}	3.95^{bc}	49.67^{cd}	11.83^{qr}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	17.50^{c}	3.95^{c}

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	Per cent infested shoots*	No. of galls/ infested shoot	Per cent infested shoots	No. of galls/ infested shoot	Per cent infested shoots	No. of galls/ infested shoot	Per cent infested shoots	No. of galls/ infested shoot	Per cent infested shoots	No. of galls/ infested shoot
Sammar Bahist Chausa	5.33^{ab}	1.07^{ab}	0.00^{a}	0.00^{a}	17.67^{abc}	8.65^{f}	0.00^{a}	0.00^{a}	5.75^{a}	2.43^{b}
Fazri Zafrani	35.67^{cd}	7.58^{d}	52.67^{cd}	11.55^{qr}	71.00^{ijk}	16.25^{j}	77.33^{ij}	16.93^{i}	59.17^{h}	13.08^{hk}
Hybrid-115	12.67^{ab}	1.43^{ab}	41.00^{cd}	8.17^{mn}	0.00^{a}	0.00^{a}	23.00^{bc}	5.90^{cd}	19.17^{c}	3.88^{c}
Sahabale	0.00^{a}	0.00^{a}	79.33^{e}	15.92^{w}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	19.83^{c}	3.98^{c}
Sesar	21.33^{ab}	2.95^{bc}	81.67^{e}	17.28^{xy}	0.00^{a}	0.00^{a}	73.00^{i}	20.38^{j}	44.00^{f}	10.15^{g}
Surkhavarn	9.00^{ab}	1.58^{ab}	43.33^{cd}	9.48^{o}	1.00^{ab}	0.50^{a}	26.00^{c}	5.20^{c}	19.83^{c}	4.19^{c}
Jalal	0.00^{a}	0.00^{a}	61.67^{d}	16.00^{x}	0.00^{a}	0.00^{a}	33.67^{d}	8.43^{f}	23.83^{cd}	6.11^{de}
Pairi	6.00^{ab}	0.65^{ab}	53.33^{cd}	12.48^{s}	28.33^{ef}	10.53^{gh}	65.00	16.68^{i}	38.17^{ef}	10.09^{fgh}
Fernandin	8.00^{ab}	2.10^{ab}	71.67^{de}	12.90^{st}	28.00^{ef}	9.42^{fg}	24.00^{bc}	5.62^{c}	32.92^{de}	7.51^{e}
Papaya Khas- I	6.00^{ab}	0.85^{ab}	70.00^{de}	17.55^{z}	15.67^{abc}	4.85^{d}	0.00^{a}	0.00^{a}	22.92^{c}	5.81^{c}
Khiros Patti	1.00^{ab}	0.20^{a}	45.00^{cd}	8.82^{n}	0.00^{a}	0.00^{a}	25.67^{bc}	5.53^{c}	17.92^{c}	3.64^{c}
HARP Selection	23.00^{bc}	2.90^{bc}	69.00^{de}	18.23^{xy}	35.00^{fg}	10.95^{g}	29.67^{cd}	5.27^{c}	39.17^{ef}	9.34^{fg}
Asduith	22.00^{bc}	4.35^{bc}	58.00^{cd}	11.73^{rs}	0.00^{a}	0.00^{a}	44.67^{e}	11.50^{g}	31.17^{d}	6.90^{d}
Vanraj	9.00^{ab}	1.55^{ab}	86.67^{e}	20.22^{xy}	28.00^{ef}	8.68^{f}	0.00^{a}	0.00^{a}	30.92^{de}	7.61^{e}
Hur	7.00^{ab}	0.45^{ab}	34.67^{bc}	2.50^{cd}	0.00^{a}	2.00^{bc}	0.00^{a}	0.00^{a}	10.42^{b}	1.24^{ab}
Benisan	1.00^{ab}	0.25^{a}	27.33^{bc}	2.33^{cd}	0.00^{a}	1.50^{bc}	0.00^{a}	0.00^{a}	7.08^{b}	1.02^{ab}
Bombay Selection	7.00^{ab}	2.00^{ab}	73.33^{de}	18.98^{xy}	16.67^{abc}	4.15^{d}	70.00^{hi}	18.07	41.75^{f}	10.80^{gh}
Indonesia	42.00^{cd}	8.85^{cd}	75.00^{de}	19.65	45.33^{gh}	13.77^{i}	24.00^{bc}	5.82^{cd}	46.58^{f}	12.02
Amir Pasand	0.00^{a}	0.00^{a}	48.67^{c}	11.45^{qr}	24.67^{de}	6.93^{e}	0.00^{a}	0.00^{a}	18.33^{c}	4.60^{c}
Buponix	20.00^{bc}	2.75^{b}	86.67^{e}	19.52	28.33^{ef}	10.25^{g}	71.67^{hi}	16.13^{i}	51.67^{g}	12.16^{hi}
Alif Laila	47.60^{de}	9.50^{cd}	60.00^{cd}	11.07^{q}	21.67^{de}	6.37^{e}	70.33^{h}	17.67	49.90^{g}	11.15^{gh}
Baramasia Yogada	33.80^{cd}	4.61^{bc}	66.33^{de}	14.78^{v}	23.00^{de}	7.95^{ef}	47.33^{ef}	9.98^{f}	42.62^{f}	9.33^{fg}
Papaya Khas- II	45.45^{de}	8.50^{cd}	71.00^{de}	16.75^{y}	1.33^{ab}	0.60^{ab}	0.00^{a}	0.00^{a}	29.45^{de}	6.46^{de}
SE(m)	3.75	0.85	6.20	0.15	2.25	0.36	2.12	0.42	2.24	0.45
LSD (*P = 0.05*)	10.42	2.36	17.26	0.45	6.27	1.05	5.90	1.17	6.24	1.25
F calculated	17.63	15.62	11.79	1238.21	106.67	660.02	262.72	360.22	303.90	568.51
Error degree of freedom	396	396	396	396	396	396	396	396	396	396

*Value following different letter down the column are significantly different using Tukey's HSD test

proportional to initiation of inflorescence in shoot gall psylla infested mango trees (Raina and Srivastava, 2018). Therefore in the present study, 100 mango genotypes in four years were screened on the basis of per cent shoot infested and number of galls formed on infested shoot to understand the relative preference of *A. cistellata* to various mango genotypes. Earlier some preliminary work on varietal screening against *A. cistellata* has been done in Himachal Pradesh and Uttarakhand (Gupta *et al*., 1994; Singh, 2000). Singh (2000) studied the varietal preference based on two years data and screen out the 113 mango varieties against *A. cistellata* infestation and reported that 20 varieties viz., Annanas, Awain, Baramalda, Chinnaswaranrekaha, Delicious, Gulabkhas, K.O.7, K.O.-11, Makaram, Maharaja of Mysore, Mohamddi, Mundappa, Nowneetum, N x Panchadharakalsa, Panchadhara Kalsa, Police, Sonakullu, Salem Banglora and Vellakachi were categorized as resistant in field conditions.

Table 2. Categorizations of evaluated mango germplasms based on shoot gall psylla, *Apsylla cistellata* infestation during 2011-12 to 2014-15

Resistant category	Genotype/s	Number of genotypes
Category I (Resistant)	Hyder Sahab, Lucknow Selection, Mulgoa Hill, Arka Neelkiran, Lal Sinduria, Swarnajahangir, Hamlet, Hybrid- 11/4, Himayuddin, Hur, Benisan	11
Category II (Moderately resistant)	Kohitur, Bhadaiya Sukul, Arka Anmol, Kesar, Dilsad, Hybrid-51, Neelgoa, Kalipari, Sammar bahist chausa, Sahable, Surkhavarn, Khiros Patti, Amir Pasand	13
Category III (Susceptible)	Jahangir-I, Kala Pahar, Piyara Phulo, Bag-e-bahar, Sari Khas, Jarda, Mundappa Black, Black Andrew, Anfas, Kalapaddy, H/51/1, Iturba, Swarnrekha-1, Ratna, Neelphanso, Nileshwari, Barbelia, Dashehari Mahmooda, Bennet Alphanso, Ratnagiri Alphanso, Amini, Sabari, Bara Sinduria, Jawahar, Sindhu, Hybrid/27, Sabari-1, Chinnarasam, Swarnaguddi, Pedarasam, Totapuri, Nileshan, Neelam, Chandrakiran, Alampur Benisan, Dudhia Malgoa, Jahangir-II, Champa, Kesington, Intemax, Illaichi, Lahutia, Neeluddin, Hybrid-51, Jarda-II, Mohan Thakur, Swarnarekha-2, Hybrid-13, Hybrid-14, Hybrid-165, Hybrid-20, Pulihara, Peter, Rajapuri, Fazri Zafrani, Hybrid-115, Sesar, Jalal, Pairi, Fernandin, Papaya Khas-I, HARP selection, Asduith, Vanraj, Bombay Selection, Indonesia, Buponix, Alif Laila, Baramasia Yogada, Papaya Khas-II	70
Category IV (Susceptible)	Vastara, Hathi Jhool, Jhapatta, Mithua Bihar, Bhatuhi, Gulabi	6

We conclude that mango genotype, Himayuddin, Lal Sinduria, Mulgoa Hill and Hybrid- 11/4 grouped as resistant sources against *A. cistellata* in field condition based on four years continuous data observations.

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RESEARCH ARTICLE

Genetic Divergence Studies and Heterotic Grouping in Maize Inbred Lines using Microsatellite Markers

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Information on genetic diversity and relationship among different maize genotypes is very important in hybrid maize breeding program. The purpose of present study was to elucidate the nature and extent of differentiation and divergence among 18 inbred lines of maize based on the analysis of targeted microsatellite sites. Using 28 primer pairs, altogether 296 allelic variants including 145 shared and 151 unique alleles were detected amongst amplified products and a total of 49 loci were assigned with an average of 6.04 alleles per locus. Polymorphic information content of microsatellite primer pairs ranged from 0.34 (umc1304) to 0.93 (umc1179) with mean of 0.77 per primer. A remarkably higher level of genetic differentiation and divergence was revealed by the use of 28 microsatellite markers, which allowed unique genotyping and unambiguous classification of the maize inbred lines under evaluation. Among the inbred lines under molecular characterization, CML163 and CML467 appeared as the most diverse genotypes. Using the matrix of genetic similarity, the cluster analysis grouped the eighteen inbred lines into four heterotic groups. The markers utilized in the present study were sufficient for discrimination and unambiguous classification of inbred lines.

Key Words: Genetic diversity, inbred lines, microsatellite, maize, heterotic group

Introduction

Globally, maize is the third most important cereal after wheat and rice. It is cultivated in wide range of environments than wheat and rice because of its greater adaptability. Among the cereals, the productivity of maize is recorded to be the highest as compared to rice and wheat. Genetic diversity is an essential element for the genetic improvement and development of new inbred lines, hybrids and synthetic cultivars of maize. Assigning the parental lines into different heterotic groups is fundamental for the maximum exploitation of heterosis through hybrid cultivar development in a cross-pollinated crop like maize. Different methodologies have been used to characterize genetic diversity in the maize germplasm, which are morphological characters (Goodman *et al*., 1977), pedigree analysis (Duvick 1984), heterosis (Smith *et al*., 1989) and detection of variation at DNA level using markers. Morphological differences are usually determined by a small number of genes and may not be representative of genetic divergence in entire genome (Singh *et al.,* 1999; Brown-Guedira *et al.,* 2000).

Recently, molecular markers, which provide reliable and complementary information, have been used by the researchers for the purpose of characterization of inbred lines, assessment of genetic diversity and classification of inbred lines into heterotic groups. Molecular markers developed for the differentiation of genotypes and assessment of genetic diversity are reliable and remain unaffected across different growth stages, seasons, locations and agronomic practices (Efendi *et al*., 2015). Since expression is not influenced by environmental factors, the most important advantage offered by molecular marker is that the actual level of genetic difference can be determined between different genotypes including inbred lines. Amongst the molecular markers, microsatellite markers are regarded as useful tool to assess the genetic diversity among the different maize inbred lines and maize genetic resources. When microsatellites are individually amplified by means of the polymerase chain reaction using a pair of flanking unique oligonucleotides as primers, they almost invariably show extensive polymorphism due to site-specific length

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variation as a consequence of the occurrence of different numbers of repeat units.Microsatellites are widely used in maize, as these markers are genetically co-dominant, hyper-variable, highly polymorphic, abundant, robust, reproducible, distributed throughout the genome and amenable to automation (Dubreuil *et al*., 2006; Prasanna *et al*., 2010). Keeping into consideration that the use of microsatellite markers can help in assessing the nature and extent of genetic diversity among inbred lines, assigning inbred lines efficiently to heterotic groups and making the choice of heterotic parents to develop new hybrids, the present study has been conducted.

Materials and Methods

DNA extraction and Primer based amplification

The total genomic DNA was isolated from leaf samples of eighteen inbred lines (Table 1) of maize which were harvested from 4-5 leaf seedlings using standardized maize genomic DNA extraction protocol (Punya *et al*., 2017). 28 microsatellite primer pairs were chosen from MaizeDB (http://www.maizegdb.org/ssr.php) (Table 2). PCR amplification were performed in 15µl volumes containing 2µl of genomic DNA, 1U *Taq* DNA polymerase, 3µl dNTP (1mM), 1.5µl primer, 2.8µl nuclease free water,1.3µl $MgCl_2$ (10mM). Amplification consisted of initial denaturation for 5 min at 94^oC, 30 cycles of 94^0C (1 min), annealing at 52^0C-60^0C (1 min) and 72^0C (2 min), followed by a final extension at 72^0C (7 min). Amplification products were separated by electrophoresis in horizontal gel system at 110 V for 1 h 30 min on 2% agarose gel stained with ethidium bromide (10mg/ml) using 0.5X TBE buffer. The amplified products were visualized with the help of a gel documentation system (Alpha Innotech, USA) and the size of fragments was estimated with the help of 50bp ladder (Fermentas).

Table 1. List of inbred lines used in the present study alongwith their source

Sl. No.	Inbreds	Source
1.	CML 467	CIMMYT, Mexico
2.	CML 468	CIMMYT, Mexico
3.	CML 469	CIMMYT, Mexico
4.	CML 470	CIMMYT, Mexico
5.	CML 471	CIMMYT, Mexico
6.	CML 373	CIMMYT, Mexico
7.	CML 115	CIMMYT, Mexico
8.	CML 196	CIMMYT, Mexico
9.	CML 465	CIMMYT, Mexico
10.	LM 13	SRI, Coimbatore
11.	Dholi 2012	TCA, Dholi
12.	HKI 162	CCS HAU, Hisar
13.	HKI 323-B	CCS HAU, Hisar
14.	HKI 586	CCS HAU, Hisar
15.	HKI 1105	CCSHAU, Hisar
16.	CML 161	CIMMYT, Mexico
17.	CML165	CIMMYT, Mexico
18.	CML 163	CIMMYT, Mexico

Data analysis

Gel photographs were scored manually and bands were binary coded by 1 or 0 for their presence or absence in each genotype. The microsatellite primer scores were used to create a data matrix to analyze genetic relationships using the NTSYS-pc software (Rohlf 2000). The information pertaining to allelic diversity and suitability of SSR based polymorphism for identification of polymorphic and informative markers to characterize and differentiate maize inbred lines was generated on the basis of polymorphism information content (PIC) of primer pairs. Polymorphism information content (PIC) values were calculated manually for each SSR locus according to the formula as described by Smith *et al*., 1997.

$$PIC = 1-\sum f_i^{\,2}$$

Where, f_i is the frequency of ith allele

Genetic similarities among inbred lines were calculated on the basis of presence and absence of common bands. The genetic association among inbred lines were analysed by calculating the similarity coefficients (Dice 1945) for pair wise comparisons based on the proportions of shared bands produced by primers.

$$\text{Similarity coefficient} = 2a/(2a+b+c)$$

Two dimensional plot was constructed using two principle components selected by the NTSYS-pc software 2.1 to infer the level of gene similarity among the inbred lines.

Results and Discussion

Allelic diversity and polymorphism analysis

Altogether 296 allelic variants were detected amongst amplified products generated with 28 primer pairs. Amongst the primer pairs used during amplification reaction, ten primer pairs, namely, umc1266, phi072,

Table 2. Characterization of 28 microsatellite markers used for analysis of 18 maize inbred line

Marker	Bin	Repeat type	Locus	Size range(bp)	No. of alleles	No. of alleles per locus	Unique alleles	Shared alleles	**PP**	**PIC**
phi 227562	1	ACC	2	313-352	07	3.5	2	5	28.5	0.80
bnlg 1429	1	AG(20)	2	197-226	11	5.5	4	7	36.3	0.89
umc 1297	1	(GA)6	2	68-182	13	6.5	5	8	38.4	0.79
nc 133	2	GTGTC	2	62-152	15	7.5	5	10	33.3	0.71
phi 083	2	AGCT	3	66-168	14	4.6	6	8	42.8	0.75
phi029	3	AG/AGCG	2	79-190	11	5.5	6	5	54.5	0.84
phi 053	3	ATAC	2	180-22	16	8	8	8	50.0	0.80
umc1266	3	(CAG)4	1	139-170	11	11	6	5	54.5	0.88
umc1136	3	(GCA)5	2	134-163	06	3	3	3	50.0	0.66
phi072	4	AAAC	1	145-161	06	6	2	4	33.3	0.72
phi093	4	AGCT	1	286-345	11	11	7	4	63.6	0.89
nc 130	5	AGC	1	150-171	09	9	4	5	44.4	0.87
umc1332	5	(CTA)5	2	126-167	13	6.5	6	7	46.1	0.79
umc1152	5	(TCA)4	2	76-187	11	5.5	4	7	36.3	0.58
bnlg118	5	--	2	133-177	13	6.5	8	5	61.5	0.89
bnlg1136	6	AG(14)	2	203-240	11	5.5	5	6	45.4	0.87
umc1083	6	(GA)16	3	61-144	14	4.6	5	9	35.7	0.73
phi034	7	CCT	1	138-172	11	11	7	4	63.6	0.87
phi116	7	ACTG/ACG	1	169-185	08	8	2	6	25.0	0.85
umc 1304	8	(TCGA)4	2	56-155	09	4.5	2	7	22.2	0.34
umc1161	8	(GCTGGG)5	3	63-180	12	4	3	9	25.0	0.55
phi115	8	AT/ATAC	1	290-313	07	7	4	3	57.1	0.81
phi 014	8	GGC	2	64-182	08	4	5	3	62.5	0.70
phi065	9	CACTT	2	141-189	15	7.5	10	5	66.6	0.85
phi 084	10	GAA	1	157-195	10	10	7	3	70.0	0.83
umc1367	10	(CGA)6	2	167-255	11	5.5	7	4	63.6	0.79
umc1196	10	CACACG	1	150-171	07	7	4	3	57.1	0.72
umc1179	10	(AAG)4	1	188-214	06	6	3	3	50.0	0.93
	Total		49	-	296	-	151	145	-	21.7
	Average									0.77

phi093, nc130, phi034, phi116, phi115, phi084, umc1196 and umc1179 generated only one polymorphic amplified product. Interestingly, the primer pairs phi227586, bnlg1429, umc1297, nc133, phi083, phi029, phi053, umc1136, umc1332,umc1152, bnlg118, bnlg1136, umc1083, umc1304, umc1161, phi014, phi065 and umc 1367 generated more than one amplified product due to amplification of more than one locus, which might have resulted from the co-dominant nature of the microsatellite markers . A total of 49 loci were assigned to the 28 primer pairs with an average of 6.04 alleles per locus. The number of alleles ranged from 6 in case of umc1136, phi072, umc1179 to 16 in case of phi053 with a range between 56 to 352 bp (Table 2). A total of 145 shared and 151 unique allelic were detected by using 28 primer pairs. The number of unique alleles ranged from 2 to 10 while number of shared alleles ranged from 3 to 10. Average number of alleles per locus obtained in this study is more or less similar to the results obtained in previous studies conducted by several researchers in maize (Pejic *et al*., 1998; Li *et al*., 2002; Wu *et al*., 2004; Qi-Lun *et al*., 2008; Morales *et al*., 2010). Confirming the earlier reports (Smith *et al*., 1997; Pejic *et al*., 1998; Yuan *et al*., 2000; Senior

et al., 1998), the results of the present study also indicated that the microsatellite loci with di-nucleotide repeat unit were more polymorphic loci than that with tri-nucleotide repeat unit and generated more number of allelic variants per locus. The maximum polymorphism was observed in phi084 (70%) and minimum was observed in umc1304 (22.2%) microsatellite marker.

The Polymorphism Information Content (PIC) demonstrates the informativeness of SSR loci and their potential to detect differences among the inbred lines based on their genetic differences. In the present study, PIC values of microsatellite loci ranged from 0.34(umc1304) to 0.93(umc1179) with mean of 0.77 (Table 2), reflecting the presence of high allelic variation in the marker loci. Average value calculated for this parameter in the present study is very close to the value obtained by several earlier research workers (Hoxa *et al.*, 2004; Reid *et al*., 2011). The highest PIC value was observed in umc 1179 (0.93) whereas, lowest was observed in case of umc1161 (0.55). Considering the number of alleles generated by different primer pairs in conjunction with the level of polymorphism detected in the present study, the primers umc1297, phi053, umc1266, phi093, bnlg118, phi034, phi115, phi065 and phi084 appeared to be more informative primers (Fig. 1).

Genetic similarity and cluster analysis

Genetic similarity between pair-wise combinations of entries was calculated on the basis of allelic data obtained with regard to 296 allelic variants amongst 18 maize inbred lines. The magnitude of similarity coefficient ranged from zero to 0.52 (Table 3), indicating thereby the existence of ample genetic differences. Thus, the estimates of similarity coefficients indicated a considerably greater extent of variation among the inbred lines under evaluation in the present study and provided greater confidence for the classification and assessment of genetic relationships. Similar inference has been derived in the studies conducted on the molecular markers including microsatellite marker-based divergence analysis in maize by earlier researchers (Efendi *et al*., 2015; Li *et al*., 2002; Hoxa *et al*., 2004).

By drawing the phenon line at 25 similarity units in the dendrogram (Fig.2), the eighteen entries were divided into six clusters for the purpose of deriving inference about the pattern of divergence amongst the entries at the molecular level. Out of six clusters, three clusters, namely, cluster A, cluster C and cluster F were

Table 3. Estimates of microsatellite markers based Dice similarity coefficients among eighteen maize inbred lines used in the present study

Genotypes	CML467	CML468	CML469	CML470	CML471	CML373	CML115	CML196	CML465	LM13	DH2012	HKI162	HKI323B	HKI586	HKI1105	CML161	CML165
CML468	0.30																
CML469	0.14	0.38															
CML470	0.22	0.32	0.33														
CML471	0.18	0.19	0.22	0.45													
CML373	0.08	0.17	0.32	0.26	0.50												
CML115	0.11	0.11	0.03	0.27	0.36	0.29											
CML196	0.13	0.17	0.11	0.23	0.41	0.23	0.32										
CML465	0.13	0.08	0.14	0.14	0.19	0.11	0.14	0.40									
LM13	0.10	0.08	0.05	0.08	0.18	0.14	0.20	0.27	0.30								
DH2012	0.08	0.08	0.05	0.02	0.10	0.05	0.14	0.08	0.21	0.29							
HKI162	0.08	0.05	0.08	0.08	0.05	0.05	0.05	0.14	0.14	0.19	0.22						
HKI323B	0.00	0.02	0.05	0.05	0.02	0.02	0.05	0.02	0.08	0.16	0.19	0.20					
HKI586	0.02	0.06	0.09	0.15	0.08	0.00	0.00	0.08	0.02	0.05	0.11	0.15	0.26				
HKI1105	0.00	0.06	0.03	0.12	0.05	0.03	0.09	0.09	0.09	0.08	0.08	0.18	0.03	0.19			
CML161	0.02	0.14	0.02	0.08	0.08	0.08	0.08	0.11	0.05	0.05	0.08	0.11	0.08	0.08	0.23		
CML165	0.02	0.09	0.00	0.09	0.05	0.03	0.09	0.09	0.06	0.17	0.11	0.06	0.09	0.15	0.12	0.52	
CML163	0.05	0.02	0.02	0.05	0.02	0.00	0.00	0.02	0.02	0.00	0.00	0.05	0.11	0.20	0.15	0.25	0.27

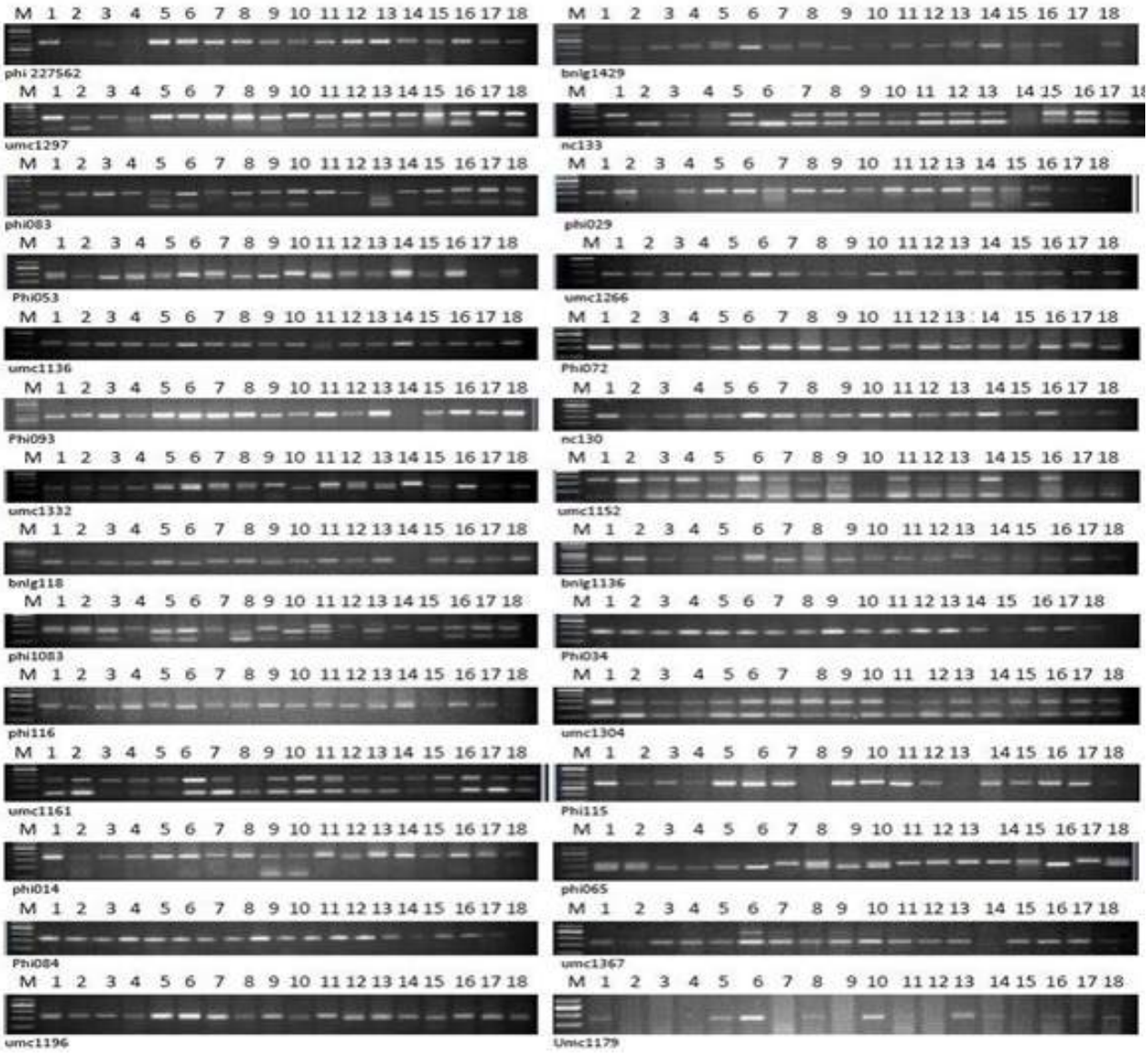

Fig. 1. Primer pairs dependent amplification of region of genomic in eighteen maize inbred lines used in the study

1. CML 467	4. CML470	7. CML115	10. LM13	13. HKI323B	16. CML161
2. CML468	5. CML471	8. CML 196	11. DH2012	14. HKI586	17. CML165
3. CML469	6. CML373	9. CML465	12. HKI162	15. HKI1105	18. CML163

tri-genotypic. Cluster B was mono-genotypic and cluster D was di-genotypic, whereas cluster E was multi-genotypic. At 50 and 75 similarity units, the cluster A was further sub-divided into cluster AI comprising of two entries, namely, CML 161and CML165, whereas cluster AII consisting of CML 163. Cluster B was mono-genotypic consisting of HKI1105. Cluster C was sub-divided into CI, CII and CIII accommodating LM13, DH2012 and HKI162, respectively. Cluster D was sub-divided into DI and DII comprising of HKI586 and HKI323B, respectively.

Keeping phenon line at 50 similarity units, the cluster E was divided into EI and EII. At 75 similarity units, sub-cluster EI was further sub-divided into sub-sub cluster EIa accommodating CML471 and CML373, sub-sub-cluster EIb containing CML470 and sub-sub-cluster EIc accommodating CML115. Similarly, EII was sub-divided into two mono-genotypic sub-sub-clusters EIIa and EIIb containing CML465 and CML196, respectively. At 50 similarity units, cluster F was divided into FI and FII. At 75 similarity units, sub-cluster FI was further sub-divided into FIa and FIb incorporating CML469

and CML468, respectively. Mono-genotypic sub-cluster FII contained CML467 (Table 4). Amongst pair-wise combinations of entries under evaluation in the present study, the magnitude of similarity coefficient between CML 165 and CML 161 was found to be the maximum, reflecting close similarity of these two inbred lines with respect to the regions of the genome targeted by the primer pairs used for amplification in the present study while CML 163 and CML467 appeared as the most diverse genotypes.

Principal component analysis

Principal component analysis was performed to examine the relationship among 18 inbred lines. Five principal components (Vectors) explained 18.64, 10.81, 8.43, 7.30 and 6.23 percentage of variability, respectively (Table 5). Principal component analysis represented the relative position and clustering pattern of inbred lines (Fig. 3), confirming the results obtained by cluster analysis. Several research workers have studied (Pebendon *et al*., 2008; Kashiani *et al*., 2013) divergence analysis on the basis of principal component analysis.

Obviously, the results of the present study clearly indicated that utilization of 28 microsatellite primer pairs in the analysis of maize inbred lines revealed a remarkably higher level of genetic polymorphism, which allowed unique genotyping of eighteen entries included in the analysis. The markers utilized in the present study were sufficient for discrimination and unambiguous classification of inbred lines.

Heterotic grouping on the basis of molecular markers

Parental line selection and breeding strategies for the successful and efficient hybrid development program are greatly facilitated by heterotic grouping of parental lines. Assigning lines to heterotic groups assists in avoiding the development and evaluation of crosses that should be discarded, allowing maximum heterosis to be exploited by crossing inbred lines belonging to different heterotic groups (Terron *et al*., 1997). Several approaches have been suggested and adopted for the classification of inbred lines into heterotic groups. Recently, microsatellite markers have been developed and used as a tool to assess the genetic diversity among inbred lines of maize and to assign them to different heterotic groups (Rajendran *et al*., 2014). The advantage of using molecular markers is the possibility of evaluating only the more promising crosses between the most divergent lines.

Table 4. Composition of clusters based on similarity coefficients for 28 primer pairs used for amplification in eighteen inbred lines of maize

Clusters identified at different phenon levels*			Entries included in each clusters
25	**50**	**75**	
A (3)	AI (2)	AI (2)	CML161,CML165
	AII (1)	AII (1)	CML163
B (1)	B (1)	B (1)	HKI1105
C (3)	CI (1)	CI (1)	LM13
	CII (1)	CII (1)	DH2012
	CIII (1)	CIII (1)	HKI162
D (2)	DI (1)	DI (1)	HKI586
	DII (1)	DII (1)	HKI323B
E (6)	EI (4)	EIa (2)	CML471,CML373
		EIb (1)	CML470
		EIc (1)	CML115
	EII (2)	EIIa (1)	CML465
		EIIb (1)	CML196
F (3)	FI (2)	FIa (1)	CML469
		FIb (1)	CML468
	FII (1)	FII (1)	CML467

Figures in parenthesis indicate number of entries in different clusters
*: Phenon levels indicate 25, 50 and 75 units of similarity coefficient

Table 5. Different characteristics features of principle components having Eigen value > 1

Component	Eigen value	Variance(%)	Cumulative Variance (%)
PC1	3.35695096	18.6497	18.6497
PC2	1.94598945	10.8111	29.4608
PC3	1.51797503	8.4332	37.8940
PC4	1.31469729	7.3039	45.1978
PC5	1.12140056	6.2300	51.4279

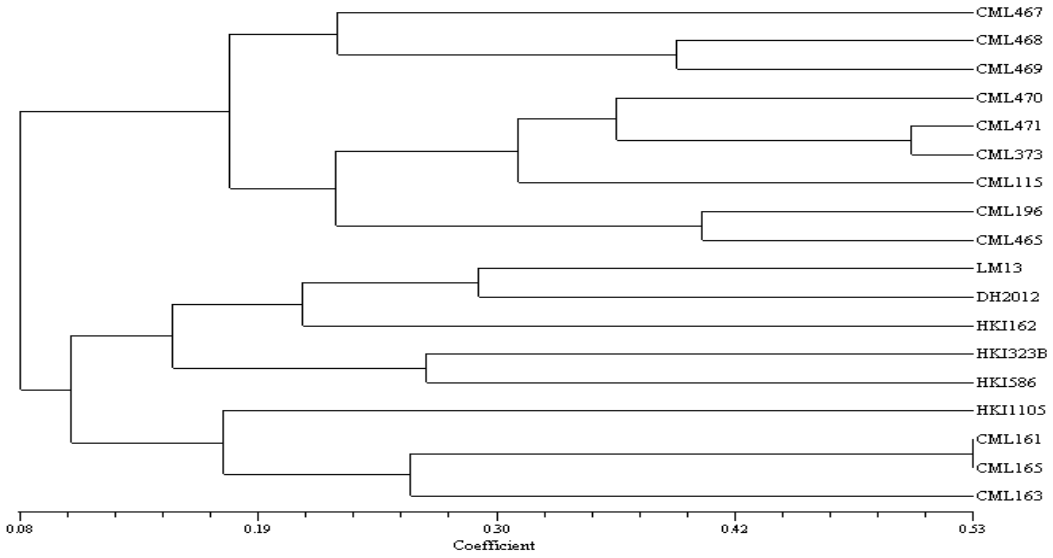

Fig. 2. Dendrogram based on Dice similarity coefficients for 28 microsatellite primer pairs among 18 maize inbred lines

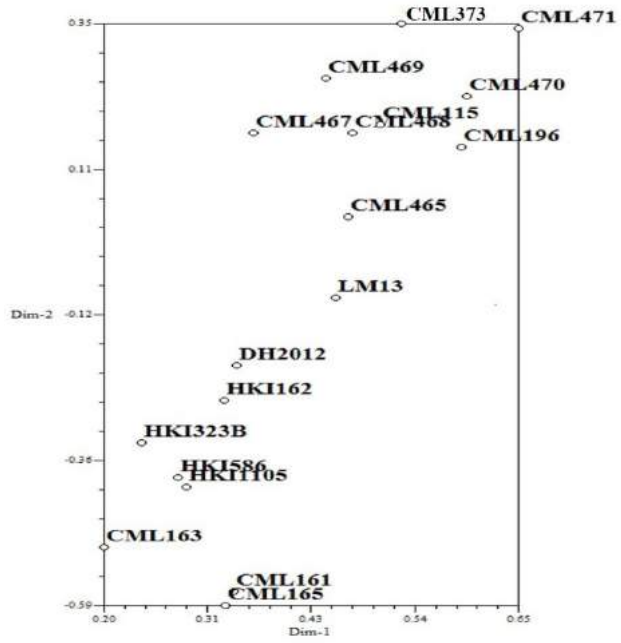

Fig. 3. Plot of the first two axes of a principal component analysis among 18 inbred lines studied

Using the matrix of genetic similarity, the cluster analysis grouped the eighteen inbred lines into four broad groups (Table 6). All the CML lines except CML161, CML165 and CML163 were included in group 1 in which there were nine inbred lines, namely, CML470, CML471, CML373, CML115, CML196 and CML465, CML467, CML468 and CML469. Heterotic group 2 consisted of inbred lines HKI323B and HKI586. Similarly, heterotic group 3 comprised of inbred lines LM13, DH2012 and HKI162. Heterotic group 4 accommodated the inbred lines HKI1105, CML161, CML165 and CML163. Microsatellite markers based discrimination and classification of inbred lines was found highly effective in heterotic grouping simply because remarkably greater number of inbred lines procured from the same source were placed in the same heterotic group.

Table 6. Heterotic grouping of inbred lines on the basis of molecular markers

Heterotic groups	Inbreds in groups
Group 1	CML470,CML471,CML373,CML115,CML196,CML465
	CML467, CML468, CML469
Group 2	HKI323B,HKI586
Group 3	LM13,DH2012,HKI162
Group 4	HKI1105,CML161,CML165,CML163

Conclusion

Understanding the genetic divergence pattern and relationship among inbred lines at genotypic level is of great importance for formulation and implementation of a systematic breeding plan for the purpose of further genetic improvement of maize. Altogether 296 allelic variants were detected amongst amplified products generated with 28 primer pairs. A total of 49 loci were assigned to 28 primer pairs with an average of 6.04 alleles per locus Considering the number of alleles generated by different primer pairs in conjunction with the level of polymorphism, the primers umc1297, phi053, umc1266, phi093, bnlg118, phi034, phi115, phi065 and phi084 appeared to be more informative primers. Among the inbred lines under molecular characterization, CML163

and CML467 appeared as the most diverse genotypes. A remarkably higher level of genetic polymorphism was revealed by the use of 28 microsatellite markers. Cluster analyses revealed that inbred lines CML 468 and CML469 are closely related to each other. Remarkably greater extent of similarity was also noticed between inbred lines HKI323B and HKI586.

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RESEARCH ARTICLE

Study on Flowering Behavior of Some Local Coconut (*Cocos nucifera* L.) Genotypes under Assam Condition

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Flowering behavior of thirteen local coconut genotypes was studied in the winter period (October-January) during 2013-14, 2014-15 and 2015-16 at ICAR-Central Plantation Crops Research Institute, Research Centre, Kahikuchi. Floral traits *viz*., inflorescence length, stalk length, number of spikelet per inflorescence, girth of stalk, length of spikelet bearing portion, number of male and female flowers, sex-ratio, duration of male and female flowers, intra-spadix overlapping and number of inflorescence produced per month were recorded. Analysis of the mean data, indicated significant differences for all recorded traits, except the number of inflorescences produced during the four months observation period. Average monthly inflorescence production per palm was higher in KKHC-4 (0.90) and KKHC-3 (0.81) genotypes.

Average female flower production (inferred from the number of female flowers per inflorescence) was found to be significantly higher in KKHC 3 (29.26) and KKHC 4 (27.69) genotypes, with lowest in KKHC 13 (6.85). Percentage of female to male flowers, among the genotypes varied from 0.60% (KKHC 3) to 0.27% (KKHC 13), with KKHC 3, KKHC 1 and KKHC 4 showing significantly higher ratio as compared to the rest of the accessions. Interestingly, though these are all tall genotypes, intra-spadix overlapping of the male and female phases was observed in all the genotypes, indicating the uniqueness of the coconut population in Assam, with higher duration of overlapping observed in KKHC 4 (3.60).

Key Words: Coconut, floral biology, traits, weather, genotypes

Introduction

Coconut (*Cocos nucifera* L.) is an important plantation crop grown in Assam mostly by small and marginal farmers. Area under coconut in Assam is estimated to be 20.71 thousand hectare with a production of 1,577.80 lakh nuts and productivity of 7,861 nuts/ha (CDB, 2018). Nagaon, Barpeta, Kamrup, Sonitpur, Nalbari, Golaghat, Cachar, Karimganj, Morigaon, Udalguri, Darang, Bongaigaon, Baksa and Shivsagar are the leading coconut growing and producing districts in Assam (Gopalakrishnan, 2013). Assam Tall is the most popular variety of coconut grown in this region. An annual average yield of about 105 nuts per palm per year has been reported in Assam Tall variety (Nath, 2017). However, winter season seems to be a limiting factor affecting coconut production (Patel, 1938). The climatic condition of Assam is characterized by warm humid sub tropical climate with prolonged summer and mild to severe winter. Winter season begins from October month with a fall in temperature below 20˚C. Coconut requires warm humid tropical climate with ideal temperature range from 26˚C to 27˚C and diurnal variation of 7-8˚C for its optimum growth and development (Marar and Pandalai, 1958; Child 1964). Effect of temperature in coconut palm has been reported by various workers (Thampan, 1981; Bhaskaran and Leela, 1983; Vanaja and Amma, 2002; Timothy, 2010). Towards development of improved varieties for specific agro-climatic zones, it is necessary to characterize the local coconut populations and identify adaptive features for utilization in the coconut improvement programme. Characterization of coconut populations/cultivars/germplasm has been undertaken based on fruit traits (Whitehead, 1968; Harries, 1978; Niral *et al.*, 2009), botanical and agronomic traits

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(Sugimura *et al*., 1997; Niral *et al.*, 2008). Descriptors of coconut germplasm have also been developed based on morphological characterization of the conserved accessions and farmers varieties (Bourdeix and Batugal, 2005; Bourdeix *et al*., 2010; Ratnambal *et al*., 1995, 2000). A better understanding of climatic factors and floral biology of coconut in specific regions will help to provide key information for choice of parents and adoption of breeding methods. Taking this into consideration, the present study was conducted to assess the flowering behavior of some local coconut genotypes in winter under Assam condition.

Material and Methods

Study was conducted for three consecutive years during 2013-14, 2014-15 and 2015-16 at ICAR-Central Plantation Crops Research Institute, Research Centre, Kahikuchi, Guwahati, Assam. Fifteen year old palms of thirteen coconut genotypes collected from Assam and field planted at the Research Centre were used for conducting the experiment. The experiment design was Randomized Block Design (RBD) with three replications. Three palms per replication were used for recording the data. Inflorescences produced in each palm were tagged during October-January and data for floral traits *viz*., inflorescence length, girth of stalk, stalk length, length of spikelet bearing portion, length of spikelet, number of spikelet per inflorescence, number of male and female flowers, number of inflorescence per palm, duration of male and female phase and intra-spadix overlapping of male and female phases were recorded as per coconut descriptors (Ratnambal *et al*., 1995, 2000). Data were statistically analyzed using MSTAT software.

Results and Discussion

Inflorescence length

Inflorescence length among the genotypes varied from 43.56cm to 67.55cm (Table 1). Genotype KKHC 4 recorded significantly higher inflorescence length (67.55cm) followed by KKHC 5 (65.54cm). Lesser inflorescence length was observed in KKHC 13 (43.56cm). Based on a study of 27 coconut accessions, comprising eight dwarfs and 19 talls, at ICAR-CPCRI Kasaragod (Niral *et al*., 2008) reported inflorescence length varying from 56-120cm, with tall accessions having relatively larger inflorescences than dwarf accessions. Further, seasonal variation in the number of spathes produced and opening of spadices has been reported (Vijayaraghavan *et al*., 1993). In the present study, most of the genotypes produced shorter length of inflorescence, possibly due to adverse growth conditions. Samanta *et al*. (2009) reported production of shorter inflorescences (39.54cm) during winter month in some coconut varieties in West Bengal.

Girth of inflorescence stalk

Girth of inflorescence stalk among the genotypes varied from 6.93cm to 9.55cm. Genotype KKHC 4 recorded the highest girth of inflorescence stalk (9.55cm), whereas KKHC 13 (6.93 cm) genotype produced inflorescence with least stalk girth. A relatively wider range of variation for inflorescence stalk girth (8.00-12.75cm) has been observed in coconut germplasm at ICAR-CPCRI Kasaragod (Niral *et al.,* 2008). The relatively smaller inflorescence stalk girth in the present study, is indicative of the relatively smaller size of the inflorescences produced.

Inflorescence stalk length

Pooled data (Table 1) showed that highest inflorescence stalk length was observed in genotype KKHC 5 (18.41cm) which was statistically at par with genotype KKHC 4 (17.32cm). Lesser inflorescence stalk length was observed in genotype KKHC 3 (12.23cm). Variation of floral traits in coconut has been reported by many workers (Panda, 1982; Patil *et al*., 1993; Kumaran *et al*., 2004; Niral *et al*., 2008), with much larger variation for stalk length. Samanta *et al*. (2009) observed seasonal variation in inflorescence stalk length with higher stalk length recorded during the month of January.

Length of spikelet bearing portion

Length of spikelet bearing portion among the genotypes varied from 28.24cm to 47.52cm. Genotype KKHC 4 (47.52cm) showed higher length of spikelet bearing portion, whereas, relatively lesser length of spikelet bearing portion was recorded in the

Table 1. Inflorescence characters of coconut genotypes

Accession	Inflore-scence length (cm)	Girth of inflore-scence stalk (cm)	Length of inflore-scence stalk (cm)	Length of spikelet bearing portion (cm)	Length of spikelet (cm)	No. of spikelets per inflore-scence	No. of male flowers per spikelet	No. of female flowers per inflore-scence	Duration of male phase (days)	Duration of female phase (days)	Flower sex-ratio (%)	Intra spadix over-lapping of male & female phases (days)	No. of inflore-scences produced/ palm
KKHC 1	57.33	8.43	16.57	38.28	30.30	33.10	142.77	23.52	31.48	5.45	0.53	1.61	0.69
KKHC 2	59.03	8.62	16.66	39.59	30.71	31.72	135.47	20.90	28.32	5.33	0.46	1.74	0.68
KKHC 3	49.85	7.78	12.23	35.20	26.77	28.70	125.72	29.26	28.86	4.76	0.60	2.21	0.81
KKHC 4	67.55	9.55	17.32	47.52	35.39	37.85	155.88	27.69	36.78	6.56	0.52	3.60	0.90
KKHC 5	65.54	7.40	18.41	44.22	33.03	37.19	149.60	20.61	35.67	5.39	0.38	2.03	0.70
KKHC 6	54.70	7.88	14.38	37.90	26.25	30.31	125.67	14.81	26.60	3.56	0.30	1.71	0.63
KKHC 7	47.83	7.62	12.67	32.59	23.78	27.13	119.27	17.22	24.47	3.90	0.40	0.85	0.57
KKHC 8	51.62	8.21	15.00	33.30	26.40	28.91	124.77	15.92	27.14	4.17	0.41	1.24	0.67
KKHC 9	53.40	8.33	14.91	35.87	27.52	31.28	132.03	18.31	30.02	4.45	0.40	1.82	0.68
KKHC 10	54.05	8.19	15.09	35.98	26.09	30.81	124.19	18.07	28.58	4.22	0.38	1.32	0.57
KKHC 11	49.17	8.06	13.08	33.77	24.80	27.19	111.96	14.89	26.74	3.69	0.37	1.21	0.63
KKHC 12	46.97	7.67	14.07	29.56	23.39	25.22	118.96	12.25	25.35	3.37	0.35	1.00	0.60
KKHC 13	43.56	6.93	13.40	28.24	19.27	22.03	94.80	6.85	21.59	2.35	0.27	0.18	0.55
SEm (+/-)	2.51	0.49	1.37	2.08	2.06	1.87	7.05	1.93	2.15	0.58	0.08	0.56	0.13
NS (0.05%)	7.35*	1.32**	4.00**	6.08**	6.01**	5.45*	20.53**	5.65*	6.27**	1.71**	0.24**	1.61*	0.37

*Significant at 5%, ** Significant at 1%

genotype KKHC 13 (28.24cm). Similar variations have been reported in coconut germplasm (Niral *et al*., 2008).

Spikelet length

Spikelet length differed significantly among the genotypes studied. KKHC 4 genotype recorded the highest spikelet length (35.39cm) as compared to other genotypes. Smaller spikelet length was recorded in genotype KKHC 13 (19.27cm). Among the conserved coconut germplasm at ICAR-CPCRI Kasaragod, spikelet length ranging from 27-55cm has been reported (Ratnambal *et al.*, 1995, 2000; Niral *et al.,* 2008). Spikelet length variation among coconut varieties and seasonal changes were also observed by Samanta *et al*. (2009).

Spikelets per inflorescence

Significant differences were observed for the number of spikelet produced per inflorescence. Genotype KKHC 4 (37.85) produced the highest number of spikelets per inflorescence, while lesser number of spikelets per inflorescence was recorded in genotype KKHC 13 (22.03). The findings are in agreement with those reported in coconut varieties by (Balakrishnan *et al*., 1988), wherein similar variation in number of spikelets (20-65) was observed.

Male flowers per spikelet

Number of male flowers per spikelet among the genotypes varied from 94.80 to 155.88. Genotype KKHC 4 (155.88) produced more number of male flowers per spikelet and was found to be statistically on par with genotypes KKHC 5 (149.60), KKHC 1 (142.77) and KKHC 2 (135.47). Lesser number of male flowers per spikelet was observed in genotype KKHC 13 (94.80). Ohler (1999) reported two to three hundred male flowers per spikelet.

Female flowers per inflorescence

Significant difference was observed for the number of female flowers produced per inflorescence. Average female flower produced among the genotypes varied from 6.85 to 29.26. Genotype

KKHC 3 produced higher number of female flowers per inflorescence. Variation with respect to female flower production among coconut varieties and environment condition has been reported by many workers (Marechal, 1928; Vanaja and Amma, 2002; Samanta *et al*., 2009; Niral *et al.,* 2017). Rao (1988) also observed seasonal variation among coconut varieties for number of female flowers produced.

Duration of male phase

Average length of male phase among the genotypes studied varied from 21.59 days to 36.78 days. Genotype KKHC 4 recorded the longest duration of male phase followed by genotype KKHC 5 (35.67). Shortest male phase duration was observed in genotype KKHC 13 (21.59 days). Sangare *et al*. (1978) and Peter (2002) reported duration of male phase varying from 19.5 to 22.7 days and 18-20 days, respectively. However, in the present study (Table 1), the relatively longer duration of male phase among the genotypes studied was observed during winter and might be related to the low temperature during the period of observation. The findings are in agreement with those reported by Rognon (1976) and Sangare *et al*. (1978) wherein they observed longer duration of male phase during cooler season than hot season.

Duration of female phase

Length of female phase in the coconut genotypes (Table 1) differed significantly among the genotypes. Genotype KKHC 4 recorded the longest duration of female phase (6.56). Ratnambal *et al*. (2003) reported longer duration of female phase in the accession Standard Kudat Tall (6.4) among the tall coconut types studied. They also reported seasonal variation changes in various coconut accessions studied.

Flower sex ratio

Percentage of female to male flowers among the genotypes varied from 0.60% (KKHC 3) to 0.27% (KKHC 13), with KKHC 3, KKHC 1 and KKHC 4 showing significantly higher sex ratio as compared to the rest of the accessions.

Intra spadix overlapping of phases

Intra-spadix overlapping of male and female phases was observed in all the genotypes studied. KKHC 4 recorded significantly higher period of intra-spadix overlapping of male and female phases (3.60) among the genotypes studied. While inter-spadix overlapping of male and female phases among tall coconut cultivars has been reported by various workers (Rognon, 1976; Samanta, 2009; Thomas and Josephrajkumar, 2013), in the present study, intra-spadix overlapping was recorded in all the genotypes. Ratnambal *et al*. (2003) observed a little influence of intra-spadix overlapping with seasonal changes in tall coconut types.

Inflorescence production

No significant difference was observed for the number of inflorescence produced during the four month period of observation among the genotypes studied. However, relatively higher number of inflorescence per palm was recorded in genotype KKHC 4 (0.90) as compared to other genotype. From the data (Table 1), it is evident that an average of one inflorescence is produced during this period and inflorescence production in the winter is low in most of the genotypes. Marar and Pandalai (1957) reported production of larger number of spadices during hot weather season as compared to other seasons. This might be attributed to low temperature (below 20˚C) and day length during the period affecting the photosynthetic efficiency (Wickremasurya, 1968; Siju Thomas *et al*., 2008). Louis (1981) also observed phenotypic variations for number of inflorescence produced in coconut varieties.

Correlation study among floral traits in coconut

Correlation coefficients among floral traits were calculated using the mean values (Table 2). Significant correlations were observed among the floral traits studied. The inflorescence length, girth of inflorescence stalk, length of spikelet bearing portion, spikelet length, spikelets per inflorescence and male flowers per spikelet showed significant correlation with the numbers of female flowers produced per inflorescence. Niral *et al*. (2008) also reported significant correlations among the floral traits, with number of female flowers in an inflorescence showing high significant positive correlation with the length of spikelet bearing position, but with

Table 2. Correlation among floral traits in coconut genotypes

Traits	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	X_{12}
X_1	1	0.63*	0.89**	0.98**	0.96**	0.97**	0.93**	0.63*	0.92**	0.88**	0.39	0.82**
X_2		1	0.46	0.64*	0.71**	0.65*	0.67*	0.61*	0.62*	0.75**	0.56*	0.75**
X_3			1	0.79**	0.82**	0.83**	0.80**	0.34	0.78**	0.71**	0.16	0.53
X_4				1	0.96**	0.97**	0.91**	0.70**	0.92**	0.89**	0.44	0.88**
X_5					1	0.97**	0.97**	0.77**	0.94**	0.96**	0.58*	0.87**
X_6						1	0.96**	0.71**	0.96**	0.91**	0.47	0.84**
X_7							1	0.76**	0.94**	0.94**	0.59*	0.85**
X_8								1	0.75**	0.87**	0.92**	0.83**
X_9									1	0.90**	0.54	0.87**
X_{10}										1	0.75**	0.86**
X_{11}											1	0.64*
X_{12}												1

X_1- Inflorescence length (cm)
X_2- Girth of inflorescence stalk (cm)
X_3- Length of inflorescence stalk (cm)
X_4- Length of spikelet bearing portion (cm)
X_5- Length of spikelet (cm)
X_6- Spikelets per inflorescence
X_7- Male flowers per spikelet
X_8- Female flowers per inflorescence
X_9- Duration of male phase (days)
X_{10}- Duration of female phase (days)
X_{11}- Flower sex-ratio (%)
X_{12}- Intra spadix overlapping of male and female phases (days)

*Significant at 0.05 level (2 tailed), **Significant at 0.01 level (2-tailed)

non significant but negative correlation with length of spikelet. Intra spadix overlapping of phases (days) showed significant positive correlation with the length of spikelet bearing portion, duration of male phase (days), duration of female phase (days) and flower sex ratio except for the inflorescence stalk length where no significant correlation was seen. The number of spikelets per inflorescence showed significant and positive correlation with the inflorescence length, girth of inflorescence stalk, inflorescence stalk length, length of spikelet bearing portion and spikelet length.

Conclusion

Seasonal changes affect the flowering and floral behavior of coconut palms. Under optimum condition, an adult, healthy coconut palm produces about 12 inflorescences per year. However, under Assam condition, winter stress has a detrimental effect on growth and development of the coconut palm and also the inflorescence production. Hence, understanding the floral biology of coconut genotypes will help the breeder in undertaking selection of desirable lines for crop improvement research and also planning crop management. In the present study, the genotype KKHC 4 recorded higher values for most of the floral traits studied *viz*., inflorescence length, girth of inflorescence stalk, length of spikelet bearing portion, length of spikelet, number of spikelets per inflorescence, number of male flowers per spikelet, duration of male phase, duration of female phase, intra spadix overlapping of male and female phase and number of inflorescences produced. However, higher number of female flowers per inflorescence and higher flower sex ratio was observed in KKHC 3. Therefore, considering the most important floral traits namely, number of inflorescences produced, number of female flowers and the sex ratio, the lines KKHC 4 and KKHC 3 appear to be promising for higher nut yield potential and are to be taken up for comparative yield evaluation trial, along with standard check varieties, in order to develop improved varieties suitable for commercial cultivation in Assam.

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SHORT COMMUNICATION

Morphological Characterization of Sweet Orange (*Citrus sinensis* Osbeck) Germplasm under Subtropical Conditions

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Characterization and evaluation of cultivars is basic key for better identification and breeding programmes. Experiment was carried out on eight cultivars in randomized block design with eight replications (one plant per replication) on five year old plants. Rootstock used was rough lemon, planted at a spacing of 3 x 6m. Plants were evaluated for their vegetative and floral characters. Vegetative characters like branch angle, shoot tip surface, shoot tip colour, vegetative life cycle, intensity of green colour, leaf lamina attachment were similar for all the genotypes depicting very less variability in qualitative characters. Significant variability was recorded among quantitative characters like rootstock diameter, leaf lamina length and width, floral characters and pollen viability.

Key Words: Floral characters, Morphological characterization, Phule mosambi, Sweet orange, Vegetative characters

Introduction

Citrus belongs to family *Rutaceae,* falls under subfamily *Aurantioideae.* There are mainly six species of the genus *Citrus* namely *C. sinensis, C. limon, C. aurantifolia, C. paradisi, C. reticulata* and *C. maxima*. Citrus covers approximately 976 thousand hectares area, with an annual production of 11717 thousand MT (NHB, 2017). Sweet orange has been reported to be originated in Southern China and, it was introduced to India during 13th century (Swingle and Reece, 1967). In India, North Eastern India is claimed as centre of origin for citrus species (Singh *et al.,* 2010).

Sweet orange (*C. sinensis*) is a hybrid between pummelo and mandarin (Wu *et al.,* 2014). The genetic diversity of *C. sinensis* is diminishing rapidly because of a number of factors, such as displacement of the natural gene pool due to selection and introduction of genotypes suitable for intensive horticulture resulting in a limited gene pool. Though, the study on different varieties of sweet oranges had already been done (Kumatkar *et al.,* 2016), still morphological characterization is initial step to check how different varieties behave differently in varied environmental conditions. Study of floral morphology is important for understanding self-incompatibility and pollen sterility, which have major role in fruit breeding and fruit set are essential pre-requisite for initiating any breeding programme. Besides, such information would also be useful in taxonomical studies (Randhawa *et al.,* 1961). As sweet orange is the most recognizable species among the citrus group (Novelli *et al.,* 2006). Within, sweet oranges there exists a large variability in characters, but much work had not been done on selections *i.e.* Kodour Sathgudi, M3, M4, M8, Phule mosambi and Shamouti. These genotypes were compared with Blood Red and Mosambi under north Indian conditions. So, the present investigation is attempted to study morphological characters of sweet orange germplasm, available at Punjab Agricultural University, Ludhiana, for assessing their genetic diversity and to find out the suitability of varieties Kodour Sathgudi, M-3, M-4, M-8, Shamouti, Phule mosambi, Blood Red and Mosambi for cultivation under Punjab conditions.

Materials and Methods

The present investigation was carried out at Punjab Agricultural University, Ludhiana to evaluate morphological characters of eight sweet orange (*C. sinensis* Osbeck) genotypes viz., Kodour Sathgudi, M-3, M-4,

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M-8, Phule mosambi, Shamouti, Blood Red and Mosambi. Source for these genotypes were Central Citrus Research Institute (CCRI), Nagpur. Age of trees was four years as it is plantation of September 2012 and they were planted at spacing of 6X3m using rough lemon as rootstock. Morphological characterization of sweet orange was done using descriptors based on International Plant Genetic Resources Institute, Italy (Anonymous, 1999).

The experiment was laid out in RBD with eight replications for each genotype and one plant per replication (Gomez and Gomez, 2010). Critical difference at 5% level of probability was computed to compare the statistical significance among different treatments. Analysis of variance was conducted for various quantitative traits using 9.3 version of SAS (Statistical analysis system) software.

The genetic diversity among sweet orange genotypes was computed on the basis of their morphological characters using computer software programme DARwin. The data were subjected to unweighted pair group's method with arithmetic mean (UPGMA) analysis to generate dendrogram (Perrier and Jacquemoud-Collet 2006).

Results and Discussion

Tree Characters

Smooth scion trunk surface and spheroid tree shape was recorded in all sweet orange genotypes**.** Tree growth habit was found erect in M-3, M-4, M-8 and Blood Red whereas tree growth was spreading in all other genotypes. Medium branching habit was observed in Shamouti, Blood Red and Mosambi whereas branch density was observed to be dense in all other genotypes. Branch angle was wide (more than 30^{o}) in all genotypes. Another study also corroborated with findings of present studies as results showed wide branch angle in all strains of Rangpur lime (Singh *et al.,* 2010). Spine density was medium in Blood Red and low in rest of the genotypes. Spine shape was recorded as straight in all genotypes. Similar results with straight shape of spine were recorded in six Rangpur lime strains (Singh *et al.,* 2010).Shoot tip colour was green and shoot tip surface was glabrous in all genotypes. Rootstock diameter was significantly higher in Kodour Sathgudi (126.29 mm) which was statistically at par with M-4 (121.50 mm) and Mosambi (120.91 mm). The minimum rootstock diameter was recorded in Blood Red (99.36 mm) which was significantly at par with Phule mosambi (102.22 mm). Ratio of scion and rootstock diameter do not vary significantly among all the genotypes. All genotypes were statistically at par with each other for scion: rootstock diameter ratio. Significantly higher spine length was recorded in Kodour Sathgudi (6.32 mm) followed by Blood Red (5.62 mm) and least was recorded in Mosambi (4.23 mm). Phule mosambi and M-3 were statistically at par with each other for spine length. However, there were no spines in Shamouti (Table 1).

Leaf Characters

Leaf lamina shape was ovate in Kodour Sathgudi, M-8, Mosambi and Phule mosambi whereas shape was elliptic in Blood Red, M-3, M-4 and Shamouti. Kodour Sathgudi, Blood Red and Shamouti had crenate shape of leaf lamina while sinuate shape was reported in all other genotypes. Leaf apex was acute in case of Blood Red and was obtuse Fin rest of the genotypes. Petiole wing was obdeltate in shape in case of Kodour Sathgudi and Blood Red where as it was obovate in rest of the genotypes. Fused petiole and lamina junction was observed in all genotypes. In similar studies, leaf petiole wing was present in all three varieties *viz*., bitter sweet orange,Yuma citrange and Sour orange (Jaskani *et al.,* 2006).

Leaf lamina length was significantly high in Shamouti (124.51 mm) followed by M-3 (113.34mm) and M-4 (105.61mm), while it was significantly lower in Kodour Sathgudi (94.16mm). M-4, M-8 and Phule mosambi were statistically at par with each other for leaf lamina length. Similarly, significant variation was recorded in leaf lamina width. Maximum leaf lamina width was observed in Shamouti (77.71mm) followed by M-3 (71.66 mm) whereas least leaf lamina width was observed in Mosambi (60.24mm). M-8 and Phule mosambi were statistically at par with each other for leaf lamina width. Non-significant difference for leaf lamina length: width ratio was observed in all cultivars except in M-3 and Phule mosambi which were at par with each other, while lowest ratio was observed in Blood Red (1.45) (Table 1). Similar studies were conducted on six Rangpur lime strains and maximum leaf lamina length: width ratio was recorded in Noreo (2.4), while minimum was in Marmalade (1.8) (Singh *et al.,* 2010). Significantly higher leaf lamina thickness was observed in Shamouti (0.49 mm) followed by M-4 (0.45 mm),

Phule mosambi (0.42 mm) and M-8 (0.42 mm) while leaf lamina thickness was observed to be significantly lower in Kodour Sathgudi (0.34 mm). However, Blood Red and M-3 were statistically at par with each other for leaf thicknes. The data in Table 1 revealed that among all sweet orange genotypes petiole wing width was recorded as significantly higher in Blood Red (4.98 mm) which was statistically at par with Shamouti (4.97 mm). Similar studies were carried out in 30 local pummelo accessions and reported that accession number five had the highest petiole wing width, while accession number 17 had minimum petiole wing width (Rahman *et al.,* 2003).

Flower Characters

Ambe bahar was observed as main flowering season. Colour of fully opened flowers was white. Similar results were reported in Assam lemons, where all open flowers were white in colour (Nath, 1999). Pale yellow coloured anthers were noted in Shamouti and Kodour Sathgudi whereas yellow colored anthers were recorded in case of Blood Red, M-3, M-4, M-8, Phule mosambi and Mosambi. Earliest flower initiation was recorded in M-3 (2nd March 2017) flowering had initiated at last in Shamouti (15th March 2017) whereas Shamouti was the first to terminate its flowering by 30th March 2017 and last to terminate was Blood Red, Kodour Sathgudi, M-3 and Phule mosambi (2nd April 2017). Maximum duration of flowering was reported in Phule mosambi (31 days) and it was minimum in case of Shamouti (15 days) (Table 2).

Significantly higher flower length was recorded in M-8 (25.84mm) whereas flower length was low in M-3 (19.10mm) which was statistically at par with Blood Red. Similar studies were carried out on 20 sweet orange genotypes. Flower length was recorded to be maximum in Campbell Valencia (41.21 mm) and minimum flower length was reported in Sanguinelli (25.69 mm) (Baswal *et al.,* 2015). Flower diameter was recorded to be highest in Mosambi (39.05 mm) followed by M-4 (36.22 mm) and Phule mosambi whereas flower diameter was recorded significantly lesser in Shamouti (23.67 mm). Similar variation in 20 sweet orange genotypes were recorded in which, maximum flower diameter was recorded in Campbell Valencia (41.21 mm) and minimum was recorded in Sanguinelli (25.69 mm) (Baswal *et al.,* 2015). Calyx diameter was significantly higher in Mosambi (7.15mm) and least in Shamouti (4.88 mm). Staminate flower percentage varied significantly in different sweet orange genotypes (Table 2). Percentage of staminate flowers was observed to be significantly higher in Phule mosambi (11.97 %) and significantly low percentage was observed in Mosambi (6.62 %). The data pertaining to perfect flower percentage of sweet orange revealed that significantly higher percentage of perfect flowers were recorded in Mosambi (93.37%) while lowest perfect flower percentage was recorded in Phule mosambi (88.02%). Similarly, pollen viability was significantly higher in Mosambi whereas significantly lower pollen viability was recorded in Shamouti (32.47). However, Blood Red and Kodour Sathgudi were statistically at par with each other for pollen viability (Table 2). Similar

Table 1. Quantitative tree characters of sweet orange genotypes

Genotypes	RD (mm)	RSRD	SL (mm)	LLL (mm)	LLW (mm)	LLLW	LT (mm)	**PWW (mm)**
Kodour Sathgudi	126.29^{a}	1.03^{a}	6.32^{a}	94.16^{e}	60.32^{e}	1.56^{ab}	0.34^{e}	3.93^{c}
M-3	109.41^{b}	1.02^{a}	4.55^{ef}	113.34^{b}	71.66^{b}	1.58^{b}	0.44^{bc}	4.35^{b}
M-4	121.50^{a}	1.06^{a}	5.33^{c}	105.61^{c}	64.66^{d}	1.63^{a}	0.45^{b}	3.96^{c}
M-8	105.78^{bc}	1.08^{a}	4.85^{d}	106.75^{c}	67.00^{cd}	1.59^{a}	0.42^{c}	3.77^{cd}
Phule mosambi	102.22^{cd}	1.09^{a}	4.56^{e}	106.67^{c}	66.40^{cd}	1.60^{a}	0.42^{c}	3.63^{d}
Shamouti	109.51^{b}	1.06^{a}	0.0	124.51^{a}	77.71^{a}	1.60^{a}	0.49^{a}	4.97^{a}
Blood Red	99.36^{d}	1.05^{a}	5.62^{b}	100.58^{d}	69.06^{bc}	1.45^{c}	0.43^{bc}	4.98^{a}
Mosambi	120.91^{a}	1.04^{a}	4.23^{f}	100.48^{d}	60.24^{e}	1.66^{a}	0.39^{d}	3.76^{cd}

Abbreviations: RD: Rootstock diameter, RSRD: Ratio of scion: rootstock diameter, SL: Spine length, LLL: Leaf lamina length, LLW: Leaf lamina width, LLLW: Leaf lamina length:width, LT: Leaf thickness, PWW: Petiole wing width

studies were conducted on seven genotypes of lemon, highest pollen viability was reported in Meyer variety (86.74%) followed by Kutdiken (69.22%) and least was in case of BATEM Sarisi (Demir *et al.,* 2015).

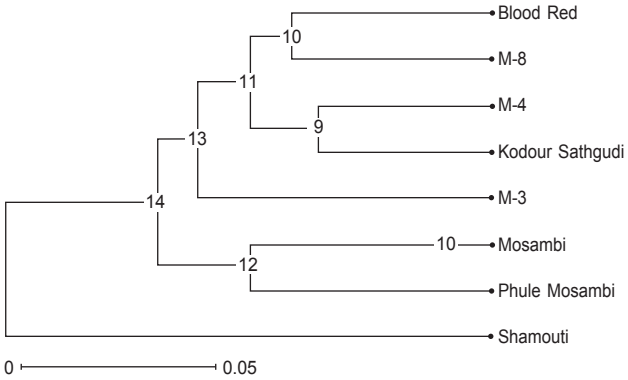

Fig. 1. Dendrogram illustrating genetic relationship among eight sweet orange genotypes generated by UPGMA tree analysis based on morphological traits. Cluster-I (Shamouti)and Cluster-II (PhuleMosambi, Mosambi, M-3, KodourSathgudi, M-4, M-8 and Blood Red).

Non-significant difference was observed in number of petals and sepals in different sweet orange genotypes. Significantly higher petal length was recorded in M-8 (21.26 mm) followed by Phule mosambi (20.05 mm) while least petal length was reported in Blood Red (16.40 mm). However, M-3 and M-4 were statistically at par with each other for petal length. Significantly higher petal width was observed in Mosambi (7.93 mm) and significantly lower petal width was recorded in Shamouti (5.28 mm). Length of style varied significantly and was recorded to be highest in Mosambi (6.69 mm) whereas significantly lesser style length was recorded in Shamouti (4.71 mm) (Table 2).

Similarly, pedicel length was highest in Blood Red (7.45mm) which was statistically at par with Mosambi (7.38mm) and lowest pedicel length was recorded in Shamouti (5.36mm). Similar variation in pedicel length was recorded. The pedicel length of Mosambi was 5.2 mm whereas it was 16.5 mm in case of Red fleshed pummelo (Singh, 2006). Highest filament length was recorded in Phule mosambi (11.16 mm) while significantly lower filament length was recorded in Shamouti (7.63 mm). Similar studies on grapefruit were conducted and variability in length of filament was reported (Baswal *et al.,* 2015). Similarly, pedicel length was highest in Blood Red (7.45 mm) which

Table 2. Quantitative flowering characters of sweet orange genotypes

Geno-types	SDF	FBDF	EDF	DF (days)	FL (mm)	FD (mm)	CD (mm)	PF (%)	SF (%)	PV (%)	PL (mm)	PW (mm)	LS (mm)	LF (mm)	PDL (mm)	NS
Kodour Sathgudi	6th March	21st-24th March	2nd April	27	18.66^{d}	27.87^{e}	5.81^{c}	93.30^{a}	6.70^{e}	53.50^{d}	15.68^{f}	6.29^{ef}	6.05^{bc}	8.35^{d}	6.67^{b}	21.62^{c}
M-3	2nd March	18th-22rd March	2nd April	30	19.10^{d}	33.54^{c}	6.05^{bc}	92.01^{cd}	7.99^{bc}	33.50^{f}	17.76^{d}	7.25^{b}	5.76^{cd}	9.06^{c}	5.97^{c}	24.00^{a}
M-4	8th March	20th-24th March	1st April	24	21.46^{c}	36.44^{b}	6.02^{bc}	92.62^{b}	7.38^{d}	58.23^{c}	18.10^{d}	6.67^{de}	5.42^{d}	9.16^{c}	5.65^{cd}	23.00^{b}
M-8	4th March	19th-24th March	1st April	28	25.84^{a}	32.82^{c}	6.26^{b}	91.62^{d}	8.38^{b}	46.37^{e}	21.26^{a}	7.16^{bc}	6.00^{bc}	10.58^{b}	6.84^{b}	22.62^{b}
Phule mosambi	3rd March	21st-26th March	2nd April	31	24.09^{b}	36.18^{b}	5.98^{bc}	88.03^{e}	11.97^{a}	68.94^{b}	20.05^{b}	6.84^{cd}	6.21^{b}	11.16^{a}	5.91^{c}	21.37^{c}
Shamouti	15th March	22nd-26th March	30th March	15	15.56^{e}	23.67^{f}	4.88^{d}	92.05^{cd}	7.95^{bc}	32.47^{g}	14.98^{f}	5.28^{g}	4.71^{e}	7.63^{e}	5.36^{d}	19.75^{d}
Blood Red	8th March	24th-28th March	2nd April	25	19.38^{d}	30.96^{d}	5.74^{c}	92.25^{bc}	7.75^{cd}	53.14^{d}	16.40^{e}	6.25^{f}	5.02^{e}	8.87^{c}	7.45^{a}	20.37^{d}
Mosambi	9th March	16th-22nd March	31st March	22	24.69^{b}	39.05^{a}	7.15^{a}	93.38^{a}	6.62^{e}	77.17^{a}	18.88^{c}	7.93^{a}	6.69^{a}	11.03^{a}	7.38^{a}	22.37^{b}

Abbreviations: SDF: start date of flowering, FBDF: full bloom date of flowering, EDF: end date of flowering, DF: duration of flowering, FL: flower length, FD: flower diameter, CD: calyx diameter, PF: perfect flower, SF: staminate flower, PV: pollen viability, PL: petal length, PW: petal width, LS: length of style, LF: length of filament, PDL: Pedicel length, NS: number of stamens.

Table 3. Distinguish characters

Genotypes	Flower length	Pollen viability	Length of filament
Phule mosambi	24.09 mm	68.94 %	11.16mm

was statistically at par with Mosambi (7.38 mm) and lowest pedicel length was recorded in Shamouti (5.36 mm) (Table 2). Similar variation in pedicel length was recorded by Singh (2006), he found pedicel length of Mosambi was 5.2 mm whereas it was 16.5 mm in case of Red fleshed pummelo. Significantly higher number of stamens was recorded in M-3 (24.00**)** and least was in Shamouti (19.75) and Blood red (20.37). Similar studies on floral morphology of mandarin genotypes was conducted and highest number of stamens were reported in Tsirang and Dagana (14.9) and lowest were (14.6) in Samste (Dorji and Yapwattanaphun, 2011).

Diversity Analysis

All the eight sweet orange genotypes were divided into two clusters. Cluster-I comprised of only one genotype (Shamouti) and in Cluster-II seven genotypes were clustered (Phule mosambi, Mosambi, M-3, Kodour Sathgudi, M-4, M-8 and Blood Red).

On the basis of this cluster analysis study, all the eight genotypes were grouped into different clusters, irrespective of their geographical origin. Similar studies on morphological variation indicated that the mandarin genotypes under study were consisted of phenotypically different individuals (Singh *et al.,* 2016). The difference in individuals could be attributed to cross pollination and mutation.

Conclusion

The study concluded that there is not much variation in qualitative parameters of all the sweet orange genotypes. However, existence of wide variations in quantitative characters was observed during the investigation. The variations are indicative of the underlined genetic diversity in sweet orange germplasm which will be very useful in citrus breeding and crop improvement programme; evergreen vegetative cycle, longer flowering period and higher pollen viability are some of the useful characters for further breeding programmes.

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SHORT COMMUNICATION

Collecting Plant Genetic Resources from South-eastern Ladakh, India

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For the first time, systematic germplasm collection trip was conducted across south-eastern Ladakh during September 2018. A total of 76 accessions belonging to 21 species including 44 accessions of wild germplasm were collected from the altitude of 3,400-5,300 meters above mean sea level. Good variability in hull-less barley, and wheat wild relatives namely *Elymus nutans* Griseb. and *Leymus secalinus* (Georgi) Tzvelev was augmented from this tract for the first time. Germplasm of *Chenopodium karoi* (Murr) Aellen was collected for the first time in India. The commonly occurring *Elymus nutans* is replaced by *E. schrenkianus* (Fisch. & C.A.Mey.) Tzvelev in altitudes above 4,700 m while *E. repens* (L.) Gould was found upto 4,300 m, which was otherwise known only below 3,000 m. This communication briefly discusses on the germplasm collected/observed and future exploration scope from this remote area.

Key Words: Cold-arid Himalaya, Crop wild relatives, Germplasm collection, Leh district, Purple barley

South-eastern part of Union Territory of Ladakh encompasses three (out of eight) tehsils of Leh district, namely, Nyoma, Kharu and Durbuk. This remote part of Ladakh shares borders with China (Tibet) in the east, Kargil district in the west, and Lahaul and Spiti district of Himachal Pradesh in the south. Broadly falling under Trans-Himalayan biogeographic zone as well as cold-arid agroclimatic zone, this region is significant for the occurrence of crop wild relatives belonging to the genera *Allium*, *Avena*, *Carum*, *Cicer*, *Elymus*, *Eremopyrum, Hordeum* and *Prunus* (Arora and Nayar, 1984). However, this part of Ladakh has not been systematically explored for plant genetic resources collection, as evident by the meagre germplasm holdings (<10 accessions) from this vast stretch of ecologically fragile area, swayed by the vagaries of climate change. Keeping this in view, an expedition for collecting germplasm of agri-horticultural crops and their wild relatives was undertaken during September 2018.

Tehsils of Nyoma, Kharu and Durbuk together comprise of 39 villages with a total population of around 25,000 (population density 3/km^2); majority of them embracing Buddhism. Upper region (>4,500 m) has permaforst climate, while lower region is slightly warmer with intense solar radiation. Rainfall is scanty, most of the precipitation is obtained through snow. Soil is mostly of rocks and sands. Landscape is arid desert-like with low temperatures leading to sparse vegetation. Nevertheless, afforestation with multipurpose tree species such as willows and poplars is common along roadsides, river banks and near oasis. Source of irrigation for crops is snow-melt *nallahs*, *kuhls* and streams. Usually spring wheat (in areas below 3,500 m) and barley are the staple crops in the areas. Common fruit trees include apple, apricot and walnut. Livestocks such as pashmina goats, sheep, cattle, horses and yaks are reared here.

Random sampling was followed while collecting germplasm in the field as well as from farm store, whereas, in case of wild germplasm, small samples from adjoining areas were often bulked. The germplasm of wild species was collected from roadsides, field margins, grasslands, salty marshes, sand dunes, screes and rock crevices. Passport data sheet was filled in as per standard format (Moss and Guarino, 1995) at each collection

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site. The collected germplasm and important herbarium voucher specimens were deposited in the National Gene Bank (NGB) and the National Herbarium of Cultivated Plants (NHCP), ICAR-NBPGR, New Delhi, respectively.

Explored areas are located at latitudes between 32°56'220" and 33°43'737" N, longitudes between 77°35'615" and 78°38'641" E, and altitudes between 3,400 and 5,300 m amsl. A total of 76 germplasm accessions (21 taxa) had been collected from 28 collection sites (Fig. 1). This includes 32 accessions of crops and 44 of crop wild relatives (Table 1). Barring barley and rapeseed, all the germplasm were augmented for the first time from this area.

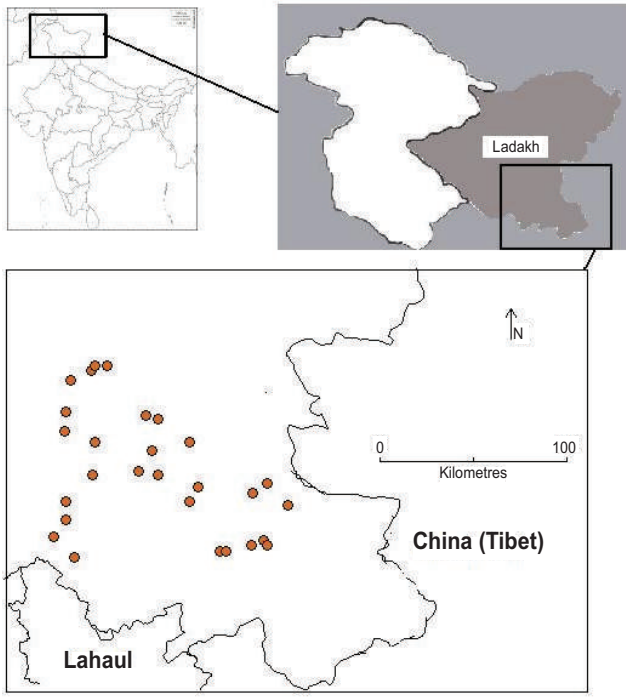

Fig. 1. Location of explored area in Ladakh, India (collection sites indicated)

In general, barley is the staple food crop of native people and is being cultivated in altitudes upto 4,400 m amsl. It is relished in the form of "*satu*". Cultivated barleys are of naked grain-type (i.e. hull-less barley); amber, black/grey and purple grain colours were present (Fig. 2A), with the preponderance of black colour. Tibetan purple barley is sparingly cultivated along with normal barley without any distinction (Fig. 2B). Normally lemma and palea of its spikelets become purple due to anthocyanin pigments, however, a variant of purple barley with green lemma awn (PRVJ-91) was also collected from Rango village. Although local people didn't distinguish grain colour variation relating to utility, literature search revealed that, in Tibet, purple barley is preferred for extracting alcoholic beverage '*chang*' owing to distinct flavour and colour (Tashi *et al*., 2012). Choo *et al*. (2005) reported that purple barley had a lower *Fusarium* head blight incidence than normal barley under high disease incidence conditions. Two-rowed barley was rarely found in Sumdo and Rango villages, where it seemed to be an accidental admixture in barley/oats crops, and matures two weeks later than naked barley. Upto 3,800 m amsl, stripe/yellow rust incidence was noticed in barley; above this altitude, incidence was not discernible; therefore, germplasm from higher altitudes, needs to be studied whether the observed field tolerance/resistance is innate or merely due to prevailing harsh weather conditions.

Only at Liktse village (3,500 m), wheat cultivation was observed, and red and amber grain types were collected; above the altitude of 3,600 m, wheat crop is replaced by barley. Both stripe/yellow and leaf rust were prevalently found in wheat crop. Rapeseed, field pea, lentil, buckwheat, potato and oats (as fodder) are the only few choices of other crops cultivated. In fieldpea, black (locally called *kalamattar*) as well as mottled brown seed types were collected. Mountain spinach, carrot, sweet chard/mangel and coriander were found only under homestead cultivation. Squashes such as zucchini (*Cucurbita pepo* L.) and scallop squash (*C. pepo* L. subsp. *ovifera* (L.) D.S. Decker) were rarely found in home gardens.

Among the wild germplasm, wild relatives of wheat and barley, i.e. *Elymus* spp. (21 acc.) and *Leymus secalinus* were predominant in the collection. *Elymus nutans* is the most common species found in field boundaries, unattended fields, ungrazed sites, roadsides and also in mountain slopes across the altitudes surveyed. *Elymus dahuricus* Turcz. ex Griseb. is rarely observed in field boundaries, orchards and roadsides. *Elymus himalayanus* (Nevski) Tzvelev is specifically common in tract between Sarchu and Pang. *Elymus longearistatus* (Boiss). Tzvelev is habitat-specific and is occasional along roadsides and hillslopes. A niche-specific species, *Elymus schrenkianus*, was found occurring gregariously in altitudes above 4,700 m amsl (Fig. 2E), replacing *E. nutans*. In Sarchu (4,100 m) and Tsaga (4,300 m), authors found *E. repens*, a rhizomatous self-incompatible species inhabiting disturbed habitats, which is the

Table 1. Germplasm collected from south-eastern Ladakh, India

Sl. No.	Species	Acc.	Remarks
Cereals		**18**	
1.	*Avena sativa* L.	3	Exclusively grown as fodder
2.	*Hordeum vulgare* L.	11	Grain colour variation noticed
3.	*Triticum aestivum* L.	4	Red and amber grain types observed
Pseudocereals		2	
4.	*Fagopyrum esculentum* Moench	1	Informed by locals as non-traditional crop
5.	*Fagopyrum tataricum* (L.) Gaertn.	1	Of rare cultivation
Grain legumes		4	
6.	*Lens culinaris* Medik.	1	Found only in Tsaga village
7.	*Pisum sativum* L. var. *arvense* (L.) Poir.	3	Characterised by pink flowers
Oilseeds		5	
8.	*Brassica napus* L.	5	Commonly cultivated as pure crop
Vegetables		**2**	
9.	*Artiplex hortensis* L.	1	Green plant type preferred
10.	*Daucus carota* L.	1	---
Spices		**1**	
11.	*Coriandrum sativum* L.	1	Small-sized seeds; highly fragrant
Crop wild relatives		**44**	
12.	*Allium przewalskianum* Regel	5	Occurs in wider ecological conditions - rocky crevices, meadows, disturbed habitats
13.	*Carum carvi* L.	4	Abundant in areas east of Nyoma
14.	*Chenopodium karoi* (Murr) Aellen	1	Big sized seeds; of potential use in chenopod improvement
15.	*Elymus dahuricus* Turcz.	2	Exhibits late spike maturity
16.	*Elymus himalayanus* (Nevski) Tzvelev	2	Characterized by thin, easily breakable rachis and straight long lemma awn
17.	*Elymus longearistatus* (Boiss.) Tzvelev	1	Has disarticulate rachis
18.	*Elymus nutans* Griseb.	10	Variability with respect to to plant stature, pigmentation, spike density and length, number of spikelets/ node, and awn length and colour
17.	*Elymus schrenkianus* (Fisch. & C.A.Mey.) Tzvelev	4	Niche-specific elegant species, characterised by pigmented robust spikes
18.	*Elymus* spp.	2	Identity of two entities yet-to-be established
19.	*Fagopyrum tataricum* (L.) Gaertn. subsp. *potaninii* Batalin	1	Ruderal type, characterised by short stature, seeds small with undulate/protruded margin
20.	*Leymus secalinus* (Georgi) Tzvelev	11	Variability with respect to pigmentation, number of spikelets/ node and plant stature
21.	*Setaria viridis* (L.) P.Beauv.	1	Common field weed; variation with respect to pigmentation

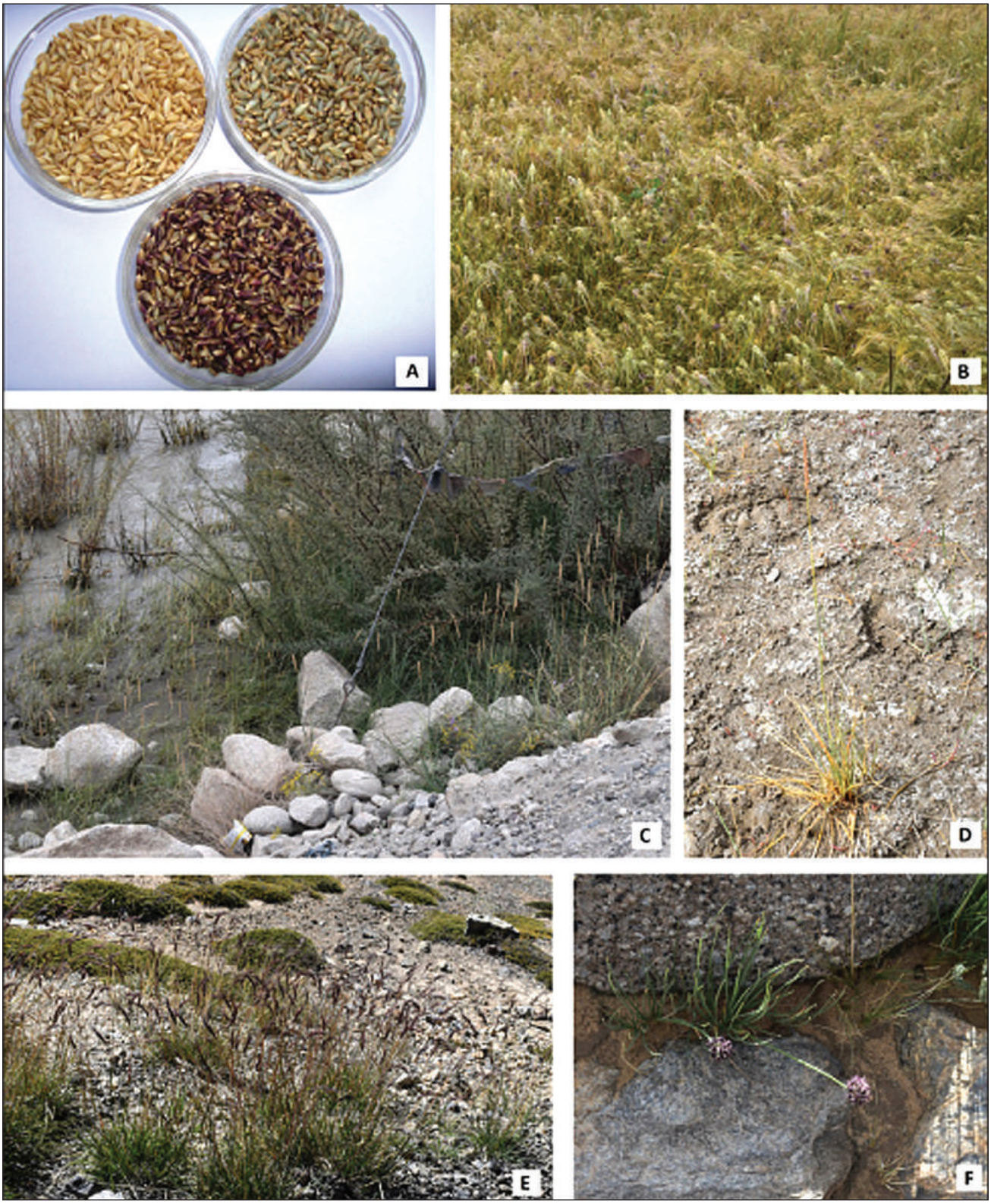

Fig. 2. A. Normal (amber), black/grey and purple grain colours in naked barley accessions collected; B. Both black/grey and purple barley grown together at Nyoma; C & D. *Leymus secalinus* naturally growing in waterlogging condition (left) and over saline encrustations (right); E & F. Natural habitat of *Elymus schrenkianus* (in Pang area) and *Allium przewalskianum* (near Tsokar Lake), respectively.

highest ever-recorded altitude for this species (generally <3,000 m, vide Pradheep *et al*., 2019). *Elymus mutabilis* (Drobow) Tzvelev was rarely observed in flowering stage at Sumdo, showing the tendency of late spike maturity (25 days) in comparison to the collected *Elymus* species.

Adaptation of *Leymus secalinus* (Georgi) Tzvelev to diverse ecological conditions such as waterlogging (Fig. 2C), saline sites (Fig. 2D), sandy as well as boulder-prone (scree) areas, hillslopes and as field weed indicate astounding plasticity. This species was found as dominant vegetation in plant community especially in the sandy areas east of Nyoma (like Loma, Muth, Rango, Dungti, Tsaga, Chushul), where delayed spike maturity (for 15-20 days) was noticed. Spikelet sterility has been observed in half of the collections, as this was reported to be a rhizomatous self-sterile species (Parmer and Sagar, 1963). The slender-spiked one (PRVJ-97), rarely found from Polokonga Pass, matches with var. *tenuis* L.B.Cai. Though widespread ecological adaptations are exhibited by this species, aphid (*Sitobion* sp.) incidence was noticed in some localities.

Other wild germplasm collections include caraway (*Carum carvi* L.), *Allium przewalskianum* Regel (locally called *kotse*; its crushed and dried leaf paste is added to traditional food, *thupka* Fig. 2F), *Fagopyrum tataricum* (L.) Gaertn. subsp. *potaninii* Batalin (progenitor of tartary buckwheat, as a rare field weed) and *Chenopodium karoi* (a common field weed; collected for the first time). Collected wild germplasm warrants detailed taxonomic studies and characterization, that would pave way for germplasm utilization especially in abiotic stress tolerance breeding programmes, besides spotting of new taxa or new distribution records to the country.

Further, explored region represents moderate level of genetic diversity in turnip, radish, apricot, seabuckthorn, *Beta vulgaris* L. (sweet chard/mangel), *Setaria viridis* (L.) P. Beauv., *Amaranthus retroflexus* L., *Chenopodium* spp., wild *Brassicae*, minor fruits (*Rubus*, *Ribes*, etc.) and wild edible plants, which may be targeted for germplasm collecting in the near future. As the choice of crops is limited in this harsh terrain, winter wheat (in snow-line area) and cereal oats in poor and marginal soils could be introduced as alternative crops to enhance the livelihood of local people. Moreover, glasshouse cultivation of vegetables such as broccoli and lettuce may be worth-considering. Future explorations can be focused on far-flung areas bordering Tibet (China) like Anlay, Demchok, Hunli, Koyul and Tsomomiri, which could not be visited during this trip.

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SHORT COMMUNICATION

Note on True Seed and Tuber Characteristics of Soh-phlang (*Flemingia procumbens* Roxb.)

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Key Words: *Flemingia procumbens*, Seed germination, Soh-phlang, Tubers

Soh-phlang (*Flemingia procumbens* Roxb.) was studied for seed germination for long time conservation. The dormancy breaking protocols were developed for germination and propagation through seed was attempted to throw light on regeneration potential of the germplasm through seed and long-term storage. Tuber size and seed size were compared and data was presented.

Introduction

Majority of the domesticated legumes are propagated by true seed. However, some of the leguminous species of well-known crops used for edible tubers viz, *Pachyrhizus erosus*, *Vigna vexillata* and *V. subterranea* that produce seeds, while many others like winged beans (*P. tetragonolobus)* also called Goa beans have edible highly nutritious seeds as well as leaves, pods and tubers.

There is a growing recognition of the fact that vegetative and seed propagation are complementary rather than competitive and are used for breeding programmes. The *ex-situ* conservation through seed bank is a crucial complement to the *in-situ* conservation and restoration of species and habitats (Chapman *et al*., 2019). Poor synchrony of flowering, flower and fruit drop during pod development and rapid shattering of ripe seed in many legumes might be the major cause of its poor seed yield and so will be difficulty in seed conservation.

Some of the less-known species of *Flemingia species are*, *F. semialata* and *F. macrophylla* are used for rearing of lac insect in Jharkhand, Chhattisgarh, West Bengal and Odisha but have remained ignored of their genetic improvement (Kumar *et al*., 2017). Through use of different plant growth regulators on morpho-physiological and biochemical characters study demonstrated improved seed set and yield in *Flemingia semialata* (Sinha *et al*., 2016).

One of the lesser-known species, *Flemingia procumbens* Roxb. locally called soh-phlang, is an indigenous species propagated only by the root tubers (Pandey *et al*., 2018). There are meager studies on the genetic resource value except a few latest sudies on nematicidal properties (Pandey *et al*., 2018; Gawade *et al*., 2019). Soh-phlang (*Flemingia procumbens* Roxb.) is propagated by tubers and has not been reported for multiplication through true seeds. The propagation and conservation aspects through seed are not worked out. There are no reports on propagation through seed nor is there any study on the genetic improvement or seed set attempted.

To support the genetic resources study on *Flemingia procumbens* Roxb. the present work was undertaken with the objective to investigate: 1) the variation in seed vs seed tubers; and 2) the seed germination methods. The information generated here will be used by the soh-phlang growers to create variability and researchers for seed conservation under long-term storage.

Materials and Methods

The mature tubers collected during an exploration to Jaintia and Khasi hills in 2017 were conserved using standard procedures for conservation (Nivedhitha *et al*., 2018). The tubers of a total of 26 accessions were replanted in 2018 at experimental farm at NBPGR, RS Umiam, Shillong in raised bunds of size 1 x 2m in the month of April 2018. The crop after 6 months yielded

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pods and mature seeds were harvested in December 2018. Data were recorded for all accessions at various stages of flowering and seeding. Healthy pods were harvested and data on seed size, number of seeds/pod, tuber number per plant was interpreted for all the accessions. Data on seed size and 100 seeds weight was noted using Vernier caliper and weighing balance respectively. Vouchers of the herbarium specimens with seeds were deposited in the NHCP (HS23620-45).

Since many accessions did not produce healthy seeds/pods, the data on germination study is taken in the pooled samples. The mature seeds were separated from the pods and classified on the basis of seed coat colour and size and divided into three lots: on the basis of seed colour and shape. The first lot with the shrivelled seeds were not included in the germination experiment as they appeared immature; only fully mature seeds of black and brown colour were subjected to germination test.

To address the germination procedures, the harvested seeds were mechanically scarified and plated on germination paper. First count was taken on the 7th day when radicle emerged and final count on 21st day. At the end, the results were expressed as percentage by number of normal and abnormal seedlings and hard, fresh and dead seeds (ISTA, 2015).

Results and Discussion

Morphological Data

The size of the flower ranged from 0.53-0.87cm. The original size of the collected tubers and those harvested for study were examined for length x diameter (Table 1a,b). The reduced diameter of the tubers may be accounted for due to the soil conditions and cultivation procedures. During pod maturation stage there was high rate of pod fall that resulted in poor pod harvest. It was a general observation that before the seeds could mature fully there was pods senescence. The number of aborted seeds in the species is directly related to domestication, which confirms that it can be one of the characters associated with the quality of fruit (Simmonds, 1997). In wild soh-phlang there are no reports on propagation methods and seed number per pod to support these observations.

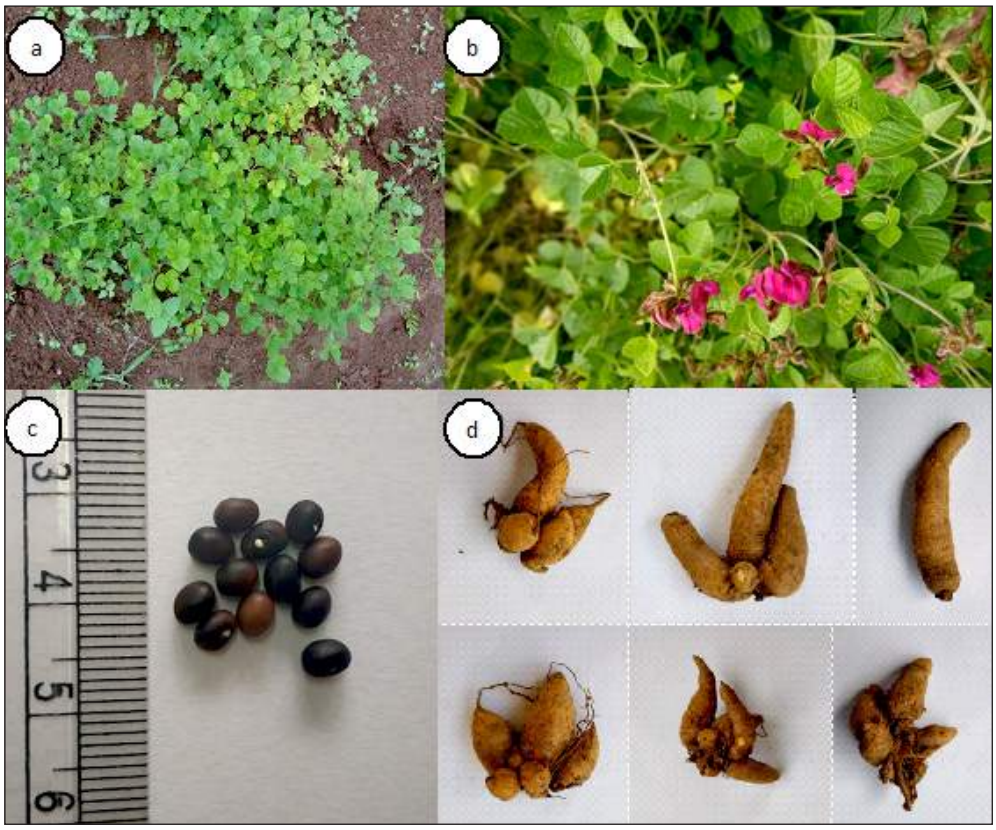

Fig. 1. Soh-phlang at various stages of study: a) crop grown in NBPGR Regional Station, Shillong experimental field; b) close-up view of vegetative and flowering stage; c) the black mature seeds harvested and d) tuber variation in harvested germplasm at NBPGR Regional Station Shillong, Umiam

Table 1a. Descriptive statistics of quantitative characters of Soh-phlang

Traits	Minimum	Maximum	Range	Mean	Std. Deviation	CV
Flower size (cm)	0.33	0.87	0.54	0.69 ± 0.02	0.13	18.28
Pod size (cm)	0.37	0.6	0.23	0.55 ± 0.01	0.06	10.87
Seed size (cm)	0.23	0.53	0.3	0.41 ± 0.01	0.06	14.39
10 seed weight (g)	0.19	0.44	0.25	0.38 ± 0.01	0.07	17.63
Tuber size (cm)	10.07	20.83	10.76	14.91 ± 0.63	3.2	21.45

Table 1b. Flower pod and seed size and average tuber size of 26 accessions of Soh-phlang

Accession No	Flower size (cm)	Pod size (cm)	Seed size (cm)	10 seed weight (g)	Average tuber size (length x width in cm)
IC627400	0.33	0.53	0.27	0.23	7.166 x 1.76
IC627401	0.47	0.50	0.40	0.40	7.16 x 2.23
IC627402	0.49	0.37	0.23	0.19	6.66 x 1.60
IC627403	0.70	0.53	0.37	0.38	7.83 x 2.66
IC627404	0.70	0.60	0.43	0.42	9.50 x 1.93
IC627405	0.67	0.60	0.40	0.38	5.66x 2.10
IC627406	0.73	0.53	0.40	0.41	6.33 x 2.16
IC627407	0.73	0.60	0.53	0.39	7.66x 1.63
IC627408	0.73	0.60	0.43	0.44	7.50 x 1.56
IC627409	0.73	0.60	0.40	0.40	6.00 x 2.10
IC627410	0.67	0.57	0.40	0.42	4.33 x 2.63
IC627411	0.67	0.50	0.37	0.39	6.50 x 2.06
IC627412	0.60	0.53	0.43	0.24	4.66 x 2.16
IC627413	0.63	0.53	0.40	0.33	6.66 x 2.06
IC627414	0.83	0.60	0.43	0.44	9.50x 2.16
IC627415	0.87	0.53	0.43	0.33	6.16 x 2.26
IC627416	0.73	0.57	0.47	0.43	5.16 x 2.16
IC627417	0.83	0.57	0.43	0.43	7.50 x 1.93
IC627418	0.70	0.60	0.43	0.33	7.33 x 2.50
IC627419	0.53	0.53	0.37	0.39	8.00 x 2.50
IC627420	0.77	0.57	0.40	0.40	7.16 x 2.50
IC627421	0.73	0.60	0.47	0.43	6.83 x 2.20
IC627422	0.73	0.57	0.43	0.36	7.33 x 2.23
IC627423	0.80	0.60	0.47	0.42	6.66 x 2.30
IC627424	0.87	0.40	0.40	0.43	8.33 x 2.00
IC627425	0.80	0.60	0.43	0.38	8.00 x 2.33
Range	0.54	0.23	0.30	0.25	10.76
Minimum	0.33	0.37	0.23	0.19	10.07
Maximum	0.87	0.60	0.53	0.44	20.83
Mean	0.69	0.55	0.41	0.38	14.91
SE	0.02	0.01	0.01	0.01	0.63
Std. Deviation	0.13	0.06	0.06	0.07	3.20
CV	18.28	10.87	14.39	17.63	21.45

There was poor seed yield in all the studied accessions; in some of them the senescence at various stage of flowering to seeding was observed in Vmiam conditions. The plants produced small pods that turned brown on ripening and after dehiscence each pod yielded one-two seeds and pod size (0.3-0.6cm). The seeds were globular, 2-3 mm in diameter and shiny brown-black. The seed ranged in size from 0.23-0.53cm. The seed coat colour varied from dark black, shiny to dull brown on maturity. The seed size and seed number was not variable; most mature pods had two seeds but in some only one seed was formed; 100 seed weight was 1.9-4.4g. The hilum was 1mm diameter, white, depressed/ sunken, and orbicular in shape. The seed with moderately hard coat, taste somewhat like rice bean, soybean, and had good palatable.

There was no correlation between the tuber size and seed size. The tubers used for study ranged in size from 4.66-9.50 cm size and 1.60-2.66 cm in diameter. As compared to the original size of tubers they were narrower in diameter only. This may be accounted for soil conditions in the experimental site which are very different from the soil conditions in the farmers' field (jhoom farming).

Seed Germination

The seeds of *F. procumbens* Roxb. were orthodox and were black-brown, non bitter, palatable like soybean, and have thick hard seed coat. In the accessions with black seed coat there was 100% germination where as in seeds with brown seed coat the germination was only 50-60%. Result suggested that seed physiological maturity played a role in this species and seed coat colour is one of the indexes of seed maturity.

The seeds in control plates did not germinate even after 20 days. Therefore, the selected seeds were scarified with due safety and germination was reported in many legumes.

The seed germination was enhanced by seed scarification of the seed coat. Mechanical scarification

is a technique to physically create scars on seed surface to increase water imbibition (Uzun and Aydin, 2004; Rostami and Shasavar, 2009; Olisa *et al*., 2010; Jayasuriya *et al*., 2012). The seed germination data could not be recorded accession-wise as number of seeds per accession required were very low to facilitate the study on dormancy breaking methods. Among the important less-known tuber legume, *Vigna vexillata* is primarily used for their storage roots, is propagated by seeds, required no scarified seeds for good germination and formed non-dehiscent pods (Karuniawan *et al*., 2006).

In related species, *Flemingia macrophylla,* the seed was hard and small, soaking seeds in cool water for 12 hours improved germination (Roshetko, 1995). Harvested pods were sun dried for 2 days are gently pound in a sack with a stick and winnowed to separated seeds from debris. Seeds were dried by pouring a small amount of nearly boiling water for 2-3 minutes on the seeds and then soaked them in cool water for 12-24 hours in warm water. This resulted in germination in 7-14 days (being careful not to damage the embryo) and soaked for a further 12 hours before sowing. https://pfaf.org/user/Plant.aspx?LatinName=Flemingia%20macrophylla.

Despite the great importance of legume seeds as human diet, establishment is difficult due to major constraints of hard seediness. Hard seed coat can cause delayed or decreased seedling emergence. Seed scarification, a technique to break the hard seed coat without lowering the quality of seeds has been studied in various crops (Stanwood, 1980; Rutar *et al*., 2001; Zeng *et al.*, 2005; Dittus and Muir, 2010).

Conclusions

Study demonstrated that the variation in tuber size is not linked to the seed size in *F. procumbens*. There was poor variation reported in the pod and seed characters of soh-phlang despite the good variation in the tuber traits (Nivedhitha *et al*., 2018). Since there is poor seed formation hence, there is need to study *in vitro* conservation methods. The study demanded further working on large sample size of variable seed maturity and also on diverse soil conditions to throw light on these findings.

The research thrusts is in the context of soh-phlang breeding and can be applied to the improvement of other clonally propagated crops. There is a need to study seed propagation techniques for commercial production in place of the stranded clonal propagation techniques.

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Plant Germplasm Registration Notice*

The Plant Germplasm Registration Committee of ICAR in its XXXIXth meeting held on January 28th, 2019 at the National Bureau of Plant Genetic Resources, New Delhi approved the registration of following 32 germplasm lines out of 52 proposals considered. The information on registered germplasm is published with the purpose to disseminate the informationto respective breeders for utilization of these genetic stocks in their crop improvement programmes. Upon request, the developer (s) / author (s) is / are obliged to distribute the material for crop improvement programme of National Agricultural Research System.

1. CSR51 (Bulk 212) (IC0619319; INGR19001), a Rice (*Oryza sativa*) Germplasm for Tolerant to Alkalinity Stresses up to pH 9.9 and Salinity Stresses up to ECe 10.0 dS/m with Long Slender Grain

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Globally, more than 800 million hectares of land are salt-affected, including both saline and alkaline soils, which are more than 6% of the world's total land area (FAO 2014). In India, 6.73 million ha are affected by salt and 3.77 million ha are affected by alkaline soils. Genetic improvement of salt tolerance in rice appears to be economically feasible and a promising strategy for maintaining stable rice production, globally. The rice (*Oryza sativa* L.) line,CSR 51 (IET 22017), a derivative of the cross CSR23/CSR27 at ICAR-CSSRI, Karnal. The line CSR 51 was developed for saline and alkaline areas where ECe ~ 10.0 dS/m and pH is up to 9.9. Of the two parents, CSR 23 is tolerant to alkalinity (pH ~9.9) and another parent CSR27 is tolerant to salinity (ECe~10.0 dS/m) with high yield potential.

Morpho-agronomic characteristics: This culture recorded good yield under salt stress situations with good initial vigour, strong culm and good tillering ability. The culture is easily distinguishable through its morphological features such as erect flag leaf with long slender grains, strong culm, well exerted panicle with late leaf senescence. This line has been evaluated in AICRIP trial (Alkaline & Inland Stress Tolerant Varietal Trial) during 2011-13. All through the testing years i.e. 2011, 2012 and 2013, IET 22017 performed well in the alkaline stress locations of Haryana (Karnal, Kurukshetra and Jind), Uttar Pradesh (Lucknow and Kanpur) and Tamil Nadu (Annmalainagar); saline stress locations were Haryana (Karnal, Rohtak and Panipat) and Karnataka (Gangavathi) and majority of occasions figured among the top ranked entries of the trial and recorded impressive yield gain over all the checks.

It's interesting to note that none of the entries have qualified in three years of testing other than the entry IET 22017. The proximity of genotype Bulk 212 to saline and alkaline environment indicates its adaptability to salt stress environments. Results of the production environments with low GE interaction can be extrapolated to other environments and used to increase rice productivity in salt-affected areas (Krishnamurthy *et al*., 2015). Across three years 2011, 2012 and 2013, CSR 51 (Bulk 212) showed superiority yield advantage of 29%, 25%, 83%, 79%, 47%, 98%, 78% and 68% CSR 36 (National check), CSR23, Jaya (Yield check), NDRK50021(qualifying variety-2),TR2005-041(qualifying variety-3), TR2005-031(qualifying variety-4) and RP4939-221-16-4-2-2-1-1(qualifying variety-5) respectively across alkalinity locations of Karnal, Kurukshetra and Jind in Haryana, Lucknow and Kanpur in Uttar Pradesh and Annamalai Nagar. Across saline affected locations of Karnal, Rohatak and Panipat in Haryana, Gangavati in Karnataka, CSR 51 (bulk212)

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showed superiority yield advantage of 27%, 95%, 10%, 44%, 37%, 29% and 53% over CSR 36 (National check), Jaya (Yield check), CSR23, NDRK50021 (qualifying variety-1), TR2005-041 (qualifying variety-2), TR2005-031 (qualifying variety-3) and RP4939-221-16-4-2-2-1-1(qualifying variety-4).

Associated Characters and Cultivated Practices: Based on three years screening in NSN1 and NSN2 IET 22017 showed moderate resistance to blast and bacterial leaf blight, green leaf hopper, gall midge and stem borer. Quality parameters studied at IIRR (DRR) showed that IET 22017 has high milling recovery (71.27%) coupled with high head rice recovery (HRR) of 69%. It has long slender grain and alkali spreading value (ASV) of 4.3. It has got intermediate amylose content (24.58%). The above values are indicative of excellent cooking quality of IET 22017.

The performance of CSR 51 (IET 22017) was consistently high under alkalinity and salinity stress locations for three successive years. The alkaline locations were Haryana (Karnal, Kurukshetra and Jind), Uttar Pradesh (Lucknow and Kanpur) and Tamil Nadu (Annmalai nagar); saline stress locations were Haryana (Karnal, Rohtak and Panipat) and Karnataka (Gangavathi). It showed yield superiority over national alkaline check (CSR 36), CSR23, local check and other qualifying checks. This was only an entry promoted to 3rd year of testing. None of the entries qualified for 3rd year of testing in both alkaline and saline stresses. Therefore, CSR 51 (IET 22017) was promising under alkalinity and salinity stresses. This Germplasm can be used as a genetic stock for future breeding programmes aiming at development of high yielding salt tolerant rice varieties for saline and alkaline soils.

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2. Robin Mutant (*Herbicide tolerant mutant*) (IC0628569; INGR19002), a Rice (*Oryza sativa*) Germplasm with Herbicide Tolerance

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Increased water and labour scarcity in major rice growing areas warrants a shift towards direct seeded rice cultivation under which management of weeds is a major issue. A few herbicide tolerant genotypes have been developed using non-transgenic approach, primarily through induced mutagenesis, in crops *viz.,* maize, canola, wheat, rice and sunflower (Anderson and Georgeson, 1989) though transgenic approach is more prevalent.

Table1. Evaluation of *Robin mutant* at Multi locations

Sl.No	Genotypes	Dose of Imazethapyr on 30 Days old seedling	Coimbatore	Cuttack	New Delhi IARI FARM July 2017	New Delhi NRCPB Botany field June 2017
1	N 22	100g ai/ha	0% survival	0% survival	0% survival	0% survival
2	"HTM Robin" mutant	100g ai/ha	100% survival	100% survival	100% survival	100% survival

In the present study, we have successfully identified an induced mutant in rice tolerant to a broad spectrum non-selective herbicide, imazethapyr, considering the ease in registering and commercializing the material developed through non-transgenic approaches.

A mutant population of about 1,00,000 single M_2 plants generated by bulking the progeny of M_1 plants raised from about 1 Kg of EMS treated N22 seeds were grown in the field at Department of Rice, Tamil Nadu Agricultural University, Coimbatore and sprayed with herbicide, Imazethapyr. Three mutant plants putatively resistant to Imazethapyr were identified from the field in the M 2 generation. However, only a single mutant plant was found to be tolerant to subsequent herbicide spray in the re-potting experiments which was named as, 'HTM-N22' later it was named as "HTM *Robin" mutant*. Tolerance against the herbicide imazethapyr exhibited by the "HTM *Robin"* mutant was confirmed across subsequent filial generations up to M_5 and found to be true breeding. The true breeding and monogenic nature of the herbicide tolerant mutant identified in the present study was evident from the results of progeny testing, inheritance studies, molecular and DUS characterization.

This mutant was evaluated along with its wild type "N 22" under four locations. The results confirmed that it was resistant to Herbicide Imazethapyr at all four locations *viz.,* Coimbatore, Cuttack, IARI New Delhi and NRCPB, New Delhi (Table 1). The novel imazethapyr tolerant mutant which has been developed indigenously has opened up the possibilities of its extensive usage, without fear of infringement of any IPR, in the public rice breeding programmes in India and elsewhere.

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3. RP 5316-RIL-243 (IC0628571; INGR19003), a Rice (*Oryza sativa*) Germplasm for Potential Donor for Resistance to Planthoppers (Brown Planthoppers and Whitebacked Planthoppers). Exhibits Resistance during Vegetative and Reproductive Stages of Crop Growth

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Planthoppers namely brown planthoppers (*Nilaparvata lugens* Stal) and whitebacked planthoppers (*Sogatella furcifera*) are the major sap sucking insects in rice causing 100% yield loss under hopper- burn.

In an ongoing breeding program at ICAR-IIRR, Hyderabad for developing promising planthopper resistant rice varieties, a RIL (F_9)designated as RP 5316–RIL-243 derived from the cross NDR 359 (Susceptible)/MO1(Resistant) was found to be promising with stable resistance against planthoppers consistently for 3 years. A set of 200 RILs were initially screened at RARS, Maruteru, a natural hotspot for infestation of planthoppers in West Godavari district along with the standard checks (Ptb 33-resistant and TN1-Susceptible) in 6 environments continuously *ie.,* (1) 2012 *rabi,* (2) 2012 *kharif,* (3) 2013 *rabi,* (4) 2013 kharif, (5) 2014 *rabi* and (6) 2014 *kharif.* Among them a RIL namely RP 5316-RIL-243 was found to withstand high insect pressure (200-520 WBPH insects and 35-40 BPH insects per hill) during *kharif*, 2012 whereas 120-459 BPH insects and 10-15 WBPH insects/ hill during rabi, 2012 (IIRR Annual report, 2012-13). Similarly it was

found resistant in the presence of as many as 350-515 BPH and 80-130 WBPH insects/hill during *kharif,* 2013, while 500-600 BPH insects and 5-10 WBPH insects/hill during rabi 2013 (IIRR Annual report, 2013-14). Also it showed resistance under heavy infestation of 390 to 450 BPH and 25 to 33 WBPH insects/hill during 2014, *kharif* and 600 to 800 BPH + 30 to 40 WBPH insects/hill during 2014, *rabi* (Fig 1). Thus it showed more or less resistant reaction with an overall mean score of 3.4±0.59 across 3 years, could survive and produced seed.

For confirmation of the observed field resistance, the same RIL was subjected to BPH and WBPH infestation separately during *kharif* and *rabi* seasons of two years (2013 and 2014) in glass house at ICAR-IIRR, Hyderabad during seedling stage using standard seed box screening method. The screening studies could detect RP 5316–RIL-243 with resistance reaction in both years with a mean damage score of 3.6 in 2013 and 3.1 in 2014 for BPH and WBPH respectively. The resistant checks, Ptb 33 and MO1 for BPH and WBPH recorded mean damage scores of 3 and 2.9 respectively. MO1, the donor parent of RIL population showed moderate resistance to BPH (DS = 3.5). Quality point of view it was superior with high head rice recovery (58%) and intermediate amylose content (23 %) and desirable ASV (5 mm).

Since RP 5316–RIL-243 displayed consistent resistance reaction for 3 years (2012 to 2014) continuously involving six seasons, it could be used as an elite resistant genetic stock to dev elop new varieties conferring combined resistance to planthoppers.

4. CHERAYI POKKALI (AC 39416A) (IC0413644; INGR19004), a Rice (*Oryza sativa*) Germplasm for Tolerant to Combined Stress of Drought and Salinity, Tolerant to Vegetative Stage Salt Stress, Tolerant to Vegetative Stage Stagnant Flooding

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Rice production is becoming more vulnerable nowadays due to climate change. Reports have shown that extreme events like longer dry spells or heavy rain have already gone up in las few years. In coastal areas,contamination of agricultural land by intrusion of seawater as a result of rise in sea level and erratic rainfall changing the micro environment in multifaceted manners. On the other hand, in rain-fed upland areas, rice is mostly cultivated as direct seeding due to lower cost of cultivation and other benefits. Water stagnation of even 5-10 cm in the field after sowing impede germination and crop growth. In reality these stresses come simultaneously either or in tandem during crop growth stages. Hence, genotype tolerant to multiple abiotic stresses can be of immense importance for its utilization in crop improvement than genotype with solitary abiotic stress tolerance trait.

Morpho-agronomic characters of AC 39416 (A): AC 39416A was used as check in the for multiple abiotic stress tolerance under multi-locational AICRIP trials during the period from 2013-2016. It was observed that AC 39416A under salinity and water stress was superior in comparison to other rice cultivars and showed moderate tolerance to anaerobic seeding condition. Based on the trial conducted at 9 locations AC 39416A along with other three lines found to be superior and were recommended as multiple stress tolerance rice genotypes based on their superior performance in terms of germination, root, shoot length and seedling vigour.

Associated characters and cultivated practices: AC 39416A is tolerant to vegetative stage salt stress. When tested for salinity stress, this genotype was found to be highly tolerant to vegetative stage salinity stress with a similar salinity evaluation score (SES) like tolerant check FL478. Detailed physiological studies also suggested highly salt tolerant nature of AC 39416A for traits like photosynthesis, chlorophyll fluorescence and Na^+/K^+ ratio (Sarkar et al. 2013; Singh and Sarkar 2014; Sarkar and Ray 2016). Anaerobic germination potential or tolerance to Germination Stage Oxygen Deficiency (GSOD) of this genotype was tested along with 2000

rice genotypes including known checks. AC 39416A showed tolerance to GSOD. The germination % of AC 39416A was 59% under anaerobic condition (10 cm of standing water above soil surface), while the maximum germination of 68% was observed in the tolerant check.

AC 39416A was also found tolerant to vegetative stage stagnant flooding (medium depth ≈ 50 cm). AC 39416A maintained greater stability of yield and yield parameters compared to other genotypes. A mere reduction of 8–10% in panicle number and <5% reduction in panicle weight (per m^2) was observed in AC39416A under vegetative stage stagnant flooding.Two-year experimentation showed that AC 39416A is tolerant to combined stresses of stagnant flooding and salinity. Even after 45 days of the imposition of combined stress of salinity (8 dS m^{-1}) and water stagnation (45 cm depth of water), the injury symptoms were negligible in AC 39416A (score 2) compared to susceptible genotype Gayatri (score 9). Testing of genotypes under combined stresses of Salinity (12 dS m^{-1}) and polyethylene induced moisture stress revealed that AC 39416A showed greater tolerance than susceptible check IR 29 and drought tolerant check CR 143-2-2.

In conclusion, we can say that AC 39416A is tolerant to drought, salinity and stagnant flooding at vegetative stage and moderately tolerant to germination stage oxygen deficiency (anaerobic seeding) and tolerance to combined stress of salinity and drought and water stagnation and salinity.

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5. DHOBANUMBERI (IC0256804) (IC0256804; INGR19005), a Rice (*Oryza sativa*) Germplasm for Resistant to Brown Plant Hopper

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The purified landrace 'Dhobanumberi (CRRI Accession number 35184)' showed resistance to Brown Plant Hopper (BPH) consistently against BPH population of NRRI, Cuttack during the years 2000, 2006 and 2015. Phenotyping showed low survivility rate and low feeding of BPH on the genotype. It was also promising in All India Coordinated Rice Improvement Programme trials (AICRIP) during 2003 and 2004. Since no separate biotype was confirmed location-wise in India, this genotype was tested against populations of different locations as mentioned in the Progress reports of the AICRIP. It was resistant to BPH in all the 5 locations tested during 2003 whereas during 2004, it showed resistant reaction at 4 locations.

Morpho-agronomic characteristics: The data on multilocation tests were generated for four years through AICRIP, ICAR-IIRR, Hyderabad in which the said entry was showing promising results against BPH. In addition, it was also identified as a potential donor for having multiple resistance to several insect pests of rice.

Associated characteristics and cultivated practices: Basing on the performance, the donor has already been utilized in breeding programme of the Institute. The breeding lines thus evolved, are also showing highly resistant reaction against BPH in multi-locational testing. It was recommended by the Institute Research Council (IRC) and Research Advisory Council (RAC) for registration as well as utilization in breeding programme for promotion to yield trial or for utilization in breeding programme for biotic stress like BPH resistance and also for resistance to multiple insect pests of rice. Basing on the recommendations, this donor has been utilized in making different cross combinations in breeding programmes of the ICAR-IRRI collaborative programmes and other rice research stations across the country.

The breeding lines thus evolved were also showing highly resistant reaction against BPH. The promising BPH-resistant breeding lines such as CR 2711-76, CR 2711-149, CR 2711-114 and CR 2711-139 evolved by using the variety as a resistant donor in the background of Tapaswini further confirms it's resistance.

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6. Khora-1 (AC 41620 (IC0574806; INGR19006), a Rice (*Oryza sativa*) Germplasm having High Anaerobic Germination

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In the era of global climate change, rice cultivation especially in the rain-fed shallow lowland ecology faces multi-facet problems. Dominated by poor and marginal farmers in this ecology, direct seeding of rice is becoming a popular option due to lower cultivation cost. But, erratic rainfall leading to water stagnation just after direct seeding of rice causes a lot of problem with excess water in the field. More often than not it creates complete submergence of the field leading to severe hypoxic conditionfor the germinating seed.Fortunately, in nature some of the rice genotypes areblessed with considerable degree of anaerobic germination potential and are tolerant to germination stage oxygen deficiency (GSOD). Interesting, the mechanism of GSOD tolerance found to be quite different than that of *SUB-1* mediated submergence tolerance at early vegetative stage. The QTLs reported for vegetative stage submergence tolerance do not work for anaerobic germination and vice-versa. Though donors for submergence tolerance had been identified long back, but till date we don't have well-established donor for GSOD tolerance. The genotype having high anaerobic germination potential would be of immense use as potential donor for GSOD tolerance trait, for rice crop improvement programme particularly for low-land ecologies.

Morpho-agronomic characters of Khora (AC 41620): AC 41620possesses an erect culm attitude. The plant is tall in nature having plant of125-130 cm and possesses weak leaf pubescence of blade surface, medium leaf sheath intensity of anthocyanin colouration and intensity of green colour, presence of leaf sheath anthocyanin colouration, leaf collar, leaf auricles and leaf ligule. The colour of the coleoptile and basal leaf sheath is green. This genotype was tested under NICRA project for 3 consecutive years (2012-13, 2013-14, 2014-15) and found that the genotype possesses high degree of anaerobic germination potential (NICRA Research Highlights 2012-13, 2013-14, 2014-15). Comparison of AC 41620 for anaerobic germination potential among 21 rice genotypes clearly showed its superior germination ability (>85%) under anaerobic condition, while the germination percentage of tolerant check viz. Rashpanjor was 75% and it was <15% in FR13A, Pokkalietc (Table 1). Further, the genotype was tested against Naveen (GSOD susceptible genotype) and found that it is having considerably higher anaerobic germination potential under 10 cm of water stagnation after sowing. The germination ability, coleoptile elongation and seedling vigour under anaerobic condition was significantly better in AC41620 as compared to Naveen (Vijayan et al. 2015; 2018).

Associated characters and cultivated practices: Our study showed a significantly different mechanism of tolerance to anaerobic germination condition exists in the genotype AC 41620. A more robust genetic regulation as evidenced from comparative transcriptomic study between anaerobic germination susceptible Naveen and tolerant AC 41620 showed, differentially regulated

genes were more in case oftolerant genotype, AC 41620 (1130 DEGs), compared to susceptible genotype, Naveen (930 DEGs). Comparisonof transcriptome profiles revealed that 604 genes were uniquely up-regulated and 164 genes were uniquely downregulatedin tolerant genotype, AC 41620, while the corresponding numbers of genes in case of susceptible genotype, Naveen were 520 and 60, respectively. Detailed pathway analysis revealed, efficient use of available resources through energy saving mode imparted anaerobic germination tolerance in AC41620. Moreover, accelerated carbon and nitrogen metabolism (higher NR and NiR activities) in AC41620 under prolonged anoxia provided improve pH-homeostasis and energy source.Interestingly, we didn't find much alteration in any of the AG-QTLs reported till date, rather the study pointed out a few newer mechanism of anaerobic germination tolerance in AC 41620, which wasn't reported in functional validation of AG-QTLs. Perhaps, this genotype can be immense use not only as donor for anaerobic germination tolerance, but also can be used for identifying robust AG-QTL for higher anaerobic germination potential in rice.

Table 1. Effect of complete submergence (10 cm standing water) on germination ability (%) of a few rice genotypes

Genotype	GP under anaerobic condition	Genotype	GP under anaerobic condition	Genotype	GP under anaerobic condition
FR13A	11.5	Ravana	37.5	Pantara	52.5
Pokkali	15.0	AC41647	42.5	AC39416A	57.5
Kamini	27.5	JRS21	45.0	AC40346	60.0
Paloi	27.5	Talmugra	47.5	AC34245	65.0
JRS155	29.0	JRS8	50.0	Rashpanjor	71.0
JRS20	32.5	JRS182	52.5	JRS196	75.0
Panikekua	37.5	AC34280	52.5	AC41620	85.5

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7. IC536365 (IC0536365; INGR19007), a Wheat (*Triticum aestivum*) Germplasm Resistant to all the Prevailing Stem, Leaf and Stripe Rust Pathotypes of India due to the Synergistic Combination of Minor and Major Rust Resistance Genes, Presence of Leaf Tip Necrosis (LTN), a Phenotypic Marker for the Presence of Minor/APR Genes

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A comprehensive germplasm evaluation study of wheat accessions conserved in the Indian National Genebank was conducted to identify sources of rust and spot blotch resistance. Genebank accessions comprising three species of wheat – *Triticum aestivum, T. durum* and *T. dicoccum* were screened sequentially at multiple disease hotspots, during the 2011-14 crop seasons, carrying only resistant accessions to the next step of evaluation. Initially, all the wheat accessions conserved in the gene bank were simultaneously regenerated and screened at Wellington (Tamil Nadu), a hotspot for stem and leaf rusts. Total of 2, 200 wheat accessions were selected based on leaf tip necrosis (LTN), a phenotypic trait linked to adult plant rust resistance genes viz., *Lr34+, Lr46+, Lr67+, Lr68* etc. having pleiotropic association with multiple disease resistance genes(Kumar*et al*., 2014). Further the LTN wheat accessions and the rest of the accessions were evaluated at Wellington. Identified 4,925 resistant accessions (including 1,526 LTN wheat accessions as well) were further evaluated at Gurdaspur (Punjab), a hotspot for stripe rust and Cooch Behar (West Bengal), a hotspot for spot blotch. Wheat accession (IC536365) displayed resistance reaction to all the three rusts in all the tested location at adult plant stage wherein stem rust pathotypes: 40A (62G29) & 40-1 (62G29-1); leaf rust pathotypes:17 (61R24), 77A(109R31), 77-5 (121R63-1), 77-7 (121R127) & 77-8 (253R31); stripe rust pathotypes: 46S119, 78S84 & I is commonly prevalent.

Wheat accessions which were found to be resistant in the field were then assayed for seedling resistance and profiled using molecular markers. Evaluation of rust resistant accessions (IC536365) for seedling resistance test (SRT) against seven virulent pathotypes (stem rust pathotypes: 40-1 (62G29-1), 11 (79G31 & 117-3 (167G3); leaf rust pathotypes: 77-5 (121R63-1) & 104-2 (21R55); stripe rust pathotypes: 46S119 & 78S84) of three rusts under artificial epiphytotic conditions in glass house showed resistance reaction. Molecular analysis to identify different combinations of genetic loci imparting resistance to leaf, stem and stripe rust using linked molecular markers for the resistant accessions (IC536365) revealed the presence of two minor/adult plant rust resistance genes (APR) for stripe rust (*Yr36* & *Yr48*) and one each for stem rust (*Sr2/Yr30/Lr27*) and leaf rust (*Lr*46/*Sr5*8/*Yr*29/*Pm*39/*Ltn2*) and two major gene for stripe rust (*Yr5* & *Yr15*) and one each for leaf rust (*Lr50*) and stem rust (*Sr13*).

Germplasm accession (IC536365) carries combination of two minor/adult plant rust resistance genes (APR) for stripe rust (*Yr36*&*Yr48*) and one each for stem rust (*Sr2/Yr30/Lr27*)and leaf rust (*Lr*46/*Sr5*8/*Yr*29/*Pm*39/*Ltn2*) and two major gene for stripe rust (*Yr5 & Yr15*) and one each for leaf rust (*Lr50*) and stem rust (*Sr13*). Moreover, APR genes have pleiotropic association with stem and stripe rust resistance along with powdery mildew which is an added advantage. Synergistic combination of minor and major rust resistance genes made the accession resistance to the prevailing rust pathotypes in India. APR genes are pathotype non-specific resistance based on horizontal type of resistance mechanism. Development

of new pathotype(s) is slow/remote compared to major gene resistance which leads to durable resistance. So, the germplasm could be vital source for imparting durable rust resistance in wheat.

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8. EC574482 (EC574482; INGR19008), a Wheat (*Triticum aestivum*) Germplasm Resistant to the Entire Prevailing Stem, Leaf and Stripe Rust Pathotypes of India due to the Synergistic Combination of Minor and Major Rust Resistance Genes. Presence of Leaf Tip Necrosis (LTN), a Phenotypic Marker for the Presence of Minor/APR Genes

Vikas VK[1*], M Sivasamy[1], Jagdish Kumar[2], Sundeep Kumar[3], P Jayaprakash[1], Sunil Archak[3], GP Singh[4], Rajbir Yadav[5], Sherry R Jacob[3], R Parimalan[3], Amit K Singh[3], Jyoti Kumari[3], SC Bhardwaj[6], Pramod Prasad[6], OP Gangwar[6], MS Saharan[5], Indoo Bhagat[7], RK Tyagi[3] and KC Bansal[3]

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Wheat accessions which were found to be resistant in the field were then assayed for seedling resistance and profiled using molecular markers. Evaluation of rust resistant accessions (EC574482) for seedling resistance test (SRT) against the stripe rust pathotypes, 46S119 & 78S84 under artificial epiphytotic conditions in glass house showed resistance reaction. Molecular analysis to identify different combinations of genetic loci imparting resistance to leaf, stem and stripe rust using linked molecular markers for the resistant ace ssions (EC574482) revealed the presence of three minor/adult plant rust resistance genes (APR) for leaf rust *Lr46*/*Sr5*8/*Yr*29/*Pm*39/*Ltn2, Lr*67/*Sr*55/*Yr*46/*Pm*46/*Ltn3* and *Lr68*) and one for stripe rust (*Yr48*) and two major genes for stripe rust (*Yr5*&*Yr15*) and one for leaf rust (*Lr50*).

Germplasm accession (EC574482) carries combination of three minor/adult plant rust resistance genes (APR) for leaf rust *Lr*46/*Sr5*8/*Yr*29/*Pm*39/*Ltn2, Lr*67/*Sr*55/*Yr*46/*Pm*46/*Ltn3* and *Lr68*) and one for

stripe rust (*Yr48*) and two major genes for stripe rust (*Yr5*&*Yr15*) and one for leaf rust (*Lr50*). Moreover, APR genes have pleiotropic association with stem and stripe rust resistance along with powdery mildew which is an added advantage. Synergistic combination of minor and major rust resistance genes made the accession resistance to the prevailing rust pathotypes in India. APR genes are pathotype non-specific resistance based on horizontal type of resistance mechanism. Development of new pathotype(s) is slow/remote compared to major gene resistance which leads to durable resistance. So, the germplasm could be vital source for imparting durable rust resistance in wheat.

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9. HI 8774 (IC0628570; INGR19009), a Wheat (*Triticum durum*) Germplasm for Resistant to Stripe Rusts with Additional Resistance to Karnal Bunt and Powdery Mildew

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Durum wheat is the second most important wheat species produced in India. Biotic stresses like stripe rusts, flag smut, Powdery mildew and Karnal bunt affect world wheat production significantly. Karnal bunt affects the wheat export prospect of the country to a great extent as it is quarantined diseases in many parts of the world. The pathogens' continued evolution and appearance of newer pathotypes leading to breakdown of new varieties in couple of years is a cause of concern and hence, broadening of genetic base for host resistance is a continuous process.

HI 8774 **(**HI 8863/HI 8498**)**,durum wheat genotype developed at ICAR-Indian Agricultural Research Institute, Regional Station, Indore was identified to be resistant to stripe rust, Karnal bunt and powdery mildew in multi-location testing *viz*., Plant Pathological Screening Nursery (PPSN), Elite PPSN and Multiple Disease Screening Nursery (MDSN) from 2015 to 2018 (Table 1). This genotypes showed high levels of adult-plant resistance to most prevalent and virulent pathotypes like 46S119 (5MS) and 78S84 (10MS) of stripe rust in isolated nurseries. In seedling test, it showed moderate resistance reaction to both these pathotypes and also showed resistance responses to many new pathotypes like 238S119, 111S68 and 38S102 (Anonymous, 2016). It also showed resistance to other diseases like Karnal bunt and powdery mildew (Table 1). HI 8774 showed significant superiority in grain yield of 7% in NIVT and AVT-I in comparison to zonal check variety HI 8498 in Central Zone (Anonymous 2015b and 2016b).It has soft grains (grain hardness index of 7) which is suitable for biscuit making (Anonymous 2016b).As the availability of multiple disease resistant high yielding genotypes is rare, HI 8774 could be utilized as a potential resistance donor to breed varieties against these multiple pathogens along with quality and high grain yield.

Table 1. Field responses of HI 8774 to stripe rust, Karnal bunt and Powdery mildew

Year of testing	Trial	Stripe rust		Karnal bunt (%)		Powdery Mildew	
		HS	ACI	HS	Avg.	HS	Avg.
2014-15	NIVT 4	20S	5.3	1.1	-	-	-
2015-16	AVT I	5S	1.2	9.3	3.8	7	5
2016-17	EPPSN	5MS	1.7	-	-	-	-
2016-17	MDSN	5S	1.0	4.0	-	4	2

(HS-Highest score, ACI-Average Coefficient of Infection)

Source: AICW&BIP - Crop Protection Report (2015-18)

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10. QLD 84 (IC0628572; INGR19010), a Wheat (*Triticum aestivum*) Germplasm with Soft Grain Genotype (Very Low Grain Hardness Index). Suitable for Better Biscuit Making

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Grain hardness is an important trait in wheat quality with a profound effect on milling, baking and end-use qualities of wheat. It is common to differentiate soft and hard wheat in the world trade for product specific utility. Soft wheat is more friable, requires less energy to mill, and produces flours and meals with finer particles and lower starch damage, which are suitable for cake and biscuit production. Soft grain textured wheat produces tender and larger biscuits.

QLD84 was developed at ICAR-Indian Institute of Wheat and Barley Research (ICAR-IIWBR) by crossing 37thIBWSN05/36thIBWSN138 (Pedigree: MIANYANG20/3/CMH84.3379/ CMH78.578// MILAN). The genotype was evaluated at 13 centers in Quality Component Screening Nursery (QCSN) for three consecutive years (2015-16, 2016-17 and 2017-18). QLD84 was found to be superior with 18 grain hardness index over the years and locations to all the testing genotypes and soft grain check variety HS490 (31: grain hardness index) (Table 1). QLD84 recorded the lowest grain hardness index in all the 13 tested centers compared to the best check variety (HS490) for low grain index. QLD84 recorded lowest grain hardness index of 19, 18 and 18, respectively during 2015-16, 2016-17 and 2017-18. Whereas, the check variety HS490 tested in 2015-16 (grain hardness index: 27) and 2017-18 (grain hardness index: 35). QLD84 was 41.9% superior over check variety HS490. Low grain hardness index is very important factor to obtain high spread factor of biscuit and better biscuit quality. Thus, QLD84 would be a potential source to be utilized in future breeding programs to develop bread wheat varieties suitable for better biscuit making.

Table 1. Grain hardness index of QLD84 at 13 locations during three years as compared to the best available check HS490

Zone	Location	QLD84			HS490 (Check)	
		2015-16	2016-17	2017-18	2015-16	2017-18
NHZ	Almora	18	16	-	24	-
	Ludhiana	19	13	-	26	-
	Durgapura	13	17	-	33	-
NWPZ	Delhi	15	19	20	27	39
	Pantnagar	18	22	-	29	-
	Karnal	21	22	-	29	-
NEPZ	Pusa	20	23	-	26	-
	Kanpur	22	-	18	21	30
CZ	Junagadh	20	16	-	25	-
	Vijapur	20	19	16	24	35
PZ	Pune	20	19	-	21	-
	Dharwad	26	11	-	37	-
	Niphad	-	-	18	-	35
Mean (National)		19	18	18	27	35
Three years mean			18		31	
%Superiority of QLD84 over best check variety HS490						41.90%

11. QLD 11 (IC0628573; INGR19011), a Wheat (*Triticum aestivum*) Germplasm with High Grain Protein Content

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Grain protein is an important trait that determines the nutritional quality and baking properties of wheat. It is one of the major pricing factors for wheat trading at global market, and is an important nutritional factor for human health. Protein is an essential component of cells and it supports muscle growth, immune and enzymatic reactions; protein-energy malnutrition causes marasmus and kwashiorkor. Grain yield and grain protein content through its contribution to end-use quality, are the two most important characters determining the economic value of a bread wheat crop.

QLD11, a promising bread wheat genotype was selected from 15th High Rainfall Wheat Screening Nursery (HRWSN) with the parentage of MURGA. The genotype was evaluated at 12 locations along with high protein check variety (UP2672) during 2015-16 and 2016-17 under Quality Component Screening Nursery (QCSN). QLD11 found to be superior with 14.48% protein content over the years and locations to all the testing genotypes and check variety UP2672 (14.06%) (Table 1). QLD11 recorded the high grain protein content in all the 12 tested centers compared to best check variety (UP2672) for high grain protein content. QLD11 was recorded high grain protein content of 14.83% and 14.13%, respectively during 2015-16 and 2016-17. Whereas, the check variety UP2672 recorded 14.39% and 13.72%, respectively during 2015-16 and 2016-17. Thus, QLD11 would be a potential source to be utilized in future breeding programs to improve grain protein content of bread wheat varieties.

Table 1. Grain protein content of QLD11 and UP2672 (C) at 12 locations during 2015-16 and 2016-17

Zone	Location	QLD11		UP2672 (C)	
		2015-16	2016-17	2015-16	2016-17
NHZ	Almora	14.60	14.46	13.27	12.64
	Ludhiana	12.70	13.02	12.73	13.26
	Durgapura	15.80	14.80	15.67	13.64
NWPZ	Delhi	15.10	14.77	14.70	14.24
	Pantnagar	14.20	11.74	12.90	11.84
	Karnal	13.80	14.94	14.00	12.85
NEPZ	Pusa	15.30	12.65	15.10	13.91
	Kanpur	14.00	-	13.83	-
CZ	Junagadh	16.00	14.79	15.33	14.99
	Vijapur	15.70	15.39	14.63	13.89
PZ	Pune	15.10	15.60	14.80	15.00
	Dharwad	15.60	13.30	15.70	14.63
Mean (National)		14.83	14.13	14.39	13.72
Two years mean		14.48		14.06	

12. DWRB191 (IC0626293; INGR19012), a Barley (*Hordeum vulgare*) Germplasm with High Grain Zinc Content

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Large portion of human diet consists of cereals which are important sources of minerals especially micro nutrients like iron (Fe), zinc (Zn), iodine (I) and selenium (Se). Identifying genotypes with higher contents of micronutrients is thus very important and useful in improving nutritional quality of crops and in turn human and livestock health. Barley flour is used with wheat and gram flour to make *chapattis* more nutritious in rural areas of north western plains particularly in Rajasthan, Uttar Pradesh and Bihar since old time. While hulless barley grains are mostly used as food in cold and arid regions of Leh and Laddakh. In this direction new initiatives were taken to develop bio-fortified hulless barley genotypes with enriched zinc content at ICAR-IIWBR, Karnal.

A hulless barley genotype DWRB191 was developed from hybridization BHS352/HBL113, which possesses high grain zinc content and desirable agro-botanic characters in hulless genetic background. A set of 18 genotypes was evaluated during 2016-17 and based on grain zinc data, disease tolerance and agro-morphological characters the genotype DWRB191 was selected. The grain zinc content was estimated by atomic absorption spectroscopy (AAS) method at ICAR-IIWBR, Karnal. The multi-location data of DWRB191with promising checks namely BHS352 and Karan 16 are presented in the Table 1.

The studies on grain zinc and iron content bio-fortification in barley are lacking under Indian conditions. Even no germplasm and variety has been registered/ release for enriched grain zinc content. The genotype DWRB191 showed grain zinc content of 44.9 ppm and while the elite hulless checks viz., BHS352 and Karan16 exhibited grain zinc content of 39.9 ppm and 36.6 ppm, respectively (Anonymous, 2018; Kumar *et al.,* 2018). The barley germplasm DWRB191 revealed 12.5% and 22.7 % superiority for grain zinc content over the checks BHS352 and Karan16, respectively.

Table 1. Grain zinc content (ppm) of DWRB191 and checks

Location	DWRB191	BHS352 (c)	Karan16 (c)
Karnal	35.2	33.2	34.0
Hisar	46.7	40.7	38.1
Durgapura	47.8	44.0	37.6
Jobner	49.7	41.5	36.5
Mean	44.9*	39.9	36.6
% advantage	-	12.5	22.7
LSD (0.05)		4.65	

*Significantly superior to the checks (CD at 5 %) & locations considered as replications

The genotype DWRB191 was observed with semi-erect growth habit and showed 1000 grain weight of 38 g (35-39 g). Barley grains are used in different industrial purposes *i.e.* food, multi-grain *atta,* health drinks etc. and the availability of high grain zinc content genotype DWRB191 will be certainly helpful for health benefits.

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13. DWRB192 (IC0626294; INGR19013), a Barley (*Hordeum vulgare*) Germplasm with High Grain Iron Content

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Barley is used for human food, cattle feed and malting purposes. Barley is called as poor man's crop due to its low input requirement and better adaptability to harsh environments and saline and alkaline soils. The hulless barley grains are mostly used as food in cold and arid regions of Leh and Laddakh and parts of plains in Rajasthan, Uttar Pradesh and Bihar etc. Barley flour in combination of wheat and gram flour is used to make nutritious *chapattis* in different parts of the country. Cereals constitute major portion of human diet and are important sources of minerals especially micro nutrients like iron (Fe), zinc (Zn), iodine (I) and selenium (Se). Therefore, the development of genotypes with higher grain iron content is one of the main objectives in the present time.

DWRB192 is a hulless barley genotype, which was developed from cross BHS352/HBL113 and possesses higher grain iron content. Different hulless barley genotypes were evaluated during 2016-17 and based on grain iron data, disease tolerance and agro-morphological characters the genotype DWRB192 was selected. The grain iron content was estimated by atomic absorption spectroscopy (AAS) method. The multi-location data of DWRB192 are presented in the Table 1 below-

Table 1. Grain iron content (ppm) of DWRB192 and checks

Location	DWRB192	BHS352 (c)	Karan16 (c)
Karnal	44.9	32.9	32.9
Hisar	43.9	39.9	39.9
Durgapura	51.0	42.9	36.9
Jobner	49.9	43.2	41.3
Mean	47.4**	39.7	37.8
% advantage	-	19.4	25.4
LSD (0.01)	6.66		

**Significantly superior to the checks (C.D. at 1 %) & locations considered as replications

Barley grains are used in different industrial purposes *i.e.* food, multi-grain *atta,* health drinks etc. and the availability of high grain iron content genotype DWRB192 will be certainly helpful for health benefits. The genotype DWRB192 showed grain iron content of 47.4 ppm and while the elite hulless checks viz., BHS352 and Karan16 exhibited grain iron content of 39.7 ppm and 37.8 ppm, respectively (Anonymous, 2018; Kumar *et al.,* 2018). The studies on grain zinc and iron content and bio-fortification in barley are lacking under Indian conditions. Even no germplasm and variety has been registered / release for enriched grain iron content. The barley germplasm DWRB192 revealed 19.4% and 25.4

% superiority for grain iron content over the checks BHS352 and Karan16, respectively. The genotype DWRB192 was observed with erect growth habit and showed 1000 grain weight of 40 g (38-41 g).

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14. GJG 0904 (IC0627616; INGR19014), a Chickpea (*Cicer arietinum*) Germplasm with Wilt Resistant, Semi Erect Plant Type, Single Pink Coloured Flower per Peduncle. Tuberculated Seed Texta with Large Sized Brown Colour Desi Type Seed

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Chickpea (*Cicer arietinum* L.) is an important pulse crop grown during *rabi* season in tropics and spring in the temperate region of the world. Chickpea is grown on about 8.39 million ha producing 7.06 million tonnes of seed with productivity of 840 kg/ha in India (Anonymous, 2017). *Fusarium oxysporum* have different races and it causes the wilt disease in chickpea and affects the all major chickpea cultivars (Gurjar *et al*., 2009). As per survey conducted by Sunit *et al*. (2012), the yield loss was up to 72.16 per cent due to this disease. This genotype was found to be resistant in wilt sick plot at ICRISAT, Jabalpur, Rahuri, Sehore and Junagadh during different years (2012-13, 2013-14, 2015-16) of testing.

The line GJG 0904**,** was developed at Pulses Research Station, Junagadh Agricultural University, Junagadh using wilt resistant source by pedigree method of selection in a segregating population of a cross (GJG 0105×Phule G 92926).

Morpho-agronomic characteristics of GJG 0904

Entry	Year	Yield (Kg/ha)	Maturity days	100 seed wt. (g)
GJG 0904	2011-12	2110	108	28.6
	2012-13	2207	105	27.0
Mean		2158.5	106.5	27.8

(Source: AICRP report 2011-12 & 2012-13)

Associated characters and cultivation practices:

- Resistant to *Fusarium* wilt disease
- Semi spreading growth habit
- Medium maturity (106.5 days)
- Single flower per peduncle
- Average Yield (2158.5 Kg/ha)
- Brown seed colour
- Large seed size (27.8 g/100 seeds)
- Angular seed shape

Recommended cultivation practices:

Seed rate: 60 Kg/ha; Spacing: 45×10 cm; Fertilizer dose: 20:40:00 N:P:K

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15. EC390977 (EC390977; INGR19015), a Soybean (*Glycine max*) Germplasm with Photoperiod Insensitivity. Source of Recessive Alleles e3 and e4, Early Maturity

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Soybean (*Glycine max* (L.) Merr.) is a photoperiod sensitive short-day plant and its different accessions starts flowering when the day length, which depends on latitudes, is less than their critical day length. Although soybean adapts to a very wide range of latitudes, its individual accessions adapt to a narrow latitudinal band. India lies in the latitudes between 6°-37° and latitude specific adaptation is observed in soybean accessions and varieties (Bhatia *et al.*, 2003). Soybean germplasm accession EC 390977 has been acquired from Taiwan and its photoinsensitive nature has been confirmed at ICAR-IISR, Indore.

At ICAR-IISR, 2071 soybean accessions were evaluated for their response to photoperiod by growing them in ambient and extended day length conditions (17 hrs). Under extended photoperiod, only 7 germplasm accessions including EC 390977 showed no significant delay in flowering and could reach to physiological maturity and hence, was identified as photoperiod insensitive(Bhatia *et al.*, 2003; Singh *et al.*, 2008a,b,c; Gupta *et al.*, 2017). Among rest of the germplasm accessions, about 30% showed delay in flowering ranging from 10 to 100 days while rest of the accessions did not flower under extended photoperiod indicating a very high degree of sensitivity to photoperiod. From 30% of the accessions that could flower under extended photoperiod, none of the accessions could reach to physiological maturity. This clearly indicated that besides flowering other reproductive stages were also sensitive to day length in soybean.

EC 390977 has been evaluated at different latitudes along with photosensitive varieties (Dharwar, Pune, Indore and Pantnagar) and has shown consistent flowering response. Delayed flowering response has been observed at higher latitudes in photosensitive varieties (Fig. 1). EC 390977 has semi-determinate growth habit with

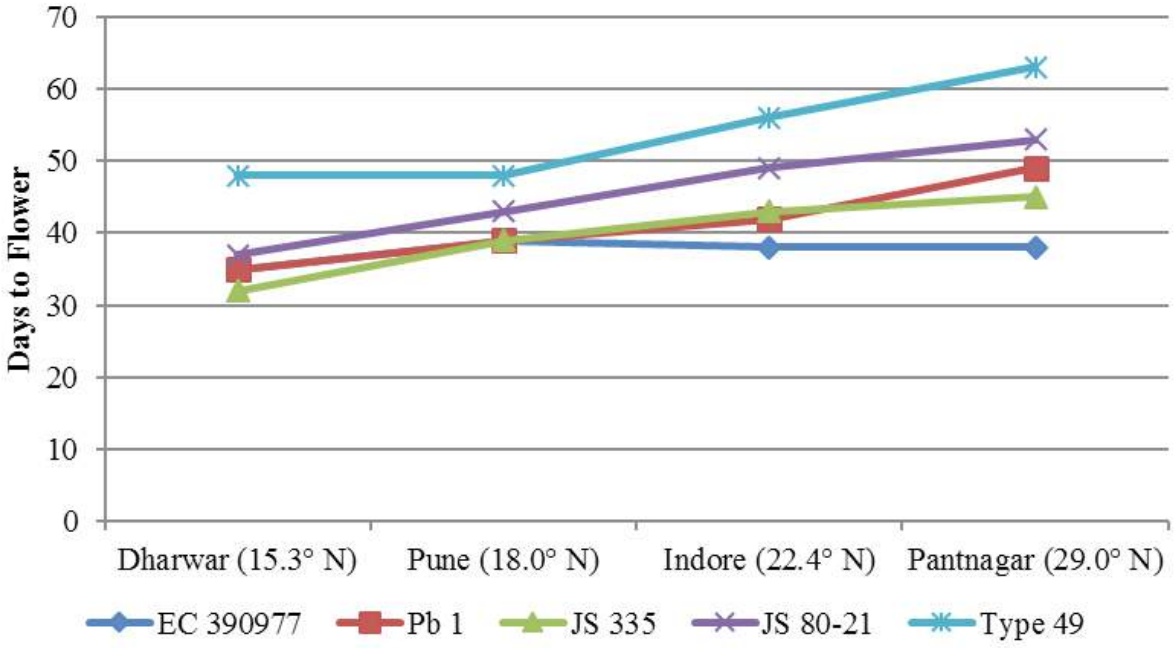

Fig. 1. Latitudinal consistency of EC 390977 in days to flowering

plant height of 60-65 cm, purple hypocotyl, purple fowers with flowering and maturity of 37-39 and 85-87 days, respectively. Its pod shatter at maturity and have tawny pubescence, seeds are yellowish green with light brown hilum. EC 390977 has been characterized for genes underlying photoperiod insensitivity, maturity and growth habit traits using sequencing and allele specific markers, and its genotype has been determined as *E1E1E2E2e3e3e4e4* (Gupta *et al.*, 2017). EC 390977 has allele specific markers for recessive alleles of *E3* and *E4* and dominant allele of *Dt1* and it has been used as a donor of these alleles for marker assisted back cross breeding in ICAR-IISR, Indore.

Genotype EC 390977 is a useful germplasm source for breeding of photo-insensitivity, earliness and semi- and indeterminate growth habit in soybean. These traits are desirable for development of soybean cultivars for growing in new areas across latitudes for wider adaptation and increasing cropping intensity & harvest index of the crop. Identified allele specific markers would be useful for gene pyramiding for earliness and photo-insensitivity using marker assisted breeding.

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16. MACS 330 (IC538550; INGR19016), a Soybean (*Glycine max* Germplasm with Photoperiod Insensitivity. Source of Recessive Alleles e2, e3-tr, Extra Early Maturity

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Soybean (*Glycine max* (L.) Merr.) is a photoperiod sensitive short-day plant and its different accessions starts flowering when the day length, which depends on latitudes, is less than their critical day length. Although soybean adapts to a very wide range of latitudes, its individual accessions adapt to a narrow latitudinal band. India lies in the latitudes between 6°-37° and latitude specific adaptation is observed in soybean accessions and varieties (Bhatia *et al*., 2003). Soybean breeding line MACS 330 was received for Initial Varietal Yield Trial in All India Coordinated Research Project on Soybean and conserved at the National genebank with National identity IC 538550. The MACS 330 is an advance generation selection from the cross Monetta × EC 95937, developed at Agharkar Research Institute, MACS, Pune.

At ICAR-IISR, 2071 soybean accessions were evaluated for their response to photoperiod by growing them in ambient and extended day length conditions (17 hrs). Under extended photoperiod, only 7 germplasm accessions including MACS 330 showed no significant delay in flowering and could reach to physiological maturity and hence, was identified as photoperiod insensitive (Bhatia *et al*., 2003; Singh *et al*., 2008a,b,c; Gupta *et al*., 2017). Among rest of the germplasm accessions, about 30% showed delay in flowering ranging from 10 to 100 days while rest of the accessions did not flower under extended photoperiod indicating a very high degree of sensitivity to photoperiod. From 30% of the accessions that could flower under extended photoperiod, none of the accessions could reach to physiological maturity. This clearly indicated that besides flowering

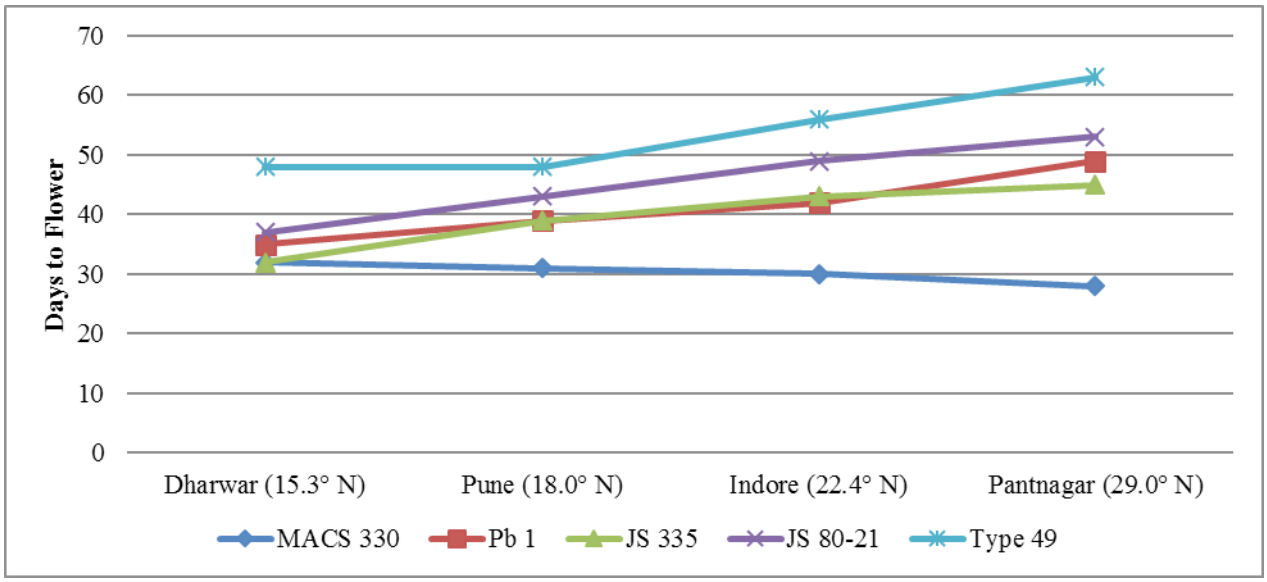

Fig. 1. Latitudinal consistency of MACS 330 in days to flowering

other reproductive stages were also sensitive to day length in soybean.

MACS 330 has been evaluated at different latitudes along with photosensitive varieties (Dharwar, Pune, Indore and Pantnagar) and has shown consistent flowering response. Delayed flowering response has been observed at higher latitudes in photosensitive varieties (Fig. 1). MACS 330 has determinate growth habit with 20-25 cm plant height, green hypocotyl, white flowers with flowering and maturity of 30 and 75 days, respectively. Its pods have tawny pubescence, seeds are yellow with gray hilum and being a breeding line it has a yield potential of up to 2 t/ha. Photoinsensitivity and determinate growth habit of MACS 330 has been confirmed through its characterization at photoperiodic, maturity and growth habit loci using sequencing and allele specific markers. Its genotype has been determined as *E1E1e2e2e3e3E4E4dt1dt1* (Gupta *et al.*, 2017). Recessive alleles of *E2* and *E3* confer earliness and photoperiod insensitivity and *dt1* makes this genotype determinate in growth habit.

Genotype MACS 330 is a useful germplasm source for breeding of photo-insensitivity, earliness and determinate growth habit in soybean. These traits are desirable for development of soybean cultivars for growing in new areas across latitudes for wider adaptation of crop, and increasing cropping intensity & harvest index. Identified allele specific markers would be useful for gene pyramiding for earliness and photo-insensitivity using marker assisted breeding.

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17. IPC-15 (DPC-15) (IC0625992; INGR19017), a Castor (*Ricinus communis*) Pistillate Line with Papaya Leaf Type, Non-spiny Capsules

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Mechanism of pistillate lines in castor is based on N, S and NES type. Majority of the pistillate lines are based on S type-dominant and epistatic in nature while NES mechanism is based on recessive genes for pistillate trait (Lavanya 2002 and Lavanya *et al.,* 2006). The present genetic stock, IPC-15 (DPC-15), is developed through conventional breeding technique *viz*., intra varietal hybridization followed by pedigree method of selection and generation advancement. Pistillate trait is from a NES source viz., NES-6, distinct with non-spiny capsules while DCS-12 is used for incorporating a distinct leaf type called "papaya leaf type" which is characterized by deep lascination of the leaf. Pistillate plants were identified in the segregating population of the cross, NES-6 x DCS-12 with the unique traits like non-spiny capsules and papaya leaf type. The pistillate trait was further stabilized by selfing the pistillate line with the male flower at the base of the spike. Heritability of the pistillate trait was observed up to 98 % in a population of nearly 60-100 plants every year. In a trial on stability of pistillate character of three pistillate lines viz., DPC-14, DPC-15 (IPC-15) and DPC-16 in three dates of sowing viz., 1st November 2006, 22nd December 2006 and 14th February 2007, the proposed pistillate line recorded mostly female plants and a few plants with 100 % monoecious and 100 % interspersed staminate flowers, necessary for the maintenance of the pistillate line (Anonymous, 2007).

IPC-15 has other distinct morphological characters like red stem, double bloom, very short plant height up to primary spike (16-18 cm) and very short primary spike (15-20 cm). The pistillate line flowers in 28-30 days and matures in 85-90 days. Primary spike emerges by 7 nodes while the secondary spikes emerge in 5-6 nodes (Table 1). The pistillate line is also distinct due to its

Table 1. Chief botanical and morpho-agronomic description of the proposed pistillate line along with DPC-9 and M-574 for comparison

Characteristic	IPC-15 (DPC-15)	DPC-9	M-574
Stem color	Red	Green	Green
Presence of bloom	Double	Zero	Triple
Type of internodes	Elongated	Elongated	Condensed
Number of nodes up to primary spike (Mean)	7	14	15
Leaf shape	Flat	Flat	Deep Cup
Leaf lascination	Deep (papaya / okra type)	Shallow	Shallow
Branching pattern	Divergent	Divergent	Convergent
Spike shape	Cylindrical	Cylindrical	Conical
Spike length of primary	Small (15-20 cm)	Long (41-60 cm)	Long (41-70 cm)
Spike compactness	Loose	Semi-compact	Semi-compact
Stigma color	Bright red	Greenish yellow	Red
Sex expression	Pistillate with ISF	Pistillate with ISF	Pistillate with ISF
Capsule: spines	Non-spiny	Spiny	Spiny
100-seed weight (g)	30	29	29
Seed: shape	Oval	Oval	Oval
Seed: base color	Brown	Light brown	Light brown
Seed: mottling	High	Low	High
Seed: caruncle	Inconspicuous	Conspicuous	Conspicuous

loose spike and non-spiny capsules. Among 34 parental lines and three checks screened, five lines viz., DPC-15, DPC-21, DPC-25, M-571 and M-574 were found highly resistant to leafhopper (grade 0) compared to susceptible checks DPC-9 and DCH-177 (grade 4.0) (DOR Annual Report, 2013-14). Molecular diversity study of 20 castor genotypes indicated that IPC-15 (DPC-15) is grouped along with pistillate lines like NES-6, NES-22 and DPC-16 which were developed using NES source of pistillateness (Ushakiran and Lavanya, 2016).

IPC-15, a genetically diverse, stable pistillate line with distinct morphological characters like papaya leaf type, non-spiny capsules, early maturity and short plant height has a good potential as a female line for the development of castor hybrids. Among the several preliminary hybrids developed using IPC-15 as female, two hybrids *viz*., PHT-12-2 (DPC-15 × DCS-105), PHT-12-3 (DPC-15 × DCS-107) with early maturity (100 days to the primary spike) and high yielding (2332 kg/ha) compared to the best check, GCH-7 (2188 kg/ha) were promising.

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18. RG-631 (IC0546708; INGR19018), a Castor (*Ricinus communis*) Germplasm Resistant to Leafhopper [(*Empoasca flavescens* (Fabr) (*Cicadellidae: Hemiptera*)]

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Castor (*Ricinus communis* L.) is an important non-edible industrial oilseed crop. India is the leading castor growing country in the world. Leafhopper, *Empoasca flavescens* Fabr. (Cicadellidae: Homoptera) is an important sucking pest and could cause yield loss to the extent of 22-89% in castor (Jayaraj, 1966; Lakshminarayana and Duraimurugan, 2014). In order to manage this pest, growing a resistant castor cultivar is an effective and economic measure. Availability of a stable resistance source is a prerequisite in leafhopper resistance breeding programmes. In order to identify sources of resistance to leafhopper, several genotypes were screened against leafhopper. RG-631, which was derived from a segregating population of a local collection, Punjab-1 through pedigree selection followed by inbreeding for about 12 years, has showed resistance to leafhopper in the first year. Its resistance reaction was tested at Palem, Yethapur and Hyderabad for four years using infester-row method under heavy leafhopper infestation condition.

Morpho-agronomic characteristics: RG-631 is a medium duration (55 days to flowering and 120-125 days to maturity), tall plant (124 cm) type having 16-17 nodes on green coloured stem with heavy triple bloom. Its capsules are green, non-spiny and non-dehiscent; its 100-seed weight is 30.3 g and oil content is 51%.

Associated character and utility: RG-631 was found to be stably resistant to leafhopper (0-1 hopper burn on 0-4 score) at Palem, Yethapur and Hyderabad locations in all the four years of testing. This would serve as a stable source of leafhopper resistance to castor breeders in All India Coordinated Research Project on Castor and other castor breeders in the country. It would also serve as genetically uniform base material to develop mapping populations for identifying molecular markers flanked to leafhopper resistance, and for developing RILs as well as for tagging the gene(s) controlling leafhopper resistance.

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19. RG-57 (IC0628058; INGR19019), a Castor (*Ricinus communis*) Germplasm Morphologically Distinct Selection from a Wild Type Collection, 1077-2 from Dantiwada, Gujarat

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India is the largest producer of castor (*Ricinus communis* L.) in the world and secured a virtual monopoly in castor production. Castor oil is the only natural oil possessing ricinoleic acid in high percentage. Ricinoleic acid in commercial cultivars is 85-88%; ricinoleic acid makes castor oil a unique source for preparation of thousands of high globally demanding industrial derivatives (Ogunniyi, 2006).An increase in ricinoleic acid from the existing level would increase value of castor oil as it would be highly beneficial to industry and the country. In order to identify sources of high ricinoleic acid type genotypes, germplasm was assayed for fatty acid profile. The accession, RG-57 giving more than 90% ricinoleic acid was further tested at four locations under rainfed and irrigated conditions for two years in RBD with two replications to test its stability for ricinoleic acid content. It was developed through progeny selection and inbreeding from a semi-wild type castor collection (1077-2) collected from Dantiwada, Gujarat.

Morpho-agronomic characteristics: RG-57 is a short plant type (78-85 cm) having green stem with bloom and 14 nodes on it. It has green spiny capsules and reaches to 50% flowering in 54-66 days and maturity in 109-126 days. Its 100-seed weight is 27-29 g and oil content is 45-47%

Associated character and utility: RG-57 possessed 91% mean ricinoleic acid content when tested for two years at four locations under rainfed and irrigated conditions indicating its high stability for high ricinoleic acid content. RG-57 is a unique source of high ricinoleic acid, and serves as a donor for high ricinoleic acid content for developing high ricinoleic type castor cultivars.

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20. DRMR 1-5 (IC0625996; INGR19020), an Indian Mustard (*Brassica juncea*) Germplasm with Double Low (<2%) Erucic Acid in Oil &< 30 µmoles Glucosinolate/g of Defatted Seed Meal). White Rust Resistant. Yellow Seed Coat

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DRMR 1-5 is a yellow seeded Indian mustard (*Brassica juncea* L.) genotype possessing white rust resistance with double low characteristics along with at par oil content in comparison to checks. DRMR 1-5 derived as single plant selection from the cross NUDHYJ-3 x Varuna, was evaluated along with 11 lines derived from the same cross and 2 checks varieties, namely Kranti (national check) and Pusa Mustard-21 (quality check) for morphological and biochemical characters in RCBD with 3 replications during 2012-13.The erucic acid content was determined by preparing methyl ester of oil sample (Sarin *et al*., 2009) in SP 2300 + 2310 SS column of Nucon model 5765 gas chromatograph. The glucosinolate content in the seed meal was determined by complex formation between glucosinolates and sodium tetra-chloropalladate solution using spectrophotometer at 405 nm as per Kumar *et al*., 2004. The oil content was determined in a Soxhlet apparatus (AOAC, 1997). DRMR 1-5 showed double low quality trait under IVT and AVT-I trial of AICRP-RM programme during

Table 1. Description of quality traits and white rust resistance of DRMR 1-5

Average over centers (Bharatpur, Ludhiana and New Delhi) and years (2012-13 to 2016-17)			
	Erucic acid (%)	Glucosinolate (µmoles/g of defatted seed meal)	Oil content (%)
Kranti (National Check)	40.71	92.5	40.17
Pusa-Mustard-21 (Quality check)	2.0	78.1	37.37
DRMR 1-5	1.29	23.2	40.72
% White Rust severity average over AICRP-RM centers and years (2012-13 to 2016-17)			
	75DAS	100DAS	Staghead
Rohini (Susceptible check)	26.0	30.8	12.9
DLSC-1 (Resistant check)	1.2	0.0	0.0
DRMR 1-5	2.8	1.6	0.0

2013-14 and 2014-15 at different locations. DRMR 1-5 also showed resistance for white rust disease caused by *Albugo candida* under both natural and artificial condition in the experiment conducted in different locations under AICRP-RM testing during 2013-14 to 2016-17, while screening against white rust disease along with other strains and checks including susceptible checks repeated after every 2 test rows. Observations on white rust disease were recorded on 75 and/or 90-100 DAS and number of stag head was recorded 15 days before harvesting. Double low quality trait for DRMR 1-5 was further confirmed during 2016-17 at DRMR, Bharatpur. DRMR 1-5 was included in the Brassica germplasm during year 2015-16 under AICRP-RM and in the year 2016-17 it was identified as white rust resistant source of *B. juncea* for use in breeding programmes. DRMR 1-5 showed 1.3 per cent erucic acid and 23.2 µmoles glucosinolate per gram of defatted seed meal with 41 per cent oil content along with resistance against white rust, in mean over locations of AICRP-RM trials and DRMR (Table 1).

Morpho-agronomic characters: Morpho-agronomic characters of the germplasm DRMR 1-5 are as follows: plant height (196.9 cm), main shoot length (62.7 cm), number of primary branches (6.1), number of siliqua on main shoot (60.5), number of siliqua per plant (822.9), 1000-seed weight (2.8 g), seed yield per plant (23.5 g), seed yield (1884 kg/ha) and oil yield (795 kg/ha). It completes 50 per cent flowering in 55 days and reaches to physiological maturity in around 132 to 138 days. Its seed coat colour is yellow.

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21. DRMRIJ 12-48 (IC0628059; INGR19021), an Indian Mustard (*Brassica juncea*) Germplasm with Multiple Disease Resistance (White Rust, Powdery Mildew and Alternaria Blight)

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White rust caused by *Albugo candida* (Pers. ex Lev.) Kuntze, Alternaria blight caused by *Alternaria brassicae* (Berk.) Sacc., and powdery mildew caused by *Erysiphe cruciferarum*are the most devastating diseases of Crucifers and cause severe yield losses in Indian mustard (*Brassica juncea*). Resistant sources are available for white rust in *Brassica juncea* but resistant sources are not available for Alternaria blight and powdery mildew in *B. juncea,* though have been reported in related / wild species. Further developing a genetic stock with resistance/ tolerance against all the three diseases will be an advantage in preventing the losses caused by these diseases. DRMRIJ 12-48 is an inbred line derived from a cross between OJR 2 and Zem 2 (exotic

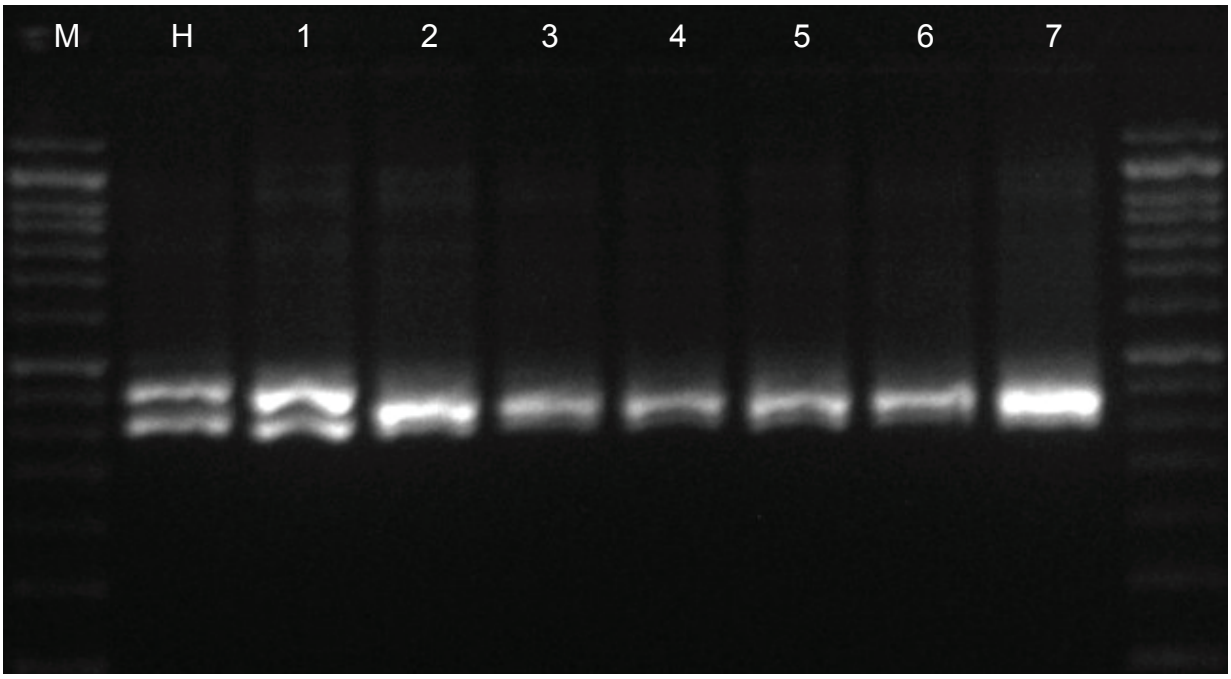

Fig. 1. Validation of IP marker (At5g41560) tagged to white rust resistance loci (AcB1-A4.1) in DRMRIJ 12-48
M: 50 bp DNA ladder; H: Heera, 1: DRMRIJ 12-48; 2: Varuna; 3: Rohini; 4: Kranti; 5: NRCHB 101; 6: Giriraj & 7: RH 749

germplasm) followed by individual plant selection. Cross was attempted during 2008-09 between OJR 2 and Zem 2.

Morpho-agronomic characteristics: Strain DRMRIJ 12-48 was characterized for agronomic traits viz., days to flower initiation (57 days), plant height (169 cm), length of main raceme (47 cm), days to flower senescence (107 days), days to maturity (136 days), oil content (38.7%), 1000-seed weight (4.7 g), number of seeds per siliquae (16.5) and siliquae length (4.4 cm). DRMRIJ 12-48 was inducted to AICRP-RM during 2014-15 and have been evaluated against white rust, alternaria blight and powdery mildew continuously for three years during 2014-15. 2015-16 and 2016-17.

Associated characters and cultivated practices: White rust reaction at leaves was recorded at two different stages viz., 75 and 100 days, while staghead formation was recorded at flowering stage. Candidate strain DRMRIJ 12-48 expressed resistant reaction against white rust at 75 days stage which was at par with resistant check BIO YSR during all three years of testing at Hisar. Disease reaction against white rust on leaves at 100 days stage was recorded at five locations viz., Bharatpur, Kangra, Kanpur, Pantnagar and Morena which included three hot spots (Kangra, Pantnagar and Morena). On the basis of mean disease index over all five locations for three years, candidate strain DRMRIJ 12-48 expressed 3.4 % which was better than the resistant check BIO YSR (8.9%) and susceptible check Varuna (42.9%) and Rohini (32.3%). A perusal of location wise disease reaction data revealed that candidate strain DRMRIJ12-48 had immune reaction at Hisar, Bharatpur, Pantnagar and Morena centres during all three years of testing. Resistant reaction against white rust was confirmed with already reported IP marker also (Fig. 1).

Reaction against alternaria blight was recorded on leaves at 100 days stage at five locations viz., Kangra, Kanpur, Morena, Hisar and Bharatpur and on pods at three locations; Kangra, Hisar and Morena over three years of testing. Disease incidence as indicated by percent Alternaria blight severity on leaves was lesser (27.2%) than both resistant (38.4% in BIO YSR) and susceptible checks (34.1 % in Varuna and 46.1% in Rohini). Considering less disease incidence on leaves on the basis of average of three years than the check cutlivars and at pods also at two locations, DRMRIJ 12-48 was found tolerant to alternaria blight.

Observations on powdery mildew incidence were recorded at Morena centre during all three years of testing, while at Kanpur for one year *i.e.* during 2016-17, hence representing performance of four environments. Percent powdery mildew severity was 0.6 % in DRMRIJ 12-48,

which was better than all existing check cultivars viz., BIO YSR (47.1%), Varuna (51.5%) and Rohini (44.0%), indicating resistant reaction of candidate strain DRMRIJ 12-48 against powdery mildew.

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22. Mutant 85 (IC0621697; INGR19022), a Linseed (*Linum usitatissimumm*) Germplasm for Early Maturity for Southern Zone

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A substantial part of rice fallows during the *rabi* (post rainy) season are left uncropped because of limited availability of soil moisture. Late maturity is a major inconvenience to utilize the residual moisture after paddy harvest, where plants must mature within a muchshorter growing season. The objective of this study was to develop early maturing linseed mutants for efficient utilization of available residual moisture after paddy harvest.

Morpho-agronomic characters: The mutant line 85 derived from 0.3 per cent EMS treatment recorded early days to fifty per cent flowering (35 days) and maturity at and 95 days respectively, compared to mean value of 40.00 days for fifty per cent flowering and 110 days for maturity in corresponding control NL- 115. This genotype with shorter duration is suited for its cultivation in paddy fallows utilizing the residual moisture.

Associated characters and cultivated practices: In the present study, promising mutants for early maturity (Mutant No. 85) derived from 0.3 per cent EMS treatment recorded an early days to 50 per cent flowering and maturity at 35 days and 95 days respectively, compared to mean value of 45.20 days for 50 per cent flowering and 104 days for maturity in corresponding control (Plate 1). This mutant has recorded significantly higher number of secondary branches per plant (75) compared to the parent with 23 secondary branches, significantly higher number of capsules per plant (230), while its non mutant parent has recorded 56 capsules. This genotype has recorded significantly higher yield of 10 q/ha compared to 7.35q/ha recorded by the initial variety NL-115. The performance of other early maturing mutants is presented in Table 1.

Table 1. Performance of early mutants for seed yield and test weight

Mutants	Seed yield (kg/ha)	Days to 50% flowering	Days to maturity	Plant height (cm)	Number of primary branches/ plant	Number of secondary branches/ plant	Number of capsules/ plant	Number of seeds/ capsule	Test weight (g)	Oil (%)
85	1000*	35*	95*	40	5	75*	230*	6	13.83	40.30
79-4	740	35*	98*	41 40	9*	73*	215*	5	11.83	40.43
60-4	655	35*	98*	38	12*	103*	438*	5	15.37	39.40
26-3	800*	35*	94*	36	19*	209*	325*	4	8.64	40.13
Control Mean	735	45.2	110	46.64	4.37	23.25	56.01	6	9.45	39.74
CD at 5%	12.23	1.78	1.895	7.989	4.121	17.871	22.34	2.027	8.21	1.94

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23. SFS-9943 (IC0625999; INGR19023), a Safflower (*Carthamus tinctorius*) Germplasm with High Oil Content (34.7%)

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Safflower (*Carthamus tinctorius* L.) is a multipurpose oilseed crop grown in winter season in India, which is the major safflower growing country in the world. The cultivars from the major safflower growing countries *viz.,* India and China are of low oil type (28-29%) (Smith, 1996). Low oil content in Indian safflower varieties has necessitated development of safflower genotypes with enhanced oil content. An inbred line, SFS-9943 derived from [(537701 × 544042) × 560165) × 537701] × 537701 was tested at 16 locations under rainfed and irrigated conditions under All India Research Project on Safflower.

Morpho-agronomic characteristics: SFS-9943 is spiny in nature, flowers are yellow at bloomand orange when dried. Seeds are white in colour (100 seed weight: 3.6 g). It reaches to 50% flowering in 88 days and maturity in 132 days.

Associated character and utility: SFS-9963 had an average oil content of 34.7% while thecheck varieties, A1, PBNS-12 and NARI-6 had 26.8, 27.4 and 29.8%, respectively. Oil content had ranged from 28.6-38.9% in SFS-9943, 23.7-29.1% in A1, 23.3-31.2% in PBNS-12 and 25.8-33.1% in Nari-6 across 16 locations. SFS-9943 had 34.6-38.9% oil content at 11 locations, 30-31.3% oil content at four locations and 28.6% at one location out of 16. It ranked 1st at 12 locations with respect to oil content among the 25 entries tested in AICRP (Safflower) (Ann. Rep. Safflower, 2015). Variation in oil content across locations was attributed to influence of environment on oil content (Mahali and Carapetian, 2013; Mustafa *et al*., 2016). SFS-9943 is anindigenous source of high oil content and would serve as a high oil parent in breeding aiming to enhance oil content in safflower.

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24. IISR 7548 (IC0619910; INGR19024), a Black Pepper (*Piper nigrum*) Germplasm for Black Pepper Cultivar with Very Long Spike (29.3 cm; reported Range is 3.7 to 17.1 cm)

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Black pepper (*Piper nigrum* L.) originated in the Western Ghats of South India. This region is endowed with many landraces besides the progenitors of cultivated black pepper. Landraces, natural mutants, improved varieties and even true seedlings constitute the primary genepool of this tropical vine (Sasikumar *et al.*, 2007, 2011; Ravindran *et al.*, 2000). The ICAR-Indian Institute of Spices Research (Kozhikode, Kerala, India), the apex organization for black pepper research, has been involved in the mapping, collection, characterization and conservation of the diversity of black pepper from its centers of diversity/ domestication of the country. A unique black pepper accession with very long spike was collected from a tea estate at Wayanad district and registered for its unique character.

Morpho-agronomic characteristics: A black pepper germplasm survey and collection programme was undertaken Coottanadu Estate, Wayanad, Kerala, bordering the evergreen forest of the Western Ghats (latitude: 11°34'42''N; longitude: 76° 06'70''E; altitude: 800m mean sea level. Collection number 7548 (IC-0619910) is given to the specimen. This accession is characterized by very long spike with a maximum length of 29.3 cm, hitherto unreported in the primary genepool of black pepper. The morphological characters of the crop based on DUS guidelines is given in Table 1.

Table 1. Morphological characters of IC- IC-0619910

Character	Remark/Value
Shoot tip color	Light purple
Leaf length (cm)	12.30 (medium)
Leaf width (cm)	5.55 (narrow)
Leaf petiole length (cm)	1.05 (short)
Spike length (cm)	29.3
Spike peduncle length (cm)	1.31 (medium)
No. of berries/spike	17.6 (less)
Piperine (%)	3.4
Essential oil (%)	2.7

Spike length is an important yield contributing trait in black pepper and amenable for selection (Sujatha and Namboodiri, 1995; Pillai *et al.,* 1979). Reported spike length in cultivated black pepper ranges from 3.7 to 17.1 cm (Ravindran *et al.,* 2000). The present new collection with unusually long spike length is thus a unique line which will be a source of new genes for spike length improvement in black pepper.

Associated characters and cultivated practices: Besides its unique feature, the accession is characterised by a loose setting; purple to light purple shoot tips; drooping/hanging lateral branches; ovate–lanceolate to ovate leaves with acuminate tips; medium-quality, round, small berries. The fresh yield per vine was 2–3 kg.

Being blessed with excellent vegetative multiplication method black pepper can be easily propagated using rooted cuttings. Runner shoots of the accession is rooted in the nursery and planted in live standards to conserve the genotype following the practice in vogue. It is being further screened against biotic and abiotic stresses.

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25. DPO185 (IC0627267; INGR19025), an Isabgol (*Plantago ovata*) Germplasm with Yellow Leaf Tip. Downy Mildew Resistance

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Isabgol (*Plantago ovata* Forsk.) belongs to *Plantaginaceae* family, is an important medicinal plant commercially grown in India. The external seed coat, 'psyllium husk' of commerce is an excellent dietary fibre and is commonly known as "Isabgol" in Hindi and "Blonde Psyllium" in English. The isabgol husk (epicarp of seed) has medicinal properties and is used against constipation and irritation of digestive tract. It is hydrophilic in nature and upon absorbing water, increases by 10-15 times in volume. Consequently, it is a very good dietary fibre, which stimulates peristalsis and helps in bowel clearance. The husk, which is about 25 to 30% of the seed, has the property of absorbing and retaining water and hence, it works as an anti-diarrheal drug. The husk is also used in calico printing, dyeing, agar-agar media preparation, gum and jelly making, as binder in tablets, as thickner and a fixative in ice-cream, confectionary and in cosmetics industries. It has been used as a deflocculant in paper and textile manufacturing, as an emulsifying agent, as binder or lubricant in meat products, and as a replacement of fat in low-calorie foods. Downy mildew (DM) disease caused by fungal pathogen *Peronospora plantaginis* Underwood., is one of the most important and destructive diseases of Isabgol. The oomycete pathogen *P. plantaginis* is a typical obligate biotroph, solely depend on the host constituents for its nutrients and survival. Development of high yielding varieties with distinct morphological marker (s) and resistance of Downy mildew are the major breeding objectives in this crop. In this direction, a mutation breeding was attempted using chemical mutagens like DES, EMS and colchicine in the popular variety GI-2. Several morphologically distinct mutants were obtained. The Isabgol genotype DPO-185 is a mutant of GI-2, a popular cultivar of Isabgol grown in India bred at ICAR-Directorate of Medicinal and Aromatic Plants Research (ICAR-DMAPR), Anand, Gujarat, India. The genotype, DPO-185 is erect, shows distinct yellow leaf tip coloration followed by tip drying during flowering, resistant to DM disease, late maturing (130-140 days) and low yielding. DOP-185 was challenged with the fungal stains causing Downy mildew disease during flowering to ascertain the disease resistance from 2014 to 2017 and was compared with DPO-14 (INGR11035), a DM susceptible and early genotype and a registered germplasm. Visual disease score was recorded 10 days after the pathogen challenge on 1 to 9 scales on the basis of area under DM infection through visual observations. The genotype DPO-185 recorded mean score of 3.3 with score ranging from 3.0 to 4.0 across seasons indicating the resistance to DM disease. DPO-185 is late maturing (130-140 days) from the date of sowing as compared to 100 days in DPO-14 and its parent GI-2 (110-120 days). The resistant genotype can be a potential allelic donor to improve host plant resistance to DM disease in Isabgol. Further, the genotype is useful for unravelling genetic mechanisms underlie DM-host interaction. Yellow leaf tip trait can be used as DUS character for identification of elite germplasm lines and cultivars.

26. DTPO6-6 (IC0627269;INGR19026), Tetraploid Isabgol (*Plantago ovata*) Germplasm

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Isabgol (*Plantago ovata* Forsk, Plantaginaceae, diploid species with 2n = 8) is an important medicinal plant cultivated in India and regularly being exported in the world market. Isabgol seed husk has the property of absorbing and retaining water which accounts for its utility in stopping diarrhea and it is a diuretic, alleviates

kidney and bladder complaints, gonorrhea, arthritis and hemorrhoids. Being an introduced crop from Mediterranean region to India, the available genetic variability is very low and hence attempt have made to induce variability through mutation and polyploidy. A stable tetraploid (2n = 4x = 16) (DTPO6-6) line of isabgol was developed from the variety GI 2 using colchicine (0.1 to 0.5%) seed treatment. The tetraploidy was confirmed throughflow cytometry, root anatomy phenotypic observation and cytology. Further, tetraploid genotype was compared with its counterpart diploid parent for qualitative and quantitative morphological traits. The tetraploids were fertile and stable over years (2010-2017). Morphologically the tetraploids were more vigorous than the diploids but were late maturing. The anatomical comparison revealed that the size xylem and phloem of stem and inflorescence stalk were larger in tetraploids than diploids. The seed yield was also higher in tetraploids than thediploids. Further, selection and breeding can be done in tetraploid lines for the qualitative and quantitative improvement of Isabgol after inter crossing between tetraploid lines.

27. DWS-10 (IC0627268; INGR19027), a Withania/Ashwagandha (*Withania somnifera*) Germplasm with Male Sterility at 10-30^{o}C at RH50-60% and 20-40^{o}C at RH40-50%

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Ashwagandha [*Withania somnifera* (L.) Dunal] belongs to family Solanaceae and its somatic chromosome number 2n=48. It is considered as wonder herb with multiple medicinal properties. It is cultivated in the North western and Central India besides some parts in peninsular region as post rainy crop. The species is annual to perennial, branched under shrub to herb of about 30 to 120 cm height. Roots are the major medicinally important part in addition to leaves and seeds. In 2009, in one of the germplasm of ashwagandha, RAS 23, a spontaneous male-sterile plant (DWS10) was found which produced male-sterile pollens under short-day (winter) condition and fertile pollens under long-day (summer) condition. The seeds obtained from this plant were grown in the next season and compare with its counter fertile plant (Parent) for morphological, anatomical, flower biology and physiological parameters. This sterile material can reproduce itself by selfing under long-day condition (summer season; temperature >35^{o}C), and becomes male sterile during winter; temperature 10-30^{o}C and RH 40-50%. Crosses can be effected during winter to produce F_1 hybrids. Significant differences between male sterile and its counter parts were observed for morphological and floral traits. Crosses were made were made between male sterile and male fertile plants during winter season. Per cent berry setting ranged from 14.29 (DWS10 x MWS204) to 54.17% (DWS10 x MWS139) and the number of seeds per berry ranged from 3.50 (DWS10 x MWS204) to 22 (DWS10 x MWS139). DWS10 can form base for commercial exploitation of hybrid vigour in Ashwagandha.

28. AKSM-08 (IC0586912; INGR19028), a Safed Musli (*Chlorophytum borivilianum*) Germplasm for High Root Weight and High Saponin

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Safed musli (*Chlorophytum borivilianum Sant*. & Fernandez) an herbaceous plant belonging to the family liliaceae commonly called as Safed musli belongs to the genus *Chlorophytum.* Mostly collected from wild sources as its tubers are in high demand in indigenous systems of medicine in the preparation of an aphrodisiac drug for curing general debility.

The present study compiled with four years data (*Kharif* 2011-15) wiht thirteen genotypes and present germplasm AKSM-08 (IC586912) have been collected from Bordi village, in Akola District in Maharashtra. These germplasm are maintained and evaluated in Nagarjun Medicinal Plants Garden, Dr.Panjabrao Deshmukh Krishi Vidyapeeth., Akola. The pure line selection method of plant breeding was applied.

Table 1. Yield performance of IC586912 accession

Year of testing	Root yield(g/plant)	AKSM-8 (IC586912) Root yield (q/ha)
2011-12	22.07	45.20
2012-13	22.53	48.96
2013-14	19.47	44.05
2014-15	18.20	37.65
Mean	20.57	43.97

Source: AICRP Report Dr. P.D.K.V., Akola.

The study revealed that, the root weight (g/plant) of AKSM-8(IC586912) was (20.57) however the root yield per hectare was significantly higher (43.97). AICRP Report of 2011-15).

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29. BMS-122/NIC-718(IC0112532; INGR19029), a Barbados Aloe (*Aloe barbadensis*) Germplasm with High Leaf Weight, High Mucilage Content

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Aloe barbadensis (2n=14) are perennial succulents or Xerophytes belongs to the family Liliaceous. Aloes have green fleshy leaves covered by thick cuticle, under which a thin vascular layer covering an inner clear pulp (Boudreau *et al*., 2013a). Though there is much diversity in Aloes the cultivators are unable to choose the best ecotype genetically differentiated is restricted to a specific habitat for commercial cultivation. Hence the present study carried out.

The present study compiled with four years data (*Kharif* 2011-15) with seventeen genotypes. In which nines were collected throughout the Maharashtra and eight genotypes were collected from National Research Centre, Boriavi (Anand), Gujarat. This germplasm were maintained and evaluated by Nagarjun Medicinal Plants Garden, Dr. Panjabrao Deshmukh Krishi Vidyapeeth., Akola. The pure line selection method of plant breeding was applied. In the present study the germplasm has been registered for its unique characteristics of high leaf weight and high mucilage content.

The study revealed that, the leaf weight (g/plant) of IC112532 (BMS-122) was (344.60), however the mucilage content was significantly higher (65.37) and also exhibits light blotches on young leaves were identified as a marker characters (AICRP Report of 2011-15).

Table 1. Yield performance of IC 112532 accession

Year of testing	IC 112532	
	Leaf weight (g/plant)	Mucilage (%)
2011-12	393.60	62.34
2012-13	291.50	62.40
2013-14	325.70	70.63
2014-15	367.60	66.15
Mean	344.60	65.37

Source: AICRP Report Dr. P.D.K.V., Akola.

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30. Nishkantak Prickleless (IC0629502; INGR19030), a *Solanum viarum* Germplasm for Prickleless, Alkaloids Content Higher than the Prickly Plant Type

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Solanum viarum Dunal (Family Solanaceae) is commonly known as tropical soda apple, while in India its local common name is 'kantha kari'. *S.viarum* is a medicinal plant having high contents of medicinally important steroidal alkaloids such as solasodine, α-solanine, solamargine, solasonine, solanidine, khasianine etc. used for commercial production of solasodine in India. These steroidal alkaloids exhibit a variety of biological activities including anti-cancerous, anti-fungal, anti-microbial, anti-viral, insecticidal, anti-inflammatory, anthelmintic, anti-herpes activities and are also major raw materials for commercial production of pharmaceutically important contraceptive steroids.

S. viarum is an economically important woody shrub of South American origin and widely distributed in the Asian subcontinent. In India,it is reported from Khasi hills, Naga hills, West Bengal, Nilgiris, Orissa, Sikkim, and the Upper Gangetic plains. The aerial parts of *S. viarum* including the calyx are packed with sharp prickles which renders harvesting of fruits diffucult and expensive. Due to nonsynchronous maturity of berries, mechanized harvesting is not possible. Therefore, the harvesting is done by hand plucking which is very difficult due to large and hard prickles. CSIR-National Botanical Research Institute, Lucknow, has obtained a prickleless spontaneous mutant under *S. viarum* breeding program (Khanna and Singh, 1985; Singh *et al*., 1998) and named as NBRI-Sel. The mutant was latterly christened 'Nishkantak'. The prickleless genotype is important not only for devoid of prickles but also for better yield and alkaloid contents. The highest alkaloid content is reported in the berries as compared to any of the other plant parts, eg., leaf, stem or root. The yellowish berries, a particular ripening stage contain the highest alkaloid contents.Germplasms of both the prickly and prickleless mutant of *S. viarum* have been conserved under *in vitro* condition for last ~35 years maintaining their prickly/prickleless nature and plants are stable under field conditions also.

The prickleless mutant, 'Nishkantak' has no prickles on the stem throughout the year but 2-4 prickles appeared on mature leaves during winter months. The finding to develop prickleless strain is novel in overcoming the constraint for adapting high density planting, easy harvesting and there by getting high yield. The berries yield is 1.29 tonnes/ hectare in prickly WT and 1.57 tonnes/ hectare in prickleless mutant.Single-nucleotide polymorphisms (SNPs) analysis measured that the prickly and prickleless genotypes were 99.92% genetically similar (Pandey *et al.,* 2018).

The various morphological characters of field-grown mature plants were recorded with five replicates for three consecutive years (2016-18). The data on different parameters of field-grown plants alongwith yield/plant were presented in Table 1. For alkaloid profiling of solasodine, solanidine and α-solanine contents different plant parts (leaf, stem and roots) of field-grown mature plants, *in vitro*-grown shoot cultures and yellowish coloured berries (II stage of ripening) were used (Table 1).

Table 1. Alkaloid contents(solasodine, solanidine, and α-solanine) in various plant parts of field and *in vitro*-grown prickly and prickleless genotypes of S. viarum

Type of Material	Alkaloids analysed (mg/g DW)	Prickly (WT)			Prickleless (Mutant)		
		*Avg±S.E.			*Avg±S.E.		
		2016	2017	2018	2016	2017	2018
Field-grown L+S+R[2]	Solasodine	1.71±0.14	1.89±0.12	1.88±0.11	2.22±0.42	2.34±0.33	2.32±0.25
	Solanidine	2.96±0.41	2.94±0.35	2.99±0.33	2.48±0.47	2.59±0.25	2.44±0.34
	α-solanine	3.96±0.25	3.99±0.28	3.84±0.31	4.09±0.31	4.12±0.53	4.21±0.22
	Total	**8.64**	**8.82**	**8.71**	**8.81**	**9.05**	**8.97**
Field-grown Berries[3]	Solasodine	1.00±0.13	1.10±0.11	1.08±0.05	1.17±0.37	1.18±0.28	1.15±0.22
	Solanidine	1.02±0.04	1.04±0.08	1.06±0.12	0.76±0.01	0.87±0.05	0.92±0.08
	α-solanine	4.91±1.23	4.99±0.09	4.88±1.22	5.61±0.87	5.66±0.12	5.45±0.99
	Total	**6.93**	**7.13**	**7.02**	**7.44**	**7.71**	**7.52**
[4]*In vitro*-grown L+S+R	Solasodine	1.34±0.07	1.31±0.05	1.25±0.03	1.90±0.24	2.01±0.33	1.98±0.21
	Solanidine	2.30±0.13	2.64±0.22	2.48±0.11	2.41±0.21	2.49±0.22	2.78±0.36
	α-solanine	1.19±0.02	1.04±0.04	1.18±0.09	2.31±0.09	2.22±0.06	2.28±0.02
	Total	**4.83**	**4.99**	**4.91**	**6.62**	**6.72**	**6.74**
Plant height (cm)		119±2.81	125±1.73	132.52±1.29	103±2.19	104.6±2.49	115±1.71
Prickles/young leaf*		13.6±0.68	14.6±1.08	17.6±0.68	nil	nil	nil
Prickles/mature leaf*		23.6±1.08	26.4±0.93	26±1.00	nil	nil	3.2±0.83
Prickles/ petiole of young leaf*		10.2±0.75	8.8±0.97	9.6±1.08	nil	nil	nil
Prickles/ petiole of mature leaf *		7.6±0.37	10.4±1.7	11±0.71	nil	nil	nil
Prickle/ Internode*		21.2± 0.11	20.4±1.44	18. 4±0.82	nil	nil	nil
Berries/ plant		57.20±3.47	52.80±3.15	54.00±3.39	63.20±0.28	64.20±2.97	65.60±1.96
Yield/plant (g)		129.04±7.83	125.56±7.50	123.01±5.98	148.27±5.11	159.34±7.38	162.03±4.85

*Average of 3 replicates ± Standard Error;
[2] L+S+R = Leaf + Stem + Roots in total
[3] Second stage of berries, i.e. yellowish green
[4] Culture incubation: 45 days

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31. Swadi Palas (IC0629501; INGR19031), a Flame of Forest (*Butea monosperma*) Germplasm with Trifoilate Leaflet and Acute Apex (occasionally penta foliate). Larger flower with Dull Orange Colour with Early Biological Maturity

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Palas (*Butea monosperma* Lam.) Taub. commonly known as 'Flame of the forest' (Sanjappa, 1987; Shah 2011). Tree is very useful for local people in terms of medicine, pharmaceutical industry and several non-

timber forest products (NTFP) (Pal and Bose, 2011; Sanjappa, 1987). Besides NTFPs, in association with *rangeeni* strain of Indian lac insect (*Kerria lacca* Kerr.) it also yields a resin called lac, extensively cultivated by tribal communitiesfrom Jharkhand, Chhattisgarh, Madhya Pradesh, West Bengal, etc. (eds. K.K. Sharma and R. Ramani, 2010) and has commercial importance. A new variant of *palas*strain from Putadag village (N 23^0 25.746' and E $85^0$43.391') in Ranchi (Jharkhand) has been identifiedfor raising *kusmi*strain of Indian lac insect (*Kerria lacca* Kerr.). This variant not only differs in terms of lac insect strain preference but also differs morphologically from commonly available Palas. Locally, it is known as *Swadi Palas* due to its preference by *kusmi*strain of lac insect. The comparative morphological account, its keys for identification is presented in Table 1. Thus, identification of *Palas* variant'*Swadi'*will increase the industrially superior *kusmi*lac production.*Kusmi* lac fetches high value in the marketdue to its superior quality over *rangeeni* lacthereby, augmenting the earningsof lac growers.

Table 1. Comparative morphological characters of Palas variant 'swadi' and common Palas

Morphological Characters	Palas variant 'Swadi'	Common Palas
Tree height (meter)	16-20	8-15
Trunk diameter (cm)	40-80	25-50
Bark colour	Whitish	Blackish-brown
Leaflets	Pinnately trifoliate (rarely 5-foliate)	Pinnately trifoliate (rarely unifoliate)
Terminal leaflet	Rhomboid	Suborbicula or obovate
Apex	Acute apex	Obtuse to round or emarginate
Pedicel length (mm)	28 to 31	17 to 22
Calyx diameter (mm)	16 to 18	14 to 16
Calyx length (mm)	16 to 21	13 to 17
Flower length (cm)	5.5 to 6.3	4.5 to 5.0 cm
Stamen length (mm)	68-78	49-62
Style (mm)	73-86	49-61
Anther length (mm)	1.3-1.74	1
Pod length	13-14 cm	10-16
Seed size (cm)	3.0-3.7 x 1.8-2.3	2.5-4 x 2-3
Flower Colour	Orange	Scarlet flowers (orange-red/Indian-orange)
Flowering time	January-February	March- April
Fruiting	March -April	April-May

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32. HTW 63 (IC36761A; INGR19032), a Wheat (*Triticum aestivum*) Germplasm for Drought Tolerance

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Wheat is grown in a wide range of environments and is affected by biotic and abiotic factors. Worldwide, it was estimated that about 65 million hectares of wheat area is under water limited environments, wheat yields are generally reduced to 50% or even less of its yield potential under normal irrigation. In India, nearly 80% wheat is cultivated under irrigated conditions, 66% of it receives only partial (1-2) irrigations, and the remaining 20% is grown under rainfed environments.Both drought and heat stress are responsible for 20-30% reduction in grain yield. Therefore, it is necessary to identify tolerant genotypes followed by introgression of the tolerance into elite agronomic background.

The genotype HTW 63 was found to be tolerant to both heat as well as drought tolerance (Sareen *et al.,* 2014). To reconfirm the drought tolerance of HTW 63,

a trial was conducted under timely sown, late sown, rainfed timely and rainfed late sown conditions at four locations (Akola, Dharwad, Karnal and Powarkheda) for two years (2015-16 and 2016-17) under NICRA project. This genotype was found tolerant to drought as well as heat under field conditions as indicated by values of stress indices DSI (-0.18), HSI (0.98) and DHSI (0.92). In addition to this, this genotype along with checks was evaluated under controlled conditions (rainout shelter for drought stress tolerance and temperature controlled glasshouse for heat tolerance). HTW 63 had shown tolerance to both drought as well as heat stress as depicted by the stress indices DSI (0.68) and HSI (0.90) (Table 1).

The performance of HTW 63 under rainfed timely sown conditions with respect to other desirable agronomic features namely: days to heading (63 days), days to maturity (114 days0, plant height (104cm) and thousand grain weight (46.6g) has also indicated its suitability to be used in crossing programmes (Table 2).

Table 1. Evaluation of HTW 63 along with checks for drought tolerance

Drought susceptibility index (DSI) of HTW 63 and checks under field (pooled over 4 locations and 2 years) and controlled (rainout shelter and glasshouse) conditions

Entry	Field conditions			Controlled conditions	
	TS IR	LS IR	LS RF	Rainout shelter	Temp controlled glasshouse
	DSI	HSI	DHSI	DSI	HSI
HTW 63 (IC 36761A)	-0.18	0.98	0.92	0.68	0.90
Checks (Registered genetic stocks for heat and drought)					
AKAW 2862-1	1.25	0.66	1.10	2.77	2.61
HTW11	1.05	1.61	1.18	1.89	0.96
HTW 6	0.82	0.80	0.89	0.45	0.69
C 306	0.52	0.64	0.94	0.35	0.93
C 306 M10	1.23	1.10	1.05	1.60	3.39
HINDI 62	0.97	1.25	1.16	0.84	2.34

*DSI- Drought susceptibility index HSI- Heat stress sensitivity index and DHSI- Drought & heat sensitivity index; TSIR – Timely sown irrigated; LSIR- late sown irrigated; LSRF- Late sown rainfed

Table 2. Agronomic characteristics of the drought tolerant genotype, HTW 63 pooled over two years under field conditions

Genotypes	Days to heading		Days to maturity		Plant height (cm)		Thousand grain weight (g)	
	IR-TS	RF-TS	IR-TS	RF-TS	IR-TS	RF-TS	IR-TS	RF-TS
HTW63	68	63	119	114	123	104	42.0	46.6
Checks								
AKW2862-1	70	66	120	114	101	79	49.5	48.2
C 306	82	76	126	121	118	105	43.4	41.8
C 306 M10	74	71	123	120	124	104	44.9	45.5
HINDI 62	87	79	129	121	99	91	36.4	42.5
HTW11	82	76	124	119	94	84	41.6	39.4
HTW6	84	77	127	122	103	86	38.8	41.3

The high level of drought tolerance in HTW 63 makes it a potential donor for use in hybridization programmes targeted to improving drought tolerance in future wheat genotypes.

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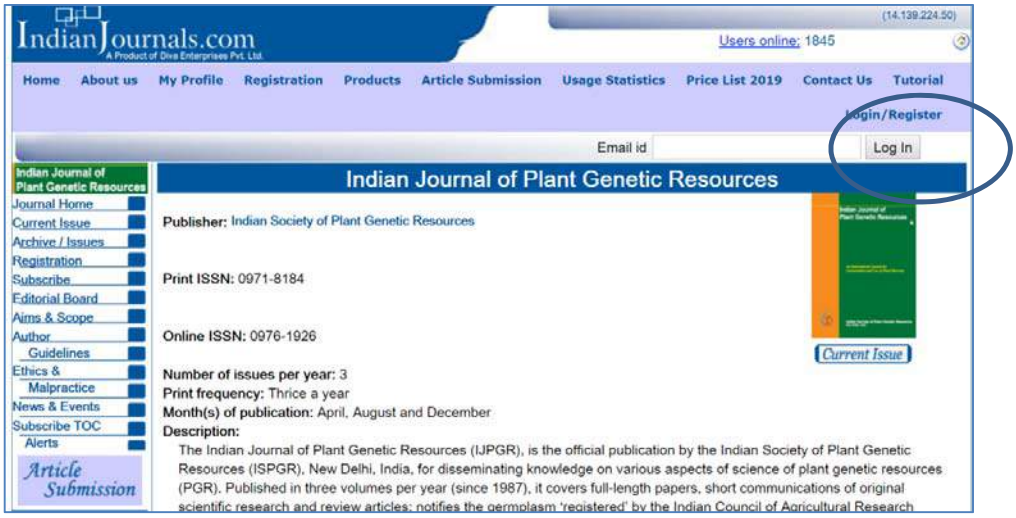

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