Vol. 31 No. 2 2018

Print ISSN: 0971-8184
Online ISSN: 0976-1926

Indian Journal of Plant Genetic Resources

An International Journal for Conservation and Use of Plant Diversity

ISPGR 1987

Indian Society of Plant Genetic Resources
New Delhi, India

INDIAN SOCIETY OF PLANT GENETIC RESOURCES

(Registration No. S/18336)

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ISSN: 0971-8184
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INDIAN SOCIETY OF PLANT GENETIC RESOURCES
NBPGR CAMPUS, NEW DELHI-110012, INDIA
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Indian J. Plant Genet. Resour. 31(2): 125-133 (2018)
DOI 10.5958/0976-1926.2018.00015.3

Medicinal Plants Used by Tribes of Andaman and Nicobar Islands: A Conservation Appraisal

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(Received: 25 July 2016; Revised: 27 April 2017; Accepted: 15 July 2017)

The present study was conducted to document the indigenous healthcare knowledge of tribes (*Nicobarese*, *Shompens*, *Jarawas*, *Onges*) inhabiting the Andaman and Nicobar group of Islands. Among these, *Nicobarese* use very high number of plant species as medicine. They collect them from surrounding natural habitat but many of these are becoming endangered. Out of these, 39 species belonging to 38 genera under different families are found to be endemic and their population is dwindling sturdily. Of these 17 species were used for curing multiple ailments, 8 species for intestinal disorders, 8 species for malarial fever, four species for 25 families asthma and cough related ailments, and one species each for cancerous blisters, arthritis and allergic ailments. The biochemical activities of these species are worth investigating. Measures of conservation have also been discussed in the present communication. Phytogeography of these species is worth studying to implement management practices for conservation.

Key Words: Andaman & Nicobar Islands, Conservation, Endemic, Medicinal plants, Tribes

Introduction

The Andaman and Nicobar Islands by virtue of unique climatic factors and location are considered as one of the hotspots of the world's biodiversity. Situated between the two major biodiversity hotspots namely the Indian sub-continent and the Malaysia-Indonesian region it is hardly surprising that the islands manifest biodiversity of extraordinary range within a limited geographical area. These islands are situated close to equator and exposed to the oceanic impacts having the tropical humid climate with the temperature ranging between 18^{o}C to 35^{o}C. About 84.4% of the total geographic area of Andaman and Nicobar Islands is under the forest cover. Of the total forest cover, 42.1% area is covered with dense forest, 34.1% by moderately dense forest, and 8.2% by open forest while mangrove constitutes 9.6% of total area (Sankaran *et al.,* 2015). The vegetation pattern includes coastal littoral, mangroves, island deciduous and evergreen forests. Floristically, there are 2654 species belonging to 1083 genera and 237 families. Out of these, 308 species are endemic. The island's tropical forests represent nature's major storehouse of chemicals and pharmacodynamic compounds used in perfumery, cosmetics and pharmaceutical industries. The forest resource of the islands has rich repository of biodiversity of medicinal plants representing a great emporium of ethnobotanical wealth which is the treasured knowledge of six different aboriginal tribes *viz*., *Nicobarese*, *Shompens*, *Jarawas*, *Sentinels*, *Onges* and *Great Andamanese* and *Karen* tribes, latter brought by British from Myanmar and Thailand border areas, settled in North Andaman. Tribals depend completely on forest for their daily needs and also use several plants as medicine. Unfortunately, the traditional healing systems and knowledge of these aboriginals have been largely eroded along with its natural resources, because of the lack of enough support and recognition, as well as the rapid destructions of their habitats through a series of unsustainable developmental activities. The human population is also increasing at an alarming rate and has already crossed island's carrying capacity (Abirami *et al*., 2012). The IUCN has proposed a Medicinal Plant Specialist Group (MPSG) aiming to preserve genetic resources of medicinal plants world over, to promote sustainable utilization of medicinal plants and to raise awareness in the public about the need of conservation of medicinal plants. The folklore medicinal uses of the tribes except for the Sentinels have been documented

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by various workers (Chander *et al.,* 2015a; Chander *et al.,* 2015b; Gupta *et al.,* 2004; Dagar and Dagar, 1996; Dagar and Dagar, 1992; Dagar and Dagar, 1991; Dagar, 1986). There exists wide biodiversity in the distribution of medicinal plants in the island and there are about 71 medicinal plants which are endemic to this island (Dagar and Dagar, 1991). The complete ethnomedicinal uses of the plants by the different tribes of the island are worth to be documented. The bioresources of the medicinal plants are to be conserved and used in a sustainable way as these resources are harvested by the island aboriginals from wild for treatment of their ailments. Study of biogeography of at least some of these species could be useful for further management practices. Hence, a serious attempt needs to be made for the conservation and sustainable use of medicinal plants keeping in view the present situation.

Materials and Methods

The present study was aimed to document the indigenous healthcare knowledge of various tribes of the Andaman and Nicobar Islands. The documented ethnobotanical information of this area was compared with the information on biodiversity and conservation of medicinal plants recorded by various workers (Watt, 1890; Kloss, 1902; Chengappa, 1953; Chak, 1967; Sangal, 1971; Singh, 1975; Bhargava, 1983; Dagar, 1986, 1987, 1991; Dutta *et al*., 1985; Chakraborty & Vasudevarao, 1988; Sreekumar, 1993; Sinha and Rao, 1996; Abirami *et al.*, 2012). In 2013-14, the first author had an opportunity to consolidate most of the research work carried out on medicinal plants in these islands. The third author had an opportunity in recording first-hand information from tribals for his research. The present status of ethnobotanical studies conducted on island flora is presented here as determined by application of International Union for Conservation of Nature (IUCN) conservation categories and criteria (IUCN, 2011).

Results and Discussion

The endemic taxa of these islands comprise 308 species belonging to 187 genera and 74 families. The degree of endemism is estimated to be 10% of the total flora. However, a total of 308 endemic taxa occurring within a small geographical area of 8,249 sq. km. is a significant feature of this peninsular flora (Pandey and Diwakar, 2008). Out of the 64 species which are listed by IUCN under the category of threatened plants, 43 species has been reported as endemic taxa of medicinal plants by Pandey and Diwakar (2008). Thirty nine species belonging to 38 genera under 25 families were found to be rare, threatened, endangered and endemic medicinal plants used by four tribes of Andaman and Nicobar islands and *Karens*, and presented alphabetically in Table 1. Out of these, 10 species are found only in Andaman group of islands, five in Nicobar group of Islands and 24 in both groups of islands. This consists of 16 trees, 11 climbers, 7 herbs and 5 shrubs. Pandey and Diwakar (2008) have also reported 43 endemic species of medicinal plants in the islands.

Ophiorrhizia nicobarica is one of the highly threatened herbs found to have analgesic and anti-inflammatory properties because of presence of biochemical active alkaloids harmalin (Chattopadhyay *et al*., 2007). Detailed work on bioactive compounds has been done by experimenting with lab animals, mice and rats (Chattopadhyay *et al*., 2007). Such work should be taken up in some more species like *Strobilanthes andamanensis* (herb), *Semecarpus kurzii* (tree), *Aristolochia tagala* (climber) and *Dioscorea vexans* (climber).

Seventeen species have been used by these tribes for curing multiple ailments (Table 1). The biochemical active compounds of these species will be of much interest to researchers. *Semecarpus kurzii*, *Alstonia kurzii*, *Aristolochia tagala*, *Myristica andamanica*, *Hedyotis paradoxa*, *Ixora grandifolia* var. *kurziana*, *Glycosmis pentaphylla*, *Lepisanthes andamanica*, *Manilkara littoralis*, *Cyrtrandroemia nicobarica*, *Amomum fenzlii* and *Kaempferia siphonantha* have been used against fever, malaria etc., because most of the time tribal people suffer from fever in these islands. They survive mainly because of application of traditional knowledge associated with these species. Therefore, further research is needed to identify some new molecules to be used in new drug formulations.

Leaves in 31 species, roots in 13 species, seeds in 7 species, bark in 6 species, fruits in 4 species, flower and all parts in 2 species are used for treatment of many diseases (Table 1). This shows that in many species large quantities of leaves are harvested for drug preparation. Roots of as many as 13 species are used for drug preparation; this destructive harvest could reduce the population, thus causes genetic erosion. Barks of six species are removed for drug preparation may also cause death of the plant.

Table 1. Endemic, Rare and Endangered Medicinal plants used by the tribals of Andaman and Nicobar Islands

S. No.	Family	Name	Habitat		Habit	Local name	Used by	Parts used	Ailment	Remarks
			A	N						
1	Acanthaceae	*Strobilanthes andamanensis* Bor	A (E)		Herb		Nicobarese	Leaves	Blood in urine, asthma, cough, bronchial complaints/ abortifacient	
2	Anacardiaceae	*Semecarpus kurzii* Engl.	A	N (E)	Tree	Pep (N) Jugane (O)	Nicobarese, Shompens	Leaves, seeds, fruits.	Injury, malaria fever, allergy, eruption, blisters, cancerous	Pericarp contains acrid juice. Trees with large leaves and orange red fruits seated on fleshy receptacle
3	Annonaceae	*Artabotrys nicobarianus* D. Das	A	N (E)	Climber		Nicobarese	Leaves, bark, seeds.	Stomach pain, fever	
4	Annonaceae	*Orophea katschallica* Kurz	A	N (E)	Tree	Tapilei-alo (N) Tonyoge (O)	Nicobarese	Leaves	Body ache	Used a bee repellent by the tribes
5	Annonaceae	*Uvaria andamanica* King	A (E)		Tree		Nicobarese	Leaves, flowers	Intestinal disorder	Flowers yellow, purple or brown.
6	Apocynaceae	*Alstonia kurzii* H.f.	A	N (E)	Tree	Tacho-roi (N)	Nicobarese Shompens	Leaves, bark, root	Fever, wash uterus after child birth, cure blood clot, internal haemorrhage, malarial fever	Many species of *Alstonia* have medicinal properties and used against treatment of malaria.
7	Arecaceae	*Calamus andamanicus* Kurz A.		N (E)	Climber	Mottabet (N)	Jarawas Nicobarese	Leaves	Used in making shafts.	Stem is used for making baskets
8	Arecaceae	*Daemonorops manii* Becc. & H.f.	A (E)		Tree	Tamoyen (O)	Onges	Leaves, root	To protect female genitals	Vegetative shoot apex is used for medicinal purpose
9	Aristolochiaceae	*Aristolochia tagala* Cham.	A	N	Climber shrub	Kom (N) Punkot (N)	Nicobarese, shompens	Root	Stomach pain, chest pain, fever, poultice in abdomen, skin disease, snake bite, malaria, dyspepsia, flatulent, colic and tonic	Root decoction is used for consumption
10	Dichapetalaceae	*Dichapetalum gelonioides* (Roxb.) Engl. subsp. *andamanicum (*King) Leenh.	A (E)				Shompens	Leaves	Asthma, cough, fever, Shompens use the juice of stem as drinking water	
11	Dioscoreaceae	*Dioscorea vexans* Prain & Burkill.	A	N (E)	Climber	Getti	Nicobarese	Tubers	Arthritis, asthma, eczema, chronic cough, diarrhoea, diabetes, regular metabolic activity	Genus *Dioscorea* is known for the presence of diosgenin which is useful in population control
12	Euphorbiaceae	*Drypetes andamanica* (Kurz) Pax & K. Hoffm.	A (E)			Toirulelu	Onge	Leaves	Chest pain	Used as an antidote for snake bite
13	Euphorbiaceae	*Glochidion calocarpum* Kurz	A	N (E)	Small tree	Angchongsi (N)	Nicobarese, Shompens	All parts	Skin disease, fever, intestinal disorder (diarrhoea and dysentery), febrifuge, wounds, domestic animal diseases	Male flowers with short pedicels, female flowers in clusters, flowers yellow
14	Euphorbiaceae	*Phyllanthus andamanicus* Balakr. & Nair	A (E)		Tree	Dadaura	Nicobarese	Leaves	Leaves are used for diuretic problem	Plant contains alkaloids, flavonoids and triterpenes.

A-Andaman Islands; N-Nicobar Islands; E-Endemic; R-Rare

S. No.	Family	Name	Habitat		Habit	Local name	Used by	Parts used	Ailment	Remarks
			A	N						
15	Hippocrateaceae	*Hippocratea andamanica* King	A (E)		Climber			Root	Ringworm, post-natal, rheumatism	Large climber with small greenish flowers in axillary cymes
16	Icacinaceae	*Codiocarpus andamanicus* (Kurz) R.A.Howard	A	N (E)	Tree	Kamarang	Nicobarese	Leaves	Renovating agent, applied on enlarged scrotum of children	Large tree with whitish longitudinally grooved fruits
17	Loganiaceae	*Strychnos andamanensis* Hill		N (E)	Climber	Lansot	Nicobarese	Leaves	Urinary troubles, constipation, stomach ache related problems	The leaves are pounded and wrapped in a piece of cloth which is dipped in boiling water, the decoction thus prepared is drunk
18	Leeaceae	*Leea grandifolia* Kurz		N (E)	Shrub	Takteyu (N)	Nicobarese	Leaves	Abdominal pain, intestinal disorders	Leaf juice taken internally
19	Asparagaceae	*Asparagus racemosus* Willd.	A		Herb	Kanyaplur/ Kanyammur (K)	Karens	Root	Jaundice	Paste of fresh root with sugar candy diluted with water taken for seven days
20	Myristicaceae	*Knema andamanica* (Warb.) de Wilde subsp. *andamanica*	A (E)		Tree		Nicobarese	Leaves seeds and root	Dysentry, mouth sores, indigestion, diarrhoea	Trees with pubescent leaves, fruits small, sub-globose and tomentose
21	Myristicaeae	*Myristica andamanica* Hook.f.	A	N (E)	Tree	Kinhanmo	Nicobarese	Fruits and leaves, seed, bark	Fever, malaria, stomach trouble	Aqueous extract of the nuts is taken by Nicobarese in stomach trouble. The powder of roasted fruit is mixed in ripe seed oil and applied on the body.
22	Myristicaceae	*Maesa andamanica* Kurz		N (E)	Shrub		Nicobarese, Shompens	Leaves, root	Skin disease	Straggling shrubs with white flowers
23	Passifloraceae	*Adenia penangiana* (Wall. ex.G.Don) de Wilde	A	N	climber	Tincham (N)	Nicobarese	Leaves, flowers, seeds	Body pain, chest pain	Twinner with yellow flowers
24	Rubiaceae	*Ophiorrhizia nicobarica* Balakr.		N (E)	Herb		Shompens	Leaves	Wounds	Herbs with large white flowers
25	Rubiaceae	*Hedyotis paradoxa* Kurz	A	N (E)	Herb		Nicobarese	Leaves	Fever, cuts, wounds	Leaves sessile, flowers dense in cyme
26	Rubiaceae	*Ixora grandifolia* Zall. & Mor. var. *kurziana* (Teysm. & Binn.) H.f.		N (E)	Shrub	Sinkoh	Nicobarese	Leaves	Fever, delivery, stomach ache	Glabrous shrubs with fragrant white flowers in cyme
27	Rubiaceae	*Lasianthus andamanicus* Hook.f.	A	N (E)			Onge	Leaves	Antidote	
28	Rubiaceae	*Psychotria platyneura* Kurz	A	N (E)	Shrub	-	Shompens		General health problem	Rare and endemic
29	Rubiaceae	*Psychotria andamanica* Kurz	A	N (E)	Shrub	-	Nicobarese, Shompens	Leaves bark, root	Body pain, chest pain, general health complaint	Large shrubs with shortly pedicelled flowers in cymes
30	Rubiaceae	*Tarenna weberaefolia* (Kurz) Balakr.	A	N (E)	Tree	-	Nicobarese	Leaves	Skin disease	Small tree with simple leaves and white flowers

A-Andaman Islands; N-Nicobar Islands; E-Endemic; R-Rare

S. No.	Family	Name	Habitat		Habit	Local name	Used by	Parts used	Ailment	Remarks
			A	N						
31	Rutaceae	*Glycosmis pentaphylla* (Retz.) DC.	A	N (E)	Tree		Onge, Nicobarese	Leaves and roots	Fever, snake bite	Unarmed small tree with 3-5 foliate leaves. Roots contain glycosmin
32	Sapindaceae	*Lepidopetalum jackianum* (Hiern.) Radlk.	A	N (E)	Tree	Lamang	Nicobarese	Leaves, bark, root	Conjunctivitis, bath the children to keep them healthy, joint pain	Hut posts, pig trays and fuel are obtained from its wood by Nicobarese. Twigs are used to handle earthen pots on fire during the process of making them pucca which is special art of the Chowra Islanders .
33	Sapindaceae	*Lepisanthes andamanica* King	A	N (E)	Tree	Tong-kal	Nicobarese	Whole plants	Fever, cough	Branches purplish brown to light silvery with many-flowered-panicles
34	Sapotaceae	*Manilkara littoralis* (Kurz) Dubbard	A	N (E)	Tree	Tengevaka (O)	Onge, Nicobarese	Leaves, fruits, bark	Delivery problem, fever	Bark yields a red dye. Fruits are edible.
35	Scrophulariaceae	*Cytandromoea nicobarica* Balakr.	A	N (E)	Herb			Leaves	Jungle fever	Flowers white in clusters, fruits enclosed by calys, seeds numerous
36	Sterculiaceae	*Sterculia parviflora* Roxb.	A	N (R)	tree	Tosamu, Kantisembal,	Nicobarese	Leaves, seeds, fruits	Injury	Small tree with velvetty fruits and black seeds
37	Vitaceae	*Tetrastigma andamanicum* (King) Suess. ex Suess.	A	N (E)	Climber			Leaves	Poultice of leaves applied to boils	Large climbing shrub
38	Zingiberaceae	*Etlingera fenzlii* (Kurz) Škornick. & M.Sabu	A	N (E)	Herb	Jungli adhrak Hami (N)	Shompens Nicobarese	Rhizome	Stomach disorders, fever ; juice of rhizome used in cough, fever, respiratory disorders and skin diseases	Perennial rhizomatous herb with many flowers in spike; common in Great Nicobar Islands
39	Zingiberaceae	*Kaempferia siphonantha* King ex Baker	A (E)		Herb	Cekur	Onge	Rhizomes	Fever, stomach pain	Widely distributed in Little Andaman and South Andaman

A-Andaman Islands; N-Nicobar Islands; E-Endemic

All these plants are medicinally important and used in cure of various ailments. Some of the important medicinal plants *viz*. *Dioscorea vexans* is used in the treatment of blood in urine, asthma and as abortifacient; *Strobilanthes andamanensis* in malarial fever; *Semecarpus kurzii* in cancerous growth, arthritis; and *Aristolochia tagala* for diabetes. Urgent conservation measures need to be taken up to increase their population in native habitat and also helpful in testing biochemically for use in new drug formulations, as these plants are being used for many generations by the aborigines in the treatment of various ailments.

The phytogeographic studies of these 39 species pinpoints to the population dynamics of this genetic wealth in tropical Andaman and Nicobar forests.

Conservation Status

The role of plants, in traditional medicinal floras has been overlooked in most areas in terms of conservation. The ethnomedicinal plant diversity can be scored as locally threatened using the IUCN Categories and Criteria (IUCN 2011). In recent years ethnomedicinal plant diversity of these islands in general is threatened due to natural disaster as well as rapid increase in population and various anthropogenic activities. Hence, there is immediate need to help conserve the existing species.

India has one of the oldest, richest and most diverse cultural traditions associated with the use of medicinal plants. This is also true with Andaman and Nicobar tribes. *Alstonia kurzii* (syn. *Alstonia scholaris*) entered IUCN red data list of threatened species therefore its conservation should be taken up as early as possible by utilizing recent advances in propagation like tissue culture and cryopreservation techniques for increasing the plant population and enriching the habitat. This particular species yields a bitter tonic and expectorant aphrodisiac.

Importance and Need for Conservation

It has been estimated that 75.90% of the rural people in the world, rely on herbal traditional medicine for their primary health care. Consequently, there is a growing interest in medicinal plants and traditional health systems. However, with the increasing use of medicinal plants and also with the accelerating destruction of natural resources, it is evident that the exploration of medicinal plants must be accompanied by conservation measures to preserve their genetic resources and to assist the rural people who have the most direct relation to the utilization of medicinal plants.

Medicinal plants are a major but neglected group of plants for which conservation is a priority. The last few decades have witnessed an unprecedented deforestation resulting in habitat loss and species depletion in tropical countries including India. Depletion of these genetic resources has resulted in significant losses, particularly in the economic productivity and this has already started reflecting in health care services and other plant based industries of biodiversity-rich developing countries.

There is an urgent need to have conservation strategies in Andaman and Nicobar islands. The traditional knowledge on the six ethnic tribes and of *Karens* of these islands should be tapped before it is vanished. This ethnomedicinal wisdom is now fast disappearing due to habitat alteration, urbanization, industrialization etc. Conservation on the rare medicinal plants of these islands should be the top-priority action for the authorities of this territory. Development of conservation strategies for threatened or endangered plants and specific projects needs to be submitted for funding by the appropriate organizations which will help in identifying the plants at risk. For ex-situ conservation field gene banks or herbal gardens needs to be established for saving endemic medicinal plants of Andaman and Nicobar islands. Besides, Medicinal Plants Conservation Areas (MPCA) and ethnobotanical conservation parks in various biodiversity rich sites should also be established.

The 'Green Health Campaign' should be initiated to promote the use of medicinal plants by rural people through nurseries. Villagers should be encouraged to procure seedlings from nearby farms and grow them in their kitchen garden, backyards as fields etc. Such gardens should be planned in Car Nicobar Island and Rutland Island for Nicobar group and Andaman group respectively.

Recommendations

To achieve this goal, the following suggestions and proposals should be implemented in future, to have a complete data on medicinal plants, their bioactive principles, validation of tribal medicine of the island along with their conservation measures.

Phytogeographic studies with special reference to endemic, endangered, rare medicinal plant species

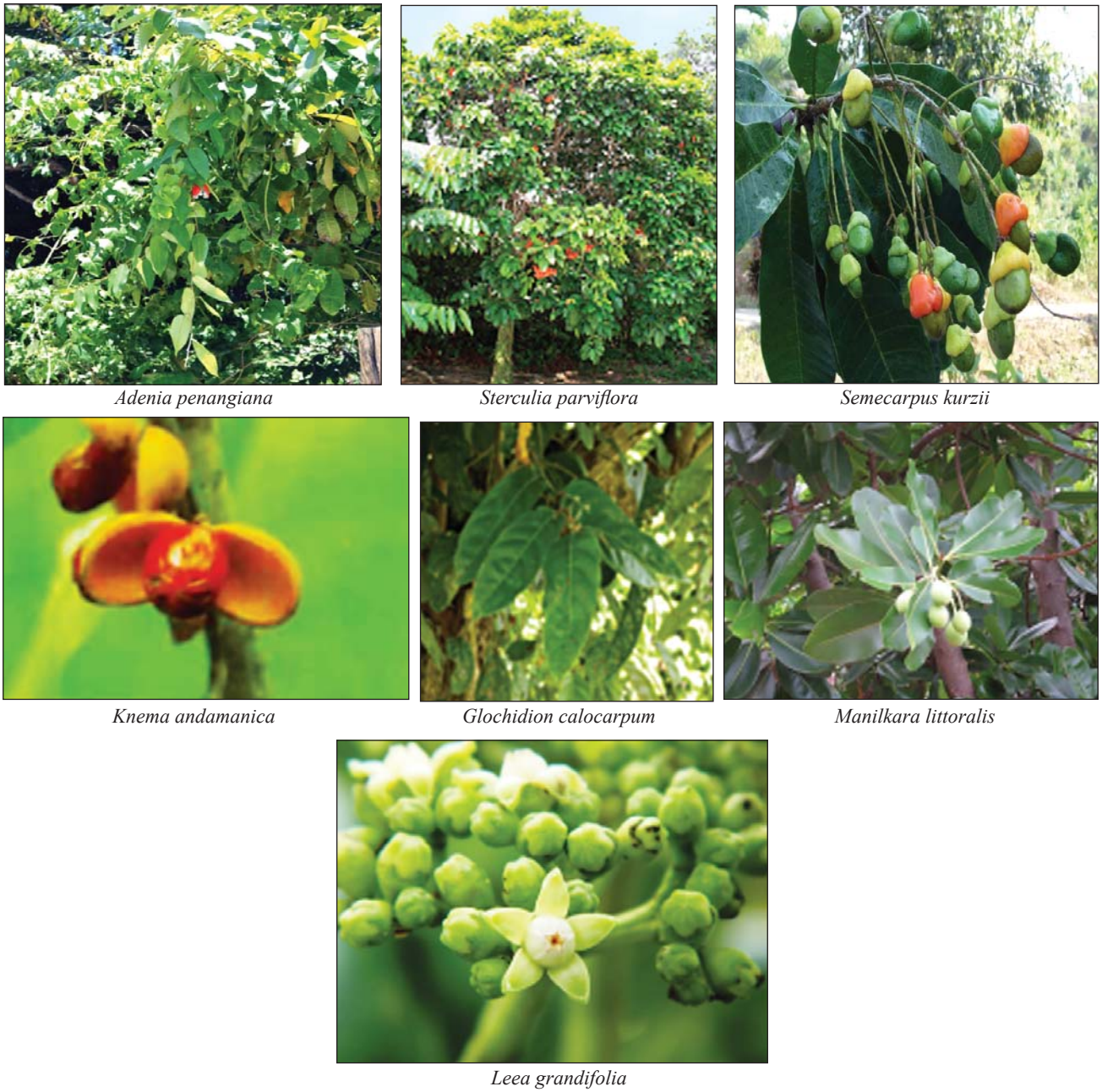

Fig. 1. Some endemic medicinal plants used by tribes of Andaman and Nicobar islands

should be taken up in Andaman and Nicobar islands for conservation and management planning.

Formulate a multidisciplinary, multi-institutional and action-oriented research programme to develop strategies for conservation of traditional life, knowledge system and resources utilization pattern by the tribals. More focus should be given on the under-explored areas/tribes for ecologically sound and economically sustainable utilization of local resources.

A network on the database of medicinal plants used by tribes of Andaman and Nicobar Islands should be developed for updating/reviewing the existing literature.

Survey and documentation of ethnomedicinally important plants should be carried out especially among the primitive and less-known tribes like *Shompens*, *Onges*, *Jarawas* and *Sentinelese* (if possible) etc. and their safety and efficiency needs to be tested for validation of claims.

MPCA and ethnomedicinal plants gardens could be set up, at least and one each in the settlement areas of *Onges*, *Nicobarese*, *Shompens*, *Great Andamanese* and *Karens*. This would help them in the conservation and sustainable utilization of the natural resources in their own surroundings. To start with, it is suggested that the medicinal plant gardens should be established at Dugong Creek for *Onges*, at Strait Island for *Great Andamanese* at Great Nicobar, near Campbell Bay National Park for *Shompens*, one each at Car Nicobar and Teressa in the Nicobar group of islands for *Nicobarese*, one in North Andaman at *Karen* community settlement.

Germplasm collections of medicinal plants in these islands could take up in network mode in collaboration with ICAR-National Bureau of Plant Genetic Resources, New Delhi, Directorate of Medicinal and Aromatic Plants Research, Anand, Botanical Survey of India (BSI), Central Island Agricultural Research Institute (CIARI), and Regional Medical Research Centre (RMRC) Departments. CIARI can liaison with the A&N Agriculture Department for the multiplication and distribution of medicinal plants to the tribal farmers/ users.

Creation of two divisions (Ethnopharmacology and Phytochemistry), either in CIARI or in RMRC would be helpful to study pharmacological and toxicological actions of plant derived-drugs and their formulations. Phytochemistry will be carried out to study the active constituents and validation of tribal claims, formulate new drugs and to do phytochemical screening to find out therapeutic efficacies, safety, etc.

For micro-propagation of medicinal plants, a cryo-preservation unit, field genebank etc. should be set up preferably in CIARI.

'Field Gene Banks' needs to be established mainly for 'betel vines' (in Great Nicobar) 'wild gingers' (in Rutland), wild species and aromatic plants (in Dhanikari) and wild mangoes (in Chidiatapu and Mt. Harriet).

Eco-rehabilitation and genepool development of selected endangered/threatened medicinal plants can be carried out. Medicinal plant conservatory can be set up with all modern amenities such as glasshouses/ greenhouses, net house etc. Bonsai (trees in trays) on rare/endangered medicinal tree species could be displayed in the conservatory. This miniature/dwarf plants would represent huge tree species of medicinal value, which are scattered in the interior forest of these far flung islands. It would aid to study and understand, to a certain extent, the morphological and other features of trees pooled out in a minimum space, helping scientists/ researchers /nature-lovers who get seldom opportunities to study them in nature due to lack of sufficient time and inaccessibility of their original habitats.

Acknowledgements

Authors are grateful to Director, ICAR-CIARI, Director, RMRC and Director, Botanical Survey of India (BSI) for their valuable suggestions and support. Authors are also thankful to Dr. PV Sree Kumar, Ex Scientist, BSI for his contribution.

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Indian J. Plant Genet. Resour. 31(2): 134-141 (2018)
DOI 10.5958/0976-1926.2018.00016.5

Multivariate Analysis to Evaluate Common and Tartary Buckwheat Germplasm in Sikkim

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(Received: 20 March 2016; Revised: 24 December 2016 Accepted: 30 November 2017)

Fifteen accessions of common buckwheat (*Fagopyrum esculentum*) and 21 of tartary buckwheat (*F. tataricum*) were studied for 15 quantitative and 21 qualitative traits to understand the nature and magnitude of genetic variability using multivariate approach. Significant variation for quantitative traits was recorded for seven traits in common buckwheat and for eleven traits in tartary buckwheat. In common buckwheat seed yield correlated positively with days to maturity while negatively associated with seed weight whereas in case of tartary type number of cymes/plant, seeds/cyme, days to flowering and days to maturity showed positive correlation with seed yield. First five and four principal components (PCs) respectively, in common and tartary type revealed majority of the variability in the accessions which further grouped the test accessions of both the species in to four clusters each. The accessions also showed considerable variation for qualitative traits.

Key Words: Buckwheat, *Fagopyrum esculentum, Fagopyrum tataricum*, Multivariate analysis

Introduction

The impact of climate change in the Himalayan region has driven agriculture to adapt to such farming practices or crops, which act as a buffer against stress or unfavorable weather conditions. Buckwheat is one of the important crops of the high altitudes and is grown under traditionally low input management conditions (Joshi and Rana, 1997; Tomotake *et al*., 2000; Tsuji and Ohnishi, 2001; Rana *et al*., 2011) and is now gaining worldwide importance due to its stress tolerant traits and excellent nutritional profile particularly due to rich amino acids, phenols (rutin) and antioxidant activities (Liu *et al*., 2008; Christa and Soral-Smientana, 2008; Tang *et al*., 2009; Gupta *et al*., 2011, Gupta *et al*., 2012; Rana *et al*., 2012). Buckwheat is consumed both as grain and green, and diverse populations exist in higher hilly tracts both for common (*Fagopyrum esculentum*) and tartary (*F. tataricum*) types. The cultivation of buckwheat had been decreasing at an alarming rate due to the influx of cash crops in the Himalayan region (Rana *et al*., 2000), however, in the recent years increasing awareness and the demand for health food has brought its cultivation back in many traditional growing areas of Himalayan region including Sikkim (Rana *et al*., 2012). It is now considered a cash crop and export commodity paying rich dividends to small holder farmers of hill regions. Buckwheat is not only grown on slopy dry lands but also as a major crop rotation crop between paddy and maize in Sikkim. The present area and production of buckwheat in Sikkim is 3,630 hectares and 3,490 tonnes with a productivity of 961.57 kg/ha (Anonymous, 2014).

The increasing demand for buckwheat seed has also compelled plant breeders to initiate hybridization work to enhance yield levels significantly. It is mentioned so far that improvement of buckwheat is confined to selection from farmer's populations only (Rana *et al*., 2000). It is known that yield and its associated traits are affected by the environment thereby suitability and adaptability of germplasm depends on local agro-ecology (Joshi and Rana, 1995; Rana *et al*., 2010). In order to meet the germplasm requirement of breeders, it is essential to evaluate the germplasm in the local environment of Sikkim and identify the diverse and promising germplasm lines, which could be used in crop improvement programmes at local level. The present study was therefore envisaged to assess the nature and magnitude of variation in buckwheat germplasm and identifying the useful genetic diversity using multivariate approach. Multivariate statistical algorithms has been suggested an important tool for classifying the germplasm and analyzing of genetic relationships among breeding material (Mohammadi and Prasanna, 2003).

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Materials and Methods

Thirty six buckwheat germplasm accessions comprising 15 common and 21 tartary buckwheat were procured from ICAR-National Bureau of Plant Genetic Resources, Regional Station, Shimla, Himachal Pradesh and also collected from different parts of Sikkim. The germplasm was evaluated at research farm of ICAR Research Complex for NEH Region, Sikkim centre, Tadong, Gangtok, Sikkim located at 27°19'0''N and 88°36'0''E at an elevation of 1320 masl. The material was grown in randomized block design with two replications during cropping season of 2012 and 2013. The data was recorded randomly on five plants based on descriptors of IPGRI (IPGRI, 1994) on 15 quantitative and 21 qualitative traits. Data on quantitative traits were analyzed for variance, correlation coefficient, Principal Component Analysis as per Pinheiro de Carvalho *et al*. (2004b). The most informative PCs were employed for clustering using Ward's hierarchical algorithm (Ward, 1963). The statistical analysis was done using SAS 9.3 software (SAS, 2012).

Results and Discussion

Morphological Characterization

The analysis of variance revealed significant differences for plant height, number of internodes, number of leaves, 1000 seed weight, days to flowering, days to maturity and seed yield in common buckwheat while tartary type differed significantly for plant height, number of branches, leaf blade length and width, 1000 seed weight, shoot diameter, petiole length, days to 50% flowering, days to maturity and seed yield. Significant differences in the buckwheat germplasm for both common and tartary buckwheat have been reported in several studies worldwide (Joshi and Paroda, 1991; Choi *et al*., 1995; Baniya *et al*., 1995 and Yan *et al*., 1992; Joshi and Rana, 1995; Senthilkumaran *et al*., 2007). The extent of variability was high in tartary type accessions, which implies wider scope of employing selection based on more number of desirable traits. Summary statistics of both the species has been shown in Table 1. Heritability estimates in case of common buckwheat were high for plant height, number of internodes, number of leaves, days to flowering, days to maturity, seed weight, seed diameter and petiole length while in tataricum type plant height, number of branches, number of leaves, leaf blade width, days to flowering, days to maturity, seed weight, seed diameter, petiole length showed high heritability. Number of cymes per plant and seeds per cyme showed low heritability in both the species, as these traits are highly influenced by the environment. Characters showing high heritability in both the species respond to selection as these are least influenced by the environment, thereby improvement in these traits can be made by employing selection on individual plants selected in both the species for respective traits.

Flowering started on 22nd day after sowing (50% flowering) in common buckwheat entries EC323730, EC125937 and EC58322 while entry EC125935 and EC58332 were earliest to attain maturity (75 days). Entry EC21874 grew tallest (90.40cm) whereas EC286396 recorded highest 1000 seed weight (33.08g). Entry PRB1 was the highest yielder (2388 kg/ha). In tartary type flowering started on 29th day after sowing in IC49669, IC108518 and Himpriya. Himpriya was earliest to attain maturity in 79 days. Maximum height attained by entry Shimla B1 (105.70 cm) whereas IC26600 recorded maximum 1000 seed weight (45.14 g). Entry IC109728 was the highest yielder (2247 kg/ha). PRB1 and VL7 were used as checks for common buckwheat whereas Himpriya and Shimla B1 for tataricum type. In case of tataricum type, accessions IC109728, IC26600, IC109729, Titey, IC15393, IC109433 and IC49671 were superior to both the checks for seed yield, however in case of common buckwheat none of the entries surpassed the checks for seed yield. Entry PRB1 and IC109728 can be recommended as high yielding varieties for buckwheat cultivation areas of Sikkim after multilocational trials.

The frequency of twenty one qualitative characters in thirty six buckwheat accessions has been shown in Table 2 which show a variable range of characters. Majority of the entries were indeterminate type with semi erect type branch shoot habit. Red stem colour predominates in twenty eight accessions. Grey, brown, black and mottled seed colour exist in different frequencies (16:8:1:11). Leaf blade shape for all the accessions were of saggitate type. Except two entries, inflorescence were of non-branched type. Triangular seed shape recorded for 15 accessions while ovate type for rest of the entries. Except four buckwheat accessions, pink margin colour of the leaf was present in all the material. Lodging resistance was recorded for majority of the accessions as a desirable character in buckwheat particularly in common type for reaping high seed yield as this species has low seed set due to genetic and climatic factors.

Table 1. Summary statistics of quantitative traits in common and tataricum buckwheat

Character	Species type	Mean	Std Dev	Std Error	Variance	CV	Range	Heritability	Genetic advance
Plant height (cm)	*F. esculentum*	80.13	6.89	0.25	47.49	8.61	67.00-93.40	0.714	8.991
	F. tataricum	86.90	12.15	1.87	147.66	13.98	65.20-105.70	0.742	16.537
No. of internodes	*F. esculentum*	7.25	0.99	0.18	0.99	13.73	5.20-9.60	0.820	1.571
	F. tataricum	13.08	1.09	0.16	1.19	8.36	9.60-15.00	0.486	0.893
No. of branches	*F. esculentum*	3.26	0.61	0.11	0.38	18.93	2.20-4.40	0.488	0.503
	F. tataricum	4.22	0.95	0.14	0.92	22.72	2.40-6.40	0.873	1.636
No. of leaves	*F. esculentum*	22.28	4.02	0.73	16.17	18.04	13.60-32.00	0.643	4.378
	F. tataricum	41.21	9.36	1.44	87.67	22.76	23.00-63.40	0.782	13.821
Leaf blade length (cm)	*F. esculentum*	6.37	0.45	0.08	0.20	7.16	5.54-7.24	0.019	0.013
	F. tataricum	5.33	0.49	0.07	0.24	9.19	4.54-6.52	0.590	0.502
Leaf blade width (cm)	*F. esculentum*	5.28	0.36	0.06	0.13	6.84	4.68-6.08	-0.612	-0.283
	F. tataricum	5.11	0.71	0.10	0.50	13.90	4.10-7.16	0.741	0.959
Length of cyme (cm)	*F. esculentum*	5.17	0.58	0.10	0.34	11.37	3.52-6.08	0.405	0.396
	F. tataricum	4.95	0.95	0.14	0.90	19.25	2.94-7.16	0.514	0.816
No. of cymes/plant	*F. esculentum*	25.15	5.62	1.02	31.59	22.34	19.00-46.60	-0.214	-1.607
	F. tataricum	32.02	6.58	1.01	43.34	20.55	19.80-49.20	0.298	2.985
Seeds/cyme	*F. esculentum*	12.35	2.47	0.45	6.12	20.03	6.40-18.40	0.321	1.283
	F. tataricum	15.37	3.02	0.46	9.16	19.68	10.60-25.80	0.028	0.118
Days to 50% flowering	*F. esculentum*	23.40	0.89	0.16	0.80	3.82	22.00-25.00	0.878	1.536
	F. tataricum	38.26	5.39	0.83	29.12	14.10	29.00-52.00	0.991	11.088
Days to maturity	*F. esculentum*	78.46	2.09	0.38	4.39	2.67	75.00-83.00	0.961	4.089
	F. tataricum	88.95	6.06	0.93	36.76	6.81	79.00-108.00	0.982	12.263
Seed yield (kg/ha)	*F. esculentum*	1521.11	391.16	71.41	153010.64	25.71	1005.56-2388.00	0.680	81.454
	F. tataricum	1543.52	504.28	77.81	254299.78	32.67	383.33-2247.00	0.532	83.011
1000 seed weight (gm)	*F. esculentum*	28.06	2.07	0.37	4.31	7.40	24.50-33.08	0.995	4.323
	F. tataricum	26.87	5.61	0.86	31.49	20.88	19.78-45.14	0.999	11.681
Stem diameter (mm)	*F. esculentum*	4.90	0.38	0.07	0.14	7.86	4.04-5.57	0.954	0.752
	F. tataricum	4.43	0.52	0.08	0.27	11.73	3.50-5.50	0.993	1.075
Petiole length (cm)	*F. esculentum*	4.23	1.24	0.22	1.55	29.47	2.12-7.60	0.998	2.606
	F. tataricum	3.68	1.05	0.16	1.11	28.73	2.10-6.50	0.997	2.194

Correlation Coefficient

The Pearson correlation coefficient traces out associations among the fifteen quantitative traits in study for both common and tataricum type as shown in Table 3a and 3b. Seed yield is the most economic character and is significantly positively correlated with days to maturity while negatively correlated with 1000 seed weight. Abundance of pollinators is an important parameter in securing yield of common buckwheat where the per cent seed set in flowers is genetically controlled by the raceme. Reproductive morphogenesis of common buckwheat depends on the resource availability and consequently the flower number and duration of the flowering period (Cawoy *et al*., 2009). Lines having flower open for longer duration might have better chances of pollinators visiting the flower as compared to short duration type. In tataricum type, seed yield showed significant positive correlation with number of cymes per plant, seeds per cyme, days to flowering and days to maturity. Days to flowering was significantly positively correlated with maturity duration, 1000 seed weight. Tataricum buckwheat is a self-compatible species with higher seed set. Selection can be employed on characters showing higher degree of correlation with seed yield for identification of superior tataricum buckwheat lines. Agronomic management including optimum date of sowing of buckwheat is beneficial for enhancing yield. The negative association of seed yield with test weight denotes that yield is compromised with

Table 2. Frequency of qualitative characters for common and tartary buckwheat accessions

Descriptors	Features	Frequency ratio
Cotyledon/seedling leaf colour	3green, 5 pink, 7 red	36:0:0
Growth and branch shoot habit	3semi-erect, 5 semi-erect longer, 7erect longer, 9 erect longer	36:0:0:0
Degree of determination	1 indeterminate, 5 intermediate, 9 determinate	33:0:3
Plant branching	1 very weak, 3 weak, 5 intermediate, 7 strong, 9 very strong	0:23:12:1:0
Stem colour	3 green, 5 pink, 7 red	0:8:28
Lodging suscept.	1 very resistant, 5 intermediate, 9 very susceptible	31:5:0
Leaf colour	3 green, 5 pink, 7 red	36:0:0
Leaf margin colour	3 green, 5 pink, 7 red	4:32:0
Leaf vein colour	3 green, 5 pink, 7 red	27:9:0
Petiole colour	3 green, 5 pink, 7 red	17:10:9
Leaf blade shape	1 ovate, 2 hastate, 3 sagittate, 4 cordate,	0:0:36:0
Compactness of inflorescence	3 cyme loose, 5 cyme semi compact, 7 cyme compact	20:13:3
Branched inflorescence	0 Yes, 1 No	2:34
Colour of inflorescence stalk	3 green, 5 pink, 7 red	2:34:0
Flower colour	1 white, 3 greenish yellow, 7 pink, 9 red	7:21:8:0
Flower abortion	3 low, 5 intermediate, 7 high	22:11:3
Seed colour	3 grey, 5 brown, 7 black, 9 mottled	16:8:1:11
Seed shape	1 triangular, 2 ovate, 3 conoidal	15:21:0
Seed surface	1 smooth, 2 irregular/wrinkled, 3 other	15:21:0
Seed quality	3 poor, 5 intermediate, 7 good	0:7:29
Threshability	3 difficult, 5 intermediate, 7 easy	9:9:18

Table 3a. Correlation matrix of quantitative traits in common buckwheat

Character	PH	NI	NOB	NOL	LBL	LBW	LOC	NOCP	SW	SD	PL	SC	DF	DM	YP
PH		**0.72**	0.28	0.29	**0.51**	**0.59**	0.39	-0.04	-0.13	0.10	-0.09	-0.00	0.16	0.26	0.04
NI			0.38	**0.47**	0.23	0.32	0.11	0.10	**-0.36**	0.15	0.09	-0.03	0.35	0.33	0.27
NOB				**0.57**	0.09	0.08	-0.13	0.29	0.07	-0.06	0.13	-0.20	0.09	0.23	0.11
NOL					0.00	0.07	-0.04	**0.45**	-0.06	-0.03	0.26	**-0.36**	**0.43**	0.38	0.16
LBL						**0.73**	**0.53**	-0.18	-0.00	0.02	0.07	0.13	0.19	0.22	0.12
LBW							**0.71**	-0.19	-0.11	0.11	-0.14	0.11	0.09	0.33	0.11
LOC								-0.17	0.07	**0.46**	0.00	**0.36**	-0.07	0.33	0.18
NOCP									0.19	0.09	-0.04	**-0.36**	0.35	**0.13**	-0.02
SW										0.02	-0.08	0.07	**-0.36**	-0.20	**-0.54**
SD											0.34	0.11	-0.16	0.19	0.25
PL												-0.20	0.24	0.05	0.27
SC													-0.08	0.07	-0.02
DF														0.43	0.28
DM															0.32

Bold figures means significant at 5% level of significance

*PH: plant height; NI: no. of internodes; NOB: no. of branches; NOL: no. of leaves; LBL: leaf blade length; LBW: leaf blade width; LOC: length of cyme; NOCP: no. of cymes per plant; SW: seed weight; SD: need diameter; PL: petiole length; SC: seeds per cyme; DF: days to 50% flowering; DM: days to maturity; YP: seed yield per plant

employing selection for higher seed weight in both the species. Selection of lines with small to medium sized achenes alongwith flowering for longer duration may enhance seed yield.

Joshi (2005) reported negative correlation of flowering days with days to maturity in tartaricum type. Rana and Sharma (2000) reported direct positive effects of days to maturity, leaf length and width, 100 seed weight, seeds per cyme and negative direct effects of number of branches, plant height, flowers per cyme, number of leaves, days to flowering and number of internodes on seed yield. Joshi and Okuna (2010) reported positive and significant association of grain yield with number of primary branches, number

Table 3b. Correlation matrix of quantitative traits in tataricum buckwheat

Character	PH	NI	NOB	NOL	LBL	LBW	LOC	NOCP	SW	SD	PL	SC	DF	DM	YP
PH		**0.32**	-0.15	-0.22	0.19	0.16	0.25	0.12	**-0.52**	0.07	0.08	**0.39**	0.06	0.07	0.21
NI			**0.41**	-0.23	-0.08	-0.20	-0.22	**0.42**	-0.29	-0.01	**-0.40**	0.10	0.20	0.00	0.11
NOB				0.02	-0.18	-0.26	-0.41	**0.53**	**0.36**	0.15	-0.10	0.03	**0.61**	**0.41**	0.08
NOL					0.22	0.29	0.29	-0.13	0.13	0.02	**-0.32**	0.19	-0.05	-0.02	0.17
LBL						**0.91**	**0.48**	-0.06	-0.05	-0.00	0.06	0.16	-0.21	-0.16	0.00
LBW							**0.60**	-0.17	-0.15	-0.11	0.09	0.13	-0.28	-0.26	-0.03
LOC								-0.23	-0.19	0.00	-0.08	0.20	-0.22	-0.28	0.12
NOCP									0.08	0.09	-0.10	0.10	**0.47**	**0.37**	**0.38**
SW										**0.44**	0.25	-0.25	**0.45**	**0.36**	0.11
SD											-0.04	0.13	**0.42**	**0.33**	0.23
PL												-0.08	0.09	0.14	-0.04
SC													0.17	0.29	**0.33**
DF														**0.81**	**0.51**
DM															0.32

Bold figures means significant at 5% level of significance

*PH: plant height; NI: no. of internodes; NOB: no. of branches; NOL: no. of leaves; LBL: leaf blade length; LBW: leaf blade width; LOC: length of cyme; NOCP: no. of cymes per plant; SW: seed weight; SD: seed diameter; PL: petiole length; SC: seeds per cyme; DF: days to 50% flowering; DM: days to maturity; YP: seed yield per plant

Table 4(a). Mean values of clusters in common buckwheat accessions

Character	Cluster I	Cluster II	Cluster III	Cluster IV
Plant height (cm)	84.26	79.92	83.10	72.10
No. of internodes	7.98	7.00	8.00	6.30
Number of branches	3.32	3.27	4.20	2.83
No. of leaves	23.18	22.28	28.10	18.87
Leaf blade length (cm)	6.70	6.19	6.21	6.24
Leaf blade width (cm)	5.51	5.27	5.15	4.98
Length of cyme (cm)	5.52	5.29	3.83	4.81
No. of cymes/plant	23.66	27.28	29.30	22.00
Seeds/cyme	13.16	12.30	8.80	12.30
Days to 50% flowering	23.80	23.25	24.00	22.83
Days to maturity	80.20	78.50	78.00	75.67
1000 seed weight (g)	26.67	29.35	26.36	28.41
Shoot diameter (mm)	5.00	5.02	4.06	4.79
Petiole length (cm)	4.32	4.11	3.10	4.70
Seed yield (kg/ha)	320.30	240.67	259.00	267.50

Table 4(b). Mean values of clusters in tataricum buckwheat accessions

Character	Cluster I	Cluster II	Cluster III	Cluster IV
Plant height (cm)	99.20	72.20	86.72	85.13
No. of internodes	13.33	11.10	12.18	13.57
Number of branches	3.63	5.50	3.24	4.68
No. of leaves	42.70	53.40	46.22	37.75
Leaf blade length (cm)	5.66	5.05	5.53	5.20
Leaf blade width (cm)	5.61	4.61	5.57	4.85
Length of cyme (cm)	5.61	4.51	5.60	4.56
No. of cymes/plant	30.53	32.60	25.10	35.23
Seeds/cyme	18.17	16.20	14.90	14.80
Days to 50% flowering	40.67	52.00	30.70	39.67
Days to maturity	91.83	107.00	82.60	89.38
1000 seed weight (g)	22.14	45.14	24.58	27.49
Shoot diameter (mm)	4.78	5.28	4.16	4.40
Petiole length (cm)	3.23	5.45	3.91	3.55
Seed yield (kg/ha)	334.00	365.50	207.60	285.75

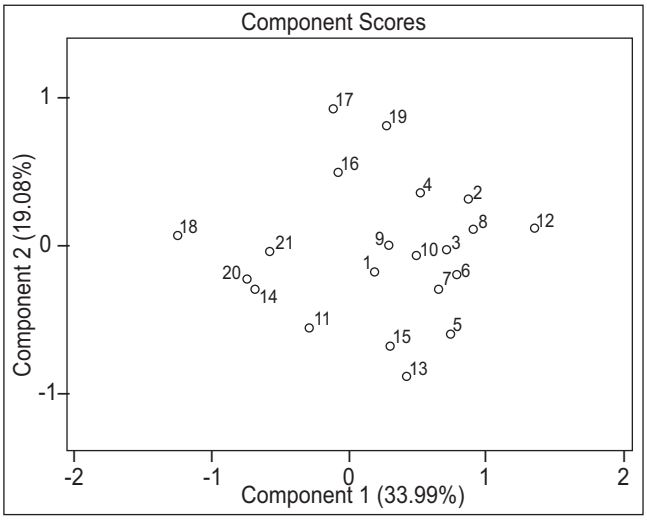

Fig. 1(a). Scatter plot of the principal component scores of esculentum buckwheat entries

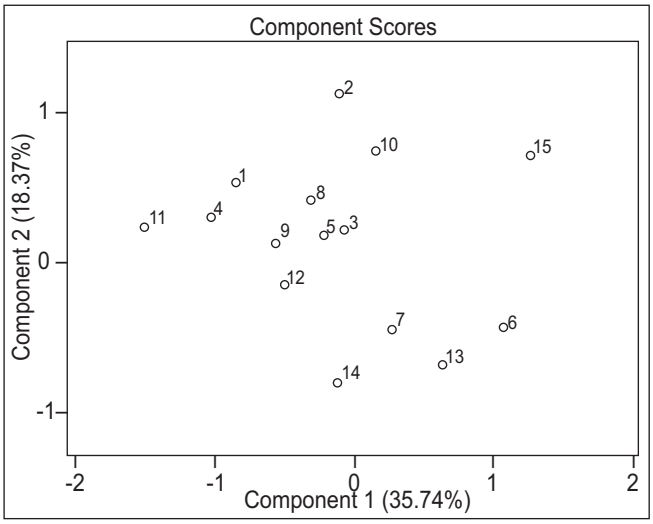

Fig. 1(b). Scatter plot of the principal component scores of tataricum buckwheat entries

of leaves, plant height, number of clusters and cyme, days to flowering, number of filled and unfilled grains, grain width and weight in 192 tartary accessions. Li *et al*., 2012 reported association of late flowering with tall plant height and higher yields in tartary buckwheat in F_3 population.

Principal Component Analysis (PCA)

The PCA simplified the complex data by transforming the number of correlated variables in to small number of most informative variables. The PCA figured out variables that account for maximum variability in both the buckwheat species. Eigen value of the correlation matrix in common buckwheat showed first five principal components (PCs) had eigen value greater than 1 and cumulatively account for 75% of the variability. The eigenvectors having high value of PC1 are number of internodes (0.39), plant height (0.36), days to maturity (0.32) and number of leaves (0.34), in PC2 are length of cyme (0.49), seeds per cyme (0.38), in PC3 1000 seed weight (0.53) and number of cymes per plant, in PC4 are shoot diameter (0.60) and petiole length (0.47) while in PC5 are days to flowering (0.55) seeds per cyme (0.54). In tataricum type eigen value of the correlation matrix showed first 4 principal components had eigen value greater than 1 and cumulatively account for 72% of the variability. The eigenvectors having high value of PC1 are number of cymes per plant (0.39) and days to flowering (0.38), in PC2 are seeds per cyme (0.55) and plant height (0.41), in PC3 are 1000 seed weight (0.51) and number of leaves (0.31) and in PC4 are petiole length (0.63) and plant height (0.37). Scatter plot of the principal components for both the species has been shown in Figure 1a and 1b. Senthilkumaran *et al*. (2008) reported first three components as most informative accounting for 59.97% and 74.36% variance in common and tataricum type buckwheat respectively. This variability provides useful information to the workers regarding most variable characters in buckwheat on which selection for buckwheat improvement can be employed.

Cluster Analysis

The first five PCs in common type and four PCs in tataricum type were used for clustering. In the former, the first five PCs divided 15 accessions in to 4 clusters. Mean value of clusters in common and tataricum buckwheat has been shown in table 4a and 4b. Cluster I consisted of five entries namely PRB1, VL7, Meethey, EC218784 and EC218742, cluster II consist of six entries EC286936, EC272442, IC188701, EC125937, EC216631, EC18864, cluster III was solitary with entry IC202226, cluster IV consists of EC323730, EC58332, EC125935. In tataricum type, Cluster I comprised of Titey, IC109433 and IC109549, cluster II comprised of IC108518, Himpriya, IC49669, Shimla B1, IC24301, cluster III was solitary with entry IC26600, cluster IV with IC49671, IC36805, IC108510, IC243184, IC202268, IC109728, IC107998, IC15393, IC107994, IC109729, IC109619 and IC108514. Wards minimum variance dendrogram of both the species has been shown in figure 2a and 2b. The grouping of clusters clearly segregate

the entries according to their metric traits. Further crop improvement work in buckwheat accessions should be carried out selecting entries inter-clusters for obtaining desirable segregates. Analysis of genetic diversity in germplasm collections aids in classification of genotypes and identification of core collection with possible utility for breeding goals (Mohammadi and Prasanna, 2003). Debnath *et al*. (2008) reported division of 21 local buckwheat germplasm in to 5 clusters using 15 quantitaive characters. Senthilkumaran *et al*. (2008) classified 30 common buckwheat in to 4 clusters and 16 tataricum in to three distinct clusters based on 14 quantitative characters. Cepkova *et al*., 2009 clustered 77 common buckwheat in to 5 major clusters and 15 tartary in to 6 clusters collections of Czech genebank collection.

Buckwheat is an underutilized crop where the varietal development work is mainly hindered by the incompatible nature of common buckwheat and availability of very few literature on its genetic improvement. However, better crop production through agronomic practices have been reported for yield improvement. In short run, selection strategies may prove to be beneficial in both the species but realizing its potential as a climate resilient crop, improvement in yield particularly in common buckwheat is needed which is only possible through breeding strategies that overcome reproductive barriers for enhancing seed yield. Breeding methodology to overcome self-incompatibility has been reported by Mukasa (2011). Alternate breeding methodology for common buckwheat (*F. esculentum* Moench) involving the use of self-incompatibility (S^h) gene derived from *F. homotropicum* Ohnishi have been reported by Mukasa *et al*. (2010). The agricultural system has been witnessing effects of climate change thereby it is prerogative to explore potential crops like buckwheat. Conserving the genetic resources alongwith catalouging and documentation after evaluation at multi locations will figure out the best adaptable lines in different locations. The *in-situ* and *ex-situ* conservation strategies needs to be strengthened and specific adaptations from diversity rich areas and their conservation on-farm is important especially in marginal and low-input areas of Himalayan region.

Acknowledgements

The authors are thankful to the Director, NBPGR and officer in charge, NBPGR, Regional Station, Shimla for providing buckwheat accessions for the study.

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Indian J. Plant Genet. Resour. 31(2): 142-151 (2018)
DOI 10.5958/0976-1926.2018.00017.7

Genetic Diversity and Identification of Potential Cold Tolerant Ecotypes from the Brazilian *Hevea* Germplasm of International Rubber Research and Development Board Collection

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Hevea brasiliensis is a commercially cultivated species for its natural rubber (NR) latex in Southeast Asian countries and its genetic base is very narrow. India has received a share of the wild *Hevea* germplasm from the 1981 IRRDB collection of Brazil. To meet the ever-increasing demand, NR cultivation has been extended to non-traditional regions in India. Eighteen wild *Hevea* accessions along with two modern clones RRII 203 and PB 235 and two check clones RRIM 600 and Haiken 1 were evaluated in RBD at Nagrakata, West Bengal, the sub-Himalayan cold-prone region of India. In *Hevea*, low temperature causes reduction in plant growth, thereby increasing its uneconomical immaturity period. The genotypes exhibited highly significant clonal differences (P=0.05) for all the growth traits. The highest mean noted for the plant height 19.62 m (RO 2727); bark thickness 5 mm (MT 900); girth of plant in the 14th year after planting 92.32 cm (MT 915); annual girth increment (cm/year) over seven years 5.61 cm (MT 915); winter girth increment (cm/year) over seven years 1.05 cm (MT 915). The 22 genotypes were grouped into 5 clusters, reflected the presence of considerable genetic diversity in the population. The top 30% of the potential accessions showing high growth vigour under cold stress were identified which could be used in reducing immaturity period in this crop. These ecotypes/selections have high potential value for the development of cold-tolerant clones to mitigate climate change for these regions and also in broadening the genetic base of the present-day cultivated rubber.

Key Words: Cold tolerance, Genetic diversity, Growth vigour, Natural rubber, Wild *Hevea brasiliensis*

Introduction

The *Hevea* rubber tree belongs to the family Euphorbiaceae and indigenous to the tropical rain forests of Central and South America. Among the ten species, *H. brasiliensis* is being cultivated in a plantation scale in the Southeast Asian countries, for its high quality natural rubber (NR) latex. NR obtained from the latex of the tree, which is harvested by tapping, is an industrial raw material and has enormous industrial applications. So commercially it is in high demand including a major automobile sector and globally short in supplies. Francois Fresneau was the first man to have set out on a carefully planned search for the tree and to have given a description of *H. brasiliensis* and of the methods of tapping and preparation of crude rubber (Schurer, 1951).

Several scientific works have contributed to a better understanding of the complex genetic composition of *Hevea*. Recently efforts have been made to understand the links between production capacity, the genetic makeup and the ecological factors. In this context, a two-pronged approaches have been made a) critical cytogeographic surveys by Baldwin (1947) and b) taxonomical studies by Seibert (1947). According to Seibert and Baldwin, everywhere, where two *Hevea* species grow in the vicinity of one another in their natural habitat, introgressive hybridization has taken place in the past and is still taking place at present. From these hybrids, geographic races have established themselves by ecotypical selection. These geographic races have a morphological stature and habit superficially identical to one of the parents, but geographically impregnated with germplasm of one or more of other *Hevea* species. Owing to this selection, they have adapted themselves to habitats different from those preferred by the parents. The geographical distribution of the genus *Hevea*, location of 1876 Wickham collection and the 1981 IRRDB collection in the Amazon rainforests of Brazil are depicted in Figure 1.

In 1876, Henry Wickham collected few seedlings from a minuscule of the genetic range of *Hevea brasiliensis* in Boim, near the Tapajos River in Brazil

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Fig. 1. Distribution, range of the genus *Hevea* and *H. brasiliensis* in Amazon forest of Brazil

(Wycherley, 1968). The entire developments in rubber of Southeast Asian countries came from these few seedlings and thus the resultant clones developed from these seedlings are popularly known as "Wickham clones". Therefore, currently the genetic base of *Hevea* in the domesticated countries is narrow. In the initial stages of crop improvement, main emphasis was on the improvement of yield, i.e. unidirectional selection for yield alone. In *Hevea* cultivated regions, the genetic advance gained in the early breeding phases seems to have slowed down in the more recent phases of breeding (Tan, 1987) for which the narrow genetic base is considered to be the chief factor.

To protect the fast depleting genetic resources from their native region in Brazil (Wycherley, 1968) and also to broaden the narrow genetic base of cultivated rubber, a large collection of wild *Hevea* germplasm was made at its center of origin in the Amazon rainforests of Brazil, by the International Rubber Research and Development Board (IRRDB) - EMBERPA during 1981 (Ong *et al*., 1983). The IRRDB expedition collection resulted in 64,736 seeds and budwood of 194 high yielding, disease free ortets. The expedition area comprised three states in Brazil *viz*. Acre (AC), Rondonia (RO) and Mato Grosso (MT), therefore ecological differences between these provenances offered chances of selection of materials suitable for diverse situations. A share of these germplasm was distributed to member countries including India. Around 4,548 accessions are being conserved in source bush nurseries in India, and are under different stages of evaluation for identification of desirable genes.

Rubber is traditionally grown profitably in the states of Kerala and Kanyakumari district of Tamil Nadu, and Dakshin Kannada and Kodagu districts of Karnataka in south India. To meet the increasing global demand for natural rubber, and also due to limited scope of further expansion in traditional area, rubber cultivation has been expanded to non-traditional regions such as Konkan region of Goa and Maharashtra and parts of Andhra Pradesh, Madhya Pradesh, Odisha, West Bengal and Notheastern (NE) states in India. Now, the rubber cultivation is extended near to sub-Himalayan range of NE India comprises Tripura, Assam, Meghalaya, Arunachal Pradesh and northern part of West Bengal. These areas are often exposed to a wide range of various biotic and abiotic stresses, such as various diseases, wind, higher altitude, and most importantly drought and temperature extremes. Various phenotypic symptoms in response to cold stress include poor germination of seeds, stunted growth, in severe cases results in leaves turning yellow and growing tips dry up and further cold stress can lead to major crop losses. Among various stress factors, low temperatures in the winter, have strongly affected growth, development and latex production of rubber trees in South central China (Priyadarshan *et al*., 2005) and in Northern part of Vietnam (Tuy *et al*., 2012). Sethuraj *et al*. (1991) and Rao *et al*. (1993) reported that cold stress is a serious threat for growth and development of *Hevea* plants and to the sustainability of crop yields in the NE India.

Therefore, the present study aimed at identifying the location specific clones which could adapt to low temperature as-well-as good performance in the cold climate. However, the wild relatives of crops are important sources of resistance to biotic and abiotic constraints. Since no information is available on wild germplasm in the sub-Himalayan region of West Bengal, the present study was undertaken in a cold-prone region to evaluate the growth performance of wild germplasm accessions in the mature phase, to quantify the degree of diversity for various growth traits in the population and to identify genetically diverse clusters and potential cold tolerant genotypes for breeding programmes.

Materials and Methods

A set of 18 wild *Hevea* accessions, two modern clones PB 235 and RRII 203 along with two check clones RRIM 600 and Haiken 1, were planted in a field trial in randomized block design during 2000, with three replications at the Regional Experiment Station of the Rubber Research Institute of India, Nagrakata (Latitude 26º43'N, longitude 88º 26'E and altitude 69 m MSL and annual rain fall 3661 mm) in Jalpaiguri district of West Bengal, sub-Himalayan region of India. The spacing adopted was 4.9 × 4.9 m with five plants per plot and the recommended cultural practices of Rubber Board were followed. Among the 18 wild germplasm accessions, five were from Acre (AC 3074, AC 3075, AC 3293, AC 3514, AC 3810), four from Mato Grosso (MT 900, MT 915, MT 1020, MT 2229) and nine from Rondonia provenance (RO 2638, RO 2727, RO 2886, RO 2901, RO 2908, RO 2948, RO 3043, RO 3169, RO 3197). On the basis of weather variables, the entire year in this sub-Himalayan region can be broadly classified into two groups/ halves *viz*., Weather Regime I and Weather Regime II (Rao and Kole, 2016). Weather Regime I comprising of non-winter period *i.e*. from April to September, mainly consisting of summer, monsoon and post monsoon seasons in this area. Mean temperature ranges from 23.3 to 32º C and 94 per cent of annual rainfall is received in this period. Weather Regime II comprising of winter period *i.e*. from October to March, mainly consisting of cool and cold winter season only. Mean temperature ranges from 13.7 to 27.7º C and it dips as low as 5º C during December to January.

Observations on the height of plant (m) were recorded by VL402 Vertex Laser-height measurer from the bud union to the tip of the tree. Bark thickness (mm) of the stem and girth (cm) of the plant was recorded at 125 cm height from the bud union. Growth during the winter cold stress period was taken as an indication of tolerance to cold. Winter growth was recorded as the girth increment (cm) of a plant during winter by computing the difference between pre- and post-winter girth, for seven consecutive years, from the seventh year after planting. Pre-winter girth was recorded in the month of September (last week) continuously from 2007 to 2013, while post-winter girth was recorded in the first week of April every year from 2007 to 2014. The post-winter girth recording commenced with the annual girth recording every year. The average annual girth increment (cm) per year over seven years was calculated using the annual girth data of seventh and fourteenth years.

The data were subjected to analysis of variance (ANOVA) for randomized block design (Panse and Sukhatme, 1989). In order to study genetic diversity, all the genotypes were clustered by hierarchical cluster analysis (SPSS, 1999; Romesburg, 2004) using six characters. The overall performance of all these accessions were assessed by rank sum method (Kang, 1988), using the traits plant height, bark thickness, annual girth in the seventh and fourteenth year after planting, mean annual girth increment (cm/year) over seven years and mean winter girth increment (cm/year) over seven years. Based on the mean value of a character, each accession was ranked, giving the highest rank to the best performer. Then the ranks across all the traits for each accession were pooled to give a rank sum. Hence the highest rank sum indicated the best performer (rank "1").

Results

The experimental site of the Nagrakata area gets an annual rainfall of 3,661 mm and about 94% of the annual rainfall is received between April and September. The maximum temperature rises as high as 32.6º C during August and the minimum temperature is as low as 8.8º C during January. From October to March, the temperature range between 20.3–15.4º C and the rainfall is less than 201 mm. In the present study, the growth behavior of different wild accessions under these environmental conditions was investigated. The genotypes exhibited highly significant clonal differences ($P = 0.05$) for all the six quantitative traits studied. The range and population mean values in comparison with the check clones for the six growth characters in the mature growth phase are depicted in Table 1.

Table 1. Mean and range of variability for various growth characters in wild *Hevea* germplasm

Characters	Wild accessions		General mean	Check clones		CD (0.05)
	Minimum	Maximum		RRIM 600	Haiken1	
Plant height (m)	14.70	19.62	17.20	16.75	16.67	1.81
Bark thickness (mm)	2.33	5.00	3.87	4.00	4.50	0.94
Annual girth (cm)- 7th year	22.38	53.12	43.44	46.83	50.02	5.81
Annual girth (cm)- 14th year	34.93	92.32	68.51	66.11	82.56	9.12
Annual girth increment (cm/year) over 7 years	1.22	5.61	3.58	2.75	4.65	0.94
Winter girth increment (cm/year) over 7 years	0.34	1.05	0.71	0.65	0.85	0.21

Plant Growth Traits

The height of the plants showed in Table 1, ranged from 14.70 m (RO 2948) to 19.62 m (RO 2727)), while the check clones-Chinese Haiken 1 and Malaysian RRIM 600 recorded height of 16.67 m and 16.75 m, respectively. Bark thickness of the tree ranged from 2.33 mm (AC 3293) to 5 mm (MT 900) with a general mean of 3.87 mm. The check clones had a bark thickness of 4-4.5 mm.

In the seventh year after planting, girth of plant ranged from 22.38 (AC 3293) to 53.12 cm (RO 2727) and the general mean was 43.32 cm. The girth of plant in the fourteenth year after planting ranged from 34.93 cm (AC 3074) to 92.32 cm (MT 915) while the best check clone Haiken 1 recorded a girth of 82.56 cm (Table 1). The wild accession MT 915 recorded the highest girth increment (5.61 cm/year) over seven years, while the check clones RRIM 600 and Haiken 1 recorded an increment of 2.75 cm and 4.65 cm, respectively.

Winter girth increment (cm/year) over seven years ranged from 0.34 cm a^{-1} (AC 3074) to 1.05 cm a^{-1} (MT 915). Certain tolerant wild accessions, namely MT 915, topped in winter girth increment rate (1.05 cm a^{-1}) closely followed by RO 2727 (0.95 cm a^{-1}) and RO 3197 (0.85 cm a^{-1}). The check clones RRIM 600 and Haiken 1 recorded a winter girth increment of 0.65 cm a^{-1} and 0.85 cm a^{-1}, respectively.

Genetic Diversity

The 22 *Hevea* genotypes were grouped into 5 clusters, in which one cluster was solitary in nature (contains only one genotype) while the other 4 were multiple-genotype clusters (Figure 2). The composition of clusters has been presented in Table 2. The similarity coefficient ranged between 0.634 and 22.295. Cluster III and IV had maximum number of 10 and 6 genotypes each followed by clusters I with 3 genotypes. Clusters II consists of 2 genotypes while clusters V was a solitary one. The accessions were ranked using the six main growth parameters *i.e*. plant height (m), bark thickness (mm), annual girth (cm) in the seventh year and fourteenth year, annual girth increment (cm a^{-1}) over seven years and winter girth increment (cm a^{-1}) over seven years for overall performance. The rank sum values ranged from 12 to 125 with a general mean of 68 (Table 3) and top 30% vigorous accessions could be identified in this study.

Discussion

In the present study, the growth behavior of different wild accessions under the environmental conditions of Nagrakata, a sub-Himalayan region of India was investigated. The genotypes exhibited highly significant clonal differences ($P = 0.05$) for all the six quantitative traits studied. The range and population mean values in comparison with the check clone for the six growth characters in the mature growth phase are depicted in Table 1 and discussed under three major grouping characters/heads. An insight into the magnitude of variability present in crop species is of utmost importance, as it provides the basis for effective selection.

Plant Growth Traits

Biological growth, which is regulated by genetics and environment, changes continuously with age, leading to an increase in volume, size, or shape of organism and *Hevea* plants are no exception to this. Apart from rubber yield, the other important aim is stand stability. The useful stand density, i.e. the number of trees tapped, tend to decrease with time in plantations. This is mostly due to damage caused by wind, uprooting and, especially trunk snapping. Wind damage could be linked to the shape of the trees, rather than to the physical properties of the wood. Plant height, also provides interesting architectural features. Wind susceptible architectures

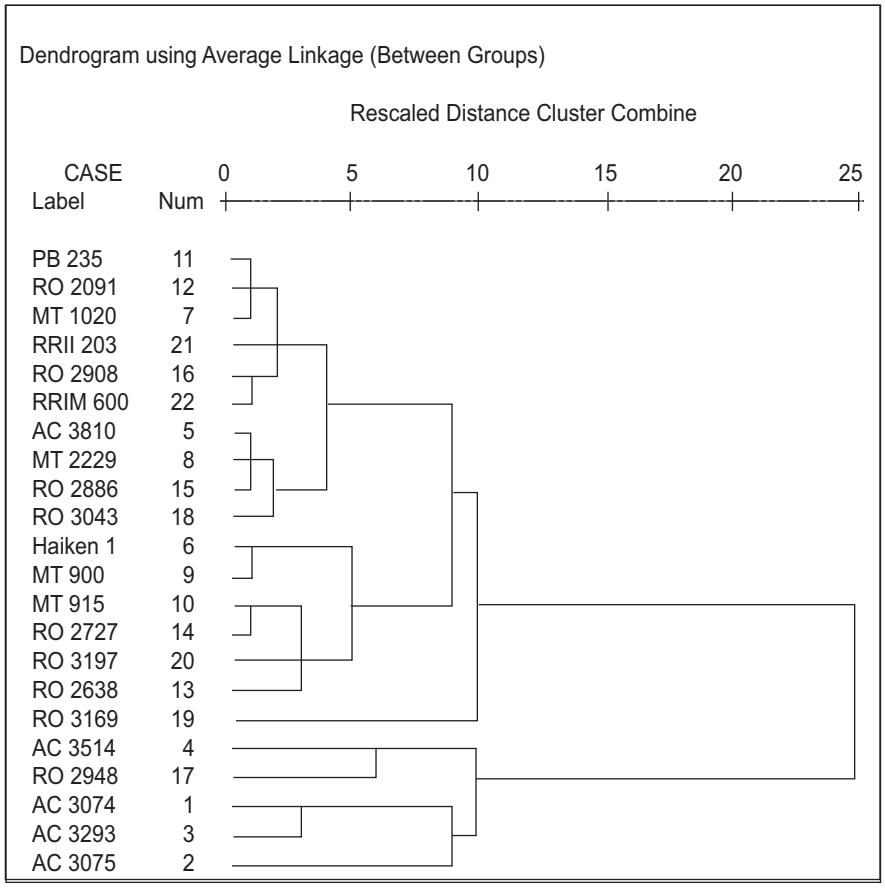

Fig. 2. Genetic distances among the wild *Hevea* germplasm and modern clones

Table 2. Distribution of 22 *Hevea* genotypes in five different clusters

Cluster No.	No. of genotypes	Name of genotype
I	3	AC 3074, AC 3075, AC 3293
II	2	AC 3514, RO 2948
III	10	AC 3810, MT 1020, MT 2229, RO2901, RO 2886, RO 2908, RO 3043, RRII 203, PB 235, RRIM 600
IV	6	Haiken 1, MT 900, MT 915, RO2638, RO 2727, RO 3197
V	1	RO 3169

are characterized by a greater trunk-crown imbalance in rubber. The wild accession RO 2727 (19.62 m) recorded the tallest plants followed by MT 915 and MT 900 (Table 1). While the popular check clones such as Chinese Haiken 1 and Malaysian RRIM 600 were extremely tall in height, and excellent in bark thickness with vigourous growth habit.

One of the main factors in the successful establishment of *Hevea* culture in the Far East was the discovery of the proper method of tapping, latex was extracted from the tree by means of cuts made through the bark. Bark consumption and thick bark is important because it minimizes wounding incidence, which is known to effect yield productivity on latter panels (Goncalves *et al*., 2006). Yield was found to be positively correlated with bark thickness and Girth. The trees of the accession MT 900 have highest bark thickness of 5 mm which is superior to the check clone Haiken 1 and RRIM 600. Bark thickness in *Hevea* is a clonal characteristics (Licy and Premakumari, 1988). Goncalves (1982) found in wild mother trees from 10 sites, pronounced difference in bark thickness between fast growing and slow growing trees in the Amazon valley basin. Similar wide variations in the bark thickness was also reported by Azwar *et al*., (1995) and Rao *et al*. (1999). Wide variations in the wild *Hevea* germplasm with reference to plant height and growth traits are reported in the traditional rubber growing regions in India (Rao *et al*., 1999; 2005; 2011; Rao and Varghese, 2011; Rao and Reghu, 2012 and Rao *et al*., 2013a).

In general, yield and vigour in *Hevea* are hardly separable (Simmonds, 1989). Girth and rate of girth increase are also used in experimental work to assess the growth performance of new planting materials. Even though in the seventh year after planting RO 2727 (53.12 cm) recorded the highest plant girth, MT 915 (92.32 cm) over took the growth of other genotypes in the fourteenth year after planting, and reached to the top position (Table 1). Girth values of this accession even significantly superior to the best check clone Haiken 1 (82.56 cm). Growth vigour in rubber tree is genetically controlled and there is a marked clonal variation with regard to girth increment under tapping and its effect on yield (Ferwerda, 1969).

The growth performance of a clone/accession can also be assessed on the basis of annual girth increment (GI) or growth rate and girth increment is seen around the year in the rubber plants (Rao and Kole, 2016). The genotype MT 915 recorded the highest girth increment (5.61 cm a^{-1}) over seven years, in comparison with the check clones RRIM 600 (2.75 cm a^{-1}) and Haiken 1 (4.65 cm a^{-1}). It is very important to note that the superiority observed in the mature phase of these accessions is in conformity with the best performance in the previous immature phase as well (Rao and Kole, 2016). Wide variations in the wild *Hevea* germplasm with respect to certain growth traits and growth-rate are reported in the traditional rubber growing regions in India (Rao *et al*., 2011; Rao and Varghese, 2011; Rao and Reghu, 2012 and Rao *et al*., 2013a) as well as in the non-traditional regions (Rao *et al*., 2006; Krishan *et al*., 2011; Rao and Kole, 2016). In general, during the non-winter season (Weather Regime I), all the wild accessions and clones under study attained maximum level of growth, as compared to the winter season (Weather Regime II).

Breeding experiments on *Hevea* always focus on genotypes with better performance in the cold climate, so as to identify the location specific clones which could adapt to low temperatures. In rubber, the threshold temperature for growth increment is believed to be in the range of 20º C (Jiang, 1988). Photosynthesis is impaired at 10º C (Huang and Zheng, 1983). The minimum temperature is as low as 5º C during December to January and the low winter temperature is conspicuous in the Nagrakata region as compared to the other rubber growing NE areas of India. So, low temperature is one of the major bottlenecks for rubber production in this region. It was reported that long durations of low temperatures affects the productivity and normal growth of *Hevea* plant (Alam *et al*., 2005). Among various stress factors, low temperatures in the winter, have strongly affected growth, development and latex production of rubber trees in South central China (Priyadarshan *et al*., 2005) and in the Northern part of Vietnam (Tuy *et al*., 2012).

The winter growth is indicative of a genotype's ability to continue its metabolic functions under stress and hence could be an indication of its tolerance to cold stress in the current study. Winter girth increment (cm/year) over seven years ranged from 0.34 cm a^{-1} (AC 3074) to 1.05 cm a^{-1} (MT 915). Certain tolerant wild accessions, namely MT 915, topped in winter girth increment rate (1.05 cm a^{-1}) closely followed by RO 2727 (0.95 cm a^{-1}), RO 3197 (0.85 cm a^{-1}) and MT 914 (0.80 cm a^{-1}) are on par with the check clones. The check clone of Malaysia- RRIM 600 and China-Haiken 1, which also reached higher growth, recorded a winter girth increment of 0.65 cm and 0.85 cm/year, respectively. These accessions which have reached high growth in the seventh year, not only performed better in low-temperature during winter period, but also showed a higher overall growth, indicating adaptability to cold stress. Similar observations were made in some wild *Hevea* accessions at immature phase (Rao and Kole, 2016). In the current study, growth of accessions during winter period was low as compared to the non-winter period and this is in agreement with an earlier report (Mondal *et al*., 1999; Das *et al*., 2011; Rao and Kole, 2016). Three wild accessions such as AC 3074, AC 3293, and AC 3075 showed negligible growth, an indication of high susceptibility to winter stress. Hence, winter growth is the critical criteria for the real assessment of a clone in terms of cold tolerance.

Genetic Diversity

The importance of cluster analysis to determine the extent of variability was reported earlier (Mahalanobis, 1936). D^2 statistics has been utilized extensively for estimating genetic diversity in a number of crop plants with diverse breeding systems (Murty and Arunachalam, 1966). Genetic diversity in germplasm lines is generally considered as an important criterion in deciding appropriate plant breeding methods for crop improvement. Precise information on the nature and magnitude of genetic diversity in the population helps the plant breeder in choosing the diverse parents for

purposeful hybridization and to maximize expression of heterosis (Arunachalam, 1981; Samsuddin, 1985).

The 22 *Hevea* genotypes were grouped into 5 clusters (Figure 2) and the distribution pattern of genotypes in various clusters reflected the considerable genetic variability/diversity present in the population under study (Table 2). There were 4 multiple-genotype clusters, while one cluster was solitary in nature. Cluster III and IV had maximum number of 10 and 6 genotypes each followed by clusters I with 3 genotypes from Acre provenance. All the three Acre provenance accessions of clusters I were poor performers for most of the growth traits. Clusters II consists of 2 genotypes from each of Acre and Rondonia provenance, while cluster V was a solitary one from Rondonia provenance. Cluster III had maximum number of diverse genotypes from all three provenances (Acre, Rondonia and Mato Grosso) of Brazil and modern clones (RRIM 600, PB 235 and RRII 203) developed from Far East includes Malaysia and India with vigorous growth traits. Cluster IV had second largest in number, comprising of genotypes from two Brazilian provenances (Rondonia and Mato Grosso) and a modern cold tolerant Chinese clone Haiken 1 with highly vigorous growth traits. This indicates that the genetic uniqueness among different ecotypes of *Hevea brasiliensis* complex.

Murty and Arunachalam (1966) have opined that hybridization programmes should be formulated in such a way that the parents belonging to different clusters with maximum divergence should be utilized to get desirable transgressive segregants. The genotypes from the cluster III and IV with more plant height, bark thickness, and other growth attributes could be utilized in hybridization programme for getting desirable segregants and high heterotic response. Crossing between widely distinct heterozygous genotypes of rubber exposes very few common recessives, so that F_1 vigour is markedly enhanced above the average, resulting in heterosis (Simmonds, 1989). The high recovery of heterotic progeny from the parents of wide divergence in *Hevea* was reported earlier (Licy *et al*., 1992; Mydin and Gireesh, 20l6). Moreover, the identification and availability of specific genotypes for use in breeding programmes assumes much significance, since wide array of diverse wild accessions and species of *Hevea* are available only in the Amazon forests of Brazil, the centre of diversity of the genus.

It was observed that the genotypes of different geographical origin were grouped together and the genotypes with same origin were included in different clusters (Table 2). The clustering pattern of the accessions showed that geographical diversity was not related with genetic diversity. This lack of correspondence between genetic and geographical diversity has also been reported in other crops (Sridhar *et al*., 2002; Tyagi and Sethi, 2011), as well as in *Hevea* (Markose, 1984; Mydin, 1992; Abraham, 2001; Rao *et al*., 2005).

Performance

By utilizing the six main growth parameters *i.e*. plant height (m), bark thickness (mm), annual girth (cm) in the seventh year and fourteenth year, annual girth increment (cm/year) over seven years and winter girth increment (cm/year) over seven years, individual performance of each accession was assessed for overall performance (Table 3). The top 30% vigorous accessions such as RO 2727, MT 915, RO 2638, RO 3197, MT 900, RO 3169, MT 2229, RO 3043, MT 1020 and AC 3810 could be identified as potentially superior for various growth traits. Balasimha *et al*. (1988) in Cocoa and Mercy (2001),

Table 3. Ranking of wild *Hevea* accessions and clones based on growth parameters

Accession/ Clone	Rank sum	Rank
RO 2727	125	1
MT 915	122	2
RO 2638	100	3
RO 3197	100	3
Haiken 1	94	5
MT 900	93	6
RO 3169	83	7
MT 2229	79	8
RO 3043	76	9
PB 235	74	10
MT 1020	73	11
RRII 203	72	12
AC 3810	68	13
RO 2901	68	13
RRIM 600	53	15
RO 2886	47	16
RO 2908	43	17
AC 3514	37	18
AC 3075	36	19
RO 2948	27	20
AC 3293	14	21
AC 3074	12	22
General mean 68		

Rao *et al*. (2006, 2011), Rao and Varghese (2011), Rao and Reghu (2012) and Rao *et al*. (2013a) also reported similar rankings in wild *Hevea* accessions, respectively while evaluating the Brazilian germplasm in India.

The high vigour of Rondonian genotypes may be due to hybrid vigour since reports on the probable hybrids of *H. brasiliensis* and *H. guianensis* in Costa Marques of Rondonia provenance (Anon. 1982) are available. Chevalier (1988) and Krishan *et al*. (2010) also reported that the intermediate position of Rondonian genotypes compared to the other two provenances. Better performance of accessions from Mato Grosso provenance also in the current study was in accordance with the earlier reports (Clement-Damange *et al*., 1990; Rao *et al*., 1999; Abraham, 2001; Krishan *et al*., 2010; Rao *et al*., 2013b). Genotypes from Acre provenance performed poorly for most of the growth traits, which is in accordance with the earlier reports.

Conclusion

Clustering of genotypes will be useful to the *Hevea* breeder in assessing the genotypes for vigour and arranging them into groups with similar growth patterns for selection. The present study confirmed the presence of wide variability in the germplasm for various growth traits. A potential group of ten accessions were identified, majority from Mato Grosso and Rondonia provenance *viz*., RO 2727, MT 915, RO 2638, RO 3197, MT 900, RO 3169, MT 2229, RO 3043, MT 1020 and AC 3810, ranked top in the cold prone sub-Himalayan region. Attainment of highest mean girth in the early mature period, which led to early tappable stage of these accessions, could be explained by its high growth rate when compared with the other wild accessions. Apparently, these genotypes also possess high timber potential. In fast growing timber species, high girth and high crotch height are proportional to high wood volume and the beneficial influence of fast growth rate on volumetric growth of tree was well known. Good growth is important in sustaining yield in high yielding clones and also reducing wind damage losses through trunk snap (Tan, 1987).

The superior performance for growth indicated that these ecotypes may be physiologically better adapted to the subtropical climate. The superiority of these selections may be explained by their inherent genetic potential and the genetic variability among accessions may be due to factors like heterogenity, selection pressure under diverse environments, genetic drift and/or geographical origin. Moreover, ecological differences between these three states/provenances (AC, RO, MT) offered chances of selection of materials suitable for diverse situations. These selected ecotypes were useful to increase the growth rate in elite clones, which leads to early maturity of the crop. Since they are genetically diverse from the cultivated clones, transgressive segregation and heterosis can be expected on crossing these accessions with elite Wickham cultivars. These ecotypes/selections possess highly potential new genes/alleles for growth and adaptation, useful in the development of cold-tolerant clones for these stress prone regions to mitigate changing climate and also in broadening the genetic base of the present-day cultivated rubber.

Acknowledgements

The authors are thankful to Dr. James Jacob, Director of Research, and Dr. CP Reghu, former Jt. Director (Germplasm), RRII, Kottayam for providing necessary support during this investigation. This paper forms part of the Ph.D. thesis work of the senior author at Visva-Bharati University, Santiniketan, West Bengal, India.

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Indian J. Plant Genet. Resour. 31(2): 152-163 (2018)
DOI 10.5958/0976-1926.2018.00018.9

Field Performance of 30-year-old Soybean Germplasm Conserved in Indian National Genebank

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(Received: 13 July 2017; Revised: 10 September 2017; Accepted: 18 September 2017)

Viability and vigour are the most important indices for assessing the seed quality. The present study was carried out to evaluate the performance of long-term conserved (30 years) soybean germplasm in the genebank. Some 100 accessions of soybean varying in seed morphological characteristics and origin were evaluated for 25 agro-morphological traits in field using standard descriptors. Field performance of freshly regenerated germplasm of the same accessions was that of originally conserved accessions. Analysis of variance showed significant difference between the regenerated and conserved accessions. Variations in the performance of genotypes with respect to field emergence, plant height, number of branches/plant, number of pods/plant, pod length, pod width, 100-seed weight and yield/plant between conserved and the regenerated accessions were observed. The mantle correlation between the two correlation matrices is very high (r = 0.936) and the mantle correlation between the distance matrices for regenerated and conserved accessions were highly significant (r = 0.957). The cophenetic correlation between the hierarchial clustering for regenerated and conserved accessions in the tanglegram was highly significant (r = 0.88) showing that the genotypic variation, initial viability and storage period has strong influence on the viability of soybean germplasm under the long-term conservation in genebank.

Key Words: Agro-morphological characterization, Genebank, Field performance, Long-term conservation, Soybean

Introduction

Soybean [*Glycine max* (L.) Merrl.] one of the most important legume crops, rich in protein and oil, has assumed an important position in Indian oilseed cultivation scenario. It occupies an area of 12.2 million hectare accounting to a production of 11.9 million tons (FAO, 2014). Soybean seed contributes 25% of the global edible oil, about two-thirds of the world's protein concentrate for livestock feeding and is a valuable ingredient in formulated feeds for poultry and fish. Soybean is grown under varied climatic conditions and geographical locations in India.

Maintenance of seed viability plays a critical role to the sustainability of *ex situ* conserved seed collections in long-term storage. For the best seed management in the genebank, the crop curators have to be careful in handling of the seeds from the receipt up to conservation in the genebank. Orthodox seeds are generally conserved in genebanks at 3-7% moisture content with ≥ 85% seed viability and a similar practice is being followed worldwide in about 1750 genebanks (Rao *et al.,* 2006; FAO, 2013). According to the set standards, long-term conserved seeds are monitored regularly every 10 years for quality assessment and regenerated as and when required. The frequency of regeneration can also be altered depending on the storability of crop species. Studies were conducted by several workers on seed monitoring during long-term conservation in several crops *e.g.* cereals (Ruiz *et al.,* 1999), threatened species (Godefroid *et al.,* 2010), other crops (Van Treuren *et al.,* 2013), rice (Hay *et al.,* 2015). A large extent of variations were observed by several workers during seed testing which may be due to the lower initial quality of seeds, differences in physiological maturity of seed at harvest, post-harvest handling procedures, storage temperatures, relative humidity of the storage environment, poor packaging, estimation error, seed proximate composition, dormancy and its genetic constitution (Godefroid *et al.,* 2010; Hay *et al.,* 2013; van Treuren *et al.,* 2013).

Genebanks, the richest reservoirs of valuable plant genetic resources, hold numerous untapped traits which are useful for crop improvement. Indian National

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Genebank (NGB), New Delhi, India, is the second largest genebank with a total holding of 0.44 millions accessions of various agri-horticultural crops including 3,702 accessions of soybean. Field evaluation of naturally aged seeds conserved in the genebank has been undertaken in soybean (Petrova and Geshava, 2017). Most of the studies on long-term conserved germplasm conducted were confined to the seed quality assessment based on seed viability and vigour (Ruiz *et al.,* 1999; Godefroid *et al.,* 2010; Abdellaoui *et al.,* 2013; van Treuren *et al.,* 2013; Hay *et al.,* 2013, 2015; Desheva *et al.,* 2017). However, information on the field performance of long-term conserved germplasm in the field is not available in literature. During monitoring, soybean germplasm conserved under long-term conditions showed lot of variation amongst accessions in terms of seed viability and vigour in laboratory conditions which cannot be precisely compared to the performance of the conserved germplasm in the field (Kulik and Yaklish, 1982; Wang *et al.,* 2004). Therefore, the present investigation is an attempt to compare the field performance of long-term conserved soybean germplasm (30 years in NGB) with the same set of freshly regenerated accessions for 25 qualitative (14) and quantitative (11) traits.

Materials and Methods

A total of 100 soybean accessions were selected on the basis of variability in seed colour, seed size and geographical origin which were collected/multiplied during 1985. These were processed as per gernebank standards and conserved under long-term conditions at -18±2^{o}C for 30 years, were used for the present study. Out of 100 germplasm accessions, 37 were indigenous collections from different regions of India and remaining were originally sourced from different countries *viz*., 19 from USA, 15 from Australia, 8 from Germany, 4 from Fiji, 3 each from Brazil and Taiwan Province of China, 2 from Bolivia and 1 each from Argentina, Canada, Hungary, Indonesia, Japan, Morocco, Papua New Guinea, South Africa and Russia (Table 1). Some 100-120 seeds of each of the above accessions were drawn from National Genebank (NGB) and equilibrated to room temperature. This was divided into two sets comprising 50-60 seeds each. One original set (conserved for 30 years in NGB; hereafterward referred to as conserved accessions) was retained as such and another set comprising 50-60 seeds was regenerated (hereafterward referred to as regenerated accessions) at ICAR-NBPGR Regional Station, Akola, during 2014-15.

The experiment to study the field performance of above accessions was conducted during *kharif* (July to October 2015) at Research Farm of ICAR-National Bureau of Plant Genetic Resources (NBPGR), Pusa Campus, New Delhi, India, located at 28^{o}35^{1}N, 77^{o}12^{1}E, altitude 228.6m.The farm is located in semi-arid, sub-tropical climate with average temperature ranging from 19 to 32^{o}C and total rainfall of 706.3 mm recorded during the cropping season (July–October 2015). The soil type was sandy loam to loamy with pH 7.5. Before sowing, FYM (farm yard manure) and recommended dose of fertilizers (20 N, 60 P, 20 K kg/ha) were applied. Four irrigations were given as and when required.

To compare the field performance of seeds of originally conserved accessions and same set of regenerated accessions (control), were grown in the field in two replicates of 30 seeds each. The experimental design was Reinforced Alpha Lattice (Gangopadhyay *et al.,* 2010) with 20 blocks in each replication; 30 seeds were sown in each row of 2 m length (spacing 45 cm × 10 cm). Each block contained 10 accessions and two national checks (SL-688 and PUSA 12). The regenerated and conserved accessions were evaluated based on the 25 standard Distinctness, Uniformity and Stability (DUS) descriptors and minimal descriptors (PPV&FRA, 2009; IBPGR, 1984). Qualitative characters included plant hypocotyl-anthocyanin pigmentation, plant growth type, leaf colour, leaf shape, plant growth habbit, flower colour, presence of pod pubescence, pod pubescence colour, pod colour, pod shattering nature, seed colour, seed lustre, seed shape and seed hilum colour. Observations on quantitative traits were recorded on five randomly selected and tagged plants in the defined block of each genotype. The observations of quantitative characters were recorded for field emergence (%), plant height (cm), days to 50% flowering (No.), days to maturity (No.), number of branches/plant, number of pods/plant, pod length (mm), pod width (mm), number of seeds/pod, 100-seed weight (g) and seed yield/plant (g).

Statistical Analyses

The analysis of variance for Reinforced Alpha Lattice Design was performed using PROC MIXED procedure of SAS® ver. 9.2 (SAS Institute, Cary, NC, USA) and least square means were computed. The rest of the analyses were performed in R package (R Core Team, 2017). Descriptive statistics were computed using 'psych' package (Revelle, 2017) and box plots

Table 1. List of selected 100 soybean accessions used to study their field performance

S. No.	Accession ID	Country of origin	S. No.	Accession ID	Country of origin
1	EC 4486	Argentina	52	IC 24058	India
2	EC 14469	Australia	53	IC 24060	India
3	EC 14479	Australia	54	IC 501186	India
4	EC 36372	Australia	55	IC 24994	India
5	EC 76756	Australia	56	IC 25763	India
6	EC 109543	Australia	57	IC 501599	India
7	EC 109517	Australia	58	IC 501154	India
8	EC 109546	Australia	59	IC 501171	India
9	EC 109549A	Australia	60	IC 501200	India
10	EC 109551	Australia	61	IC 501202	India
11	EC 109552	Australia	62	IC 501150	India
12	EC 109553	Australia	63	IC 501286	India
13	EC 109556	Australia	64	IC 501316	India
14	EC 109557	Australia	65	IC 501337	India
15	EC 109548	Australia	66	IC 501392	India
16	EC 95674	Australia	67	IC 501531	India
17	EC 93598	Bolivia	68	IC 501468	India
18	EC 93602	Bolivia	69	IC 501493	India
19	EC 93744	Brazil	70	IC 501220	India
20	EC 93745	Brazil	71	IC 501581	India
21	EC 93749	Brazil	72	IC 501592	India
22	EC 61434	Canada	73	EC 112828	Indonesia
23	EC 114522	Fiji	74	EC 62818	Japan
24	EC 114523	Fiji	75	EC 38128	Morocco
25	EC 114525	Fiji	76	EC 113401	Papua New Gyinea
26	EC 114532	Fiji	77	EC 4496	South Africa
27	EC 34342	Germany	78	EC 39489	Taiwan Province of China
28	EC 34343	Germany	79	EC 113773	Taiwan Province of China
29	EC 34374	Germany	80	EC 116055	Taiwan Province of China
30	EC 93425	Germany	81	EC 25167	United States of America
31	EC 39039	Germany	82	EC 2573	United States of America
32	EC 39020	Germany	83	EC 6103	United States of America
33	EC 39028	Germany	84	EC 61396	United States of America
34	EC 39043	Germany	85	EC 65773	United States of America
35	EC 34117	Hungary	86	EC 95280	United States of America
36	IC 15539	India	87	EC 95278	United States of America
37	IC 501434	India	88	EC 95264	United States of America
38	IC 501263	India	89	EC 95281	United States of America
39	IC 03	India	90	EC 100783	United States of America
40	IC 501267	India	91	EC 100786	United States of America
41	IC 501185	India	92	EC 100790	United States of America
42	IC 15554	India	93	EC 100794	United States of America
43	IC 16561	India	94	EC 100797	United States of America
44	IC 16573	India	95	EC 100804	United States of America
45	IC 16804	India	96	EC 106992	United States of America
46	IC 16816	India	97	EC 107006	United States of America
47	IC 16823	India	98	EC 95261	United States of America
48	IC 18751	India	99	EC 95269	United States of America
49	IC 18756	India	100	EC 37089	USSR
50	IC 21755	India	101	Pusa 12	India (National Check)
51	IC 21756	India	102	SL 688	India (National Check)

were plotted using 'ggplot2' package (Wickham, 2009). The least square means were used for accession comparison, correlation and cluster analysis. Pearson's linear correlation coefficients among all the traits were computed using the R package 'Hmisc' (Harrel and Dupont, 2017) and plotted using 'corrplot' (Wei and Simko). Principal component analysis was performed in 'stats' package (R Core Team, 2017) and plotted using 'scatterplot3d' package (Ligges, 2003). Gower distance measure implemented in the R package 'cluster' was used to obtain the genetic distance among accessions (Gower, 1971; Maechler, 2017) for hierarchical clustering by UPGMA method. Mantel correlation (Mantel, 1967) between the distance matrices for regenerated and conserved accessions were computed using the 'ade4' package (Dray *et al.,* 2007). Similarly the cophenetic correlation between the hierarchial clustering for regenerated and conserved accessions were computed and a tanglegram was plotted using the 'dendextend' package (Galili, 2015).

Results

Table 2 resets the data on agro-morphological characters of regenerated ad conserved accessions of soybean. The descriptive statistical analysis of the agro-morphological data of regenerated and conserved accessions (conserved for 30 years in NGB) showed large variation in the mean values of field emergence (%), plant height, number of pods/plant and plant yield (g). Field emergence was observed as highest in seeds of regenerated accession IC 21755 (100%) followed by EC 34342 (98%), whereas highest field emergence was recorded in seeds of conserved accessions in EC 95281 (88%) followed by EC 109556 (86%). These records correlated well with the seed germination, vigour values recorded in laboratory conditions (J Radhamani pers. comm., 2015). The field emergence of the seeds of regenerated accessions varied from 33.3 to 100%, with a mean value of 74.5%, whereas for the seeds of conserved accessions, the range of field emergence was recorded as 16.6 to 88% with a mean value of 48.86%, which is almost 35% lower than that of the seeds of regenerated accessions. The mean values of plant height and yield/plant of regenerated and conserved accessions was 39 cm and 36 cm and 8.08 g and 7.34 g, respectively. Less variation was observed in the days to 50% flowering, days to maturity, pod size, number of seeds/pod and 100-seed weight (Table 2). Number of seeds/ pod ranged between 2-3 and 100-seed weight from 3.45 g to 18.25 g in regenerated and 3.51 to 18.00 g in conserved accessions with a mean value of 10.07 g. Seed yield ranged from 3.21 to 14.02 g in regenerated and 3.03 to 13.87 g in conserved accessions with a mean value of 7.86 g. Irrespective of the regenerated or conserved accessions, the highest number of branches/ plant was recorded in EC 95280 and EC 95674 (8.1); similarly, the highest yield/plant was recorded in IC 501220 and EC 95278 (12.8 g).

The frequency distribution on 14 qualitative traits of regenerated and conserved accessions is presented in Table 3. No differences for qualitative traits between regenerated and conserved accessions were observed.

Table 2. Mean ± SE and range of agro-morphological data of regenerated accessions and conserved accessions (30 years in NGB) of soybean recorded in field

	Field emergence %		Days to 50% flowering		Days to maturity		Plant height (cm)	
	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range
Check	93.9 ± 0.5	83.3 - 100.0	50.3 ± 0.7	41.0 - 58.0	112.7 ± 0.5	105.0 - 119.0	67.6 ± 0.5	60.5 - 75.4
Regenerated	74.5 ± 1.2	33.3 - 100.0	49.0 ± 0.5	28.0 - 66.0	105.5 ± 0.5	83.0 - 121.0	39.3 ± 0.7	21.3 - 66.5
Conserved	48.9 ± 1.5	16.7 - 93.3	49.8 ± 0.5	28.0 - 64.0	106.0 ± 0.5	84.0 - 120.0	36.3 ± 0.6	20.3 - 61.3
Overall	61.7 ± 1.2	16.7 - 61.7	49.4 ± 0.4	28.0 - 49.3	105.8 ± 0.4	83.0 - 105.8	37.8 ± 0.4	20.3 - 37.8
	No. of branches/plant		No. of pods/plant		Pod length (mm)		Pod width (mm)	
Check	5.0 ± 0.03	4.0 - 5.6	64.1 ± 0.5	56.4 - 72.6	41.0 ± 0.2	36.5 - 44.9	7.231 ± 0.04	4.3 - 7.9
Regenerated	5.4 ± 0.08	3.2 - 8.6	37.5 ± 0.7	15.8 - 69.6	35.6 ± 0.3	23.4 - 44.4	7.277 ± 0.06	4.9 - 9.8
Conserved	4.9 ± 0.07	3.0 - 8.4	31.3 ± 0.6	12.3 - 55.3	34.8 ± 0.3	20.0 - 45.8	7.116 ± 0.06	4.6 - 9.7
Overall	5.2 ± 0.05	3.0 - 5.2	34.4 ± 0.5	12.3 - 34.4	35.2 ± 0.2	20.0 - 35.2	7.197 ± 0.04	4.6 - 7.2
	No. of seeds/pod		100-seed weight (g)		Yield/Plant (g)			
Check	2.9 ± 0.01	2.6 - 3.0	9.4 ± 0.1	7.9 - 10.6	8.6 ± 0.2	6.0 - 11.6		
Regenerated	2.5 ± 0.02	2.0 - 3.0	10.3 ± 0.2	3.4 - 18.2	8.1 ± 0.1	3.2 - 14.0		
Conserved	2.5 ± 0.02	2.0 - 3.0	10.1 ± 0.2	3.5 - 18.0	7.3 ± 0.1	3.0 - 13.9		
Overall	2.5 ± 0.01	2.0 - 2.5	10.2 ± 0.1	3.4 - 10.2	7.7 ± 0.1	3.0 - 7.7		

SE, Standard error, Mean values were rounded off to the nearest integer up to one place of decimal

Table 3. Frequency distribution of 14 qualitative traits of soybean germplasm

Trait	State	No. of accessions
Hypocotyl anthocyanin colour	Present	73
	Absent	27
Plant growth type	Determinate	100
Leaf colour	Green	82
	Dark green	18
Leaf shape	Pointed	92
	Round	5
	Lanceolate	3
Growth habitat	Erect	32
	Semi erect	67
	Spreading	1
Flower colour	Purple	77
	White	27
Pod Pubescence	Present	100
Pod pubescence colour	Brown	82
	Grey	18
Pod colour	Brown	71
	Yellow	26
	Black	3
Pod shattering nature	Shattering	74
	Non-shattering	26
Seed colour	Yellow	59
	Yellow green	24
	Black	10
	Brown	7
Seed shape	Elliptic	57
	Spherical	43
Seed lustre	Shiny	62
	Dull	38
Seed hilum colour	Brown	65
	Black	35

Hypocotyl anthocyanin pigmentation recorded on the 9- or 10-day-old seedlings, showed that 73% of the accessions were pigmented and 27% non-pigmented. All the 100 accessions were found to be determinate and the most of the accessions (67%) were semi-erect type. Flower colour varied from white (27%) to purple (77%). Pod colour also varied from black (3%), brown (71%) and yellow (26%). Pods of all the accessions showed pubescence, of which 82% showed brown pubescence and 18% grey. Most of the accessions (92%) exhibited pointed tips with green leaves (82%). Pod shattering habit was recorded at the physiological maturity stage and 74% of the accessions were non-shattering and remaining were of shattering type. Distinct seed coat colours were observed among the accessions; 59% accessions were observed as yellow, 26% yellow green and remaining were either black or brown. Some 67% accessions were elliptical and 43% had spherical shape. Seed of 62% of accessions were lustrous and remaing were dull in nature. In 65% of genotypes hilum colour was brown and the rest had black colour.

ANOVA of all the 11 quantitative characters with replication are non-significant between the regenerated and conserved accessions. However, block (replication) is significant for plant height. Accession-wise differences are highly significant for all the 11 quantitative characters. The interaction between regenerated *vs* conserved germplasm is significant for most of the characters but non-significant for days to 50% flowering, days to maturity and number of seeds/pod (Table 4).

Correlation Analysis

Correlations are used to quantify the relationships between different characters, which are important for selection. Comparison of the correlations between regenerated and conserved accessions revealed a similar trend of relationships. For example, significant positive correlation was observed between days to 50% flowering × days to maturity, number of branches/plant x number of pods/plant, 100-seed weight × yield/plant, plant yield x number of pods/plant. A highly negative correlation was observed between days to 50% flowering × pod width, plant height × pod width, pod length × pod width, days to 50% flowering × 100-seed

Table 4. ANOVA of quantitative traits used to evaluate the field performance of soybean germplasm

Source	DF	Field emergence (%)	Days to 50% flowering	Days to maturity	Plant height (cm)	No. of branches/ plant	No. of Pods /plant	Pod length (mm)	Pod width (mm)	No. of seed/ pod	100-seed wt (g)	Yield/ plant (g)
Replication	1	0.05^{NS}	0.74^{NS}	12.41^{NS}	1.52^{NS}	0.06^{NS}	0.06^{NS}	2.98^{NS}	0.32^{NS}	0.07^{NS}	0.03^{NS}	0.21^{NS}
Block (Replication)	38	37.04^{NS}	15.79^{NS}	17.79^{NS}	15.08*	0.11^{NS}	18.16^{NS}	7.89^{NS}	0.41^{NS}	0.03^{NS}	0.06^{NS}	0.21^{NS}
Acc. No.	201	1151.91**	101.29**	113.82**	440.94**	2.36**	459.4**	36.84**	0.81**	0.19**	17.88**	10.45**
Regenerated vs Conserved	1	25152.7**	3.4^{NS}	0.34^{NS}	251.71**	9.55**	1479.27**	32.08*	1.79*	0.01^{NS}	1.63**	19.43**
Error	239	53.46	13.61	15.84	9.2	0.11	14.76	6.9	0.42	0.03	0.05	0.22

**significant at the 0.01 probability level; *significant at the 0.05 probability level; NS, Non-significant

weight (Table 5). The mantle correlation between the two correlation matrices was very high (r = 0.936) and the mantle correlation between the distance matrices for regenerated and conserved accessions were highly significant (r = 0.957). The cophenetic correlation between the hierarchial clustering for regenerated and conserved accessions in the tanglegram was highly significant (r = 0.88) (Fig. 1).

Principal Component Analysis

Through principal component analysis, 11 principal components were extracted from the 11 quantitative traits. However, among these, the first three components explain 88.7% of the total variation and hence can be used to summarize the original 11 variables. The variability contributed by first component was 62.98% followed by second component (14.45%) and third component (11.28%) (Fig. 2a). Clustering on the basis of these three components revealed overlapping of regenerated and conserved accessions (Fig. 2b). An examination of the loading values in the eigen vectors for these principal components revealed the contribution of individual characters towards clustering. For the first component, field emergence had the highest positive loading, greater in magnitude than that for other traits. Similarly, for the second component plant height and number of pods/plant had high positive loadings. In the third component, days to maturity and days to 50% flowering had high positive loading and number of pods/plant had high negative loading (Fig. 2c). So these traits contribute more to the diversity observed in the studied accessions of soybean. Hence, the marginal grouping observed among the accessions with respect to their storage status may be due to fact that the seed vigour is an influenced trait of field emergence and other related traits such as number of pods/plant and plant height.

Discussion

Under long-term storage orthodox seeds are generally stored *ex situ* at -18±2ºC conditions. *Ex situ* storage influences the seed germination, vigour, rate of seed germination based on the seed quality, proximate composition, seed moisture content and the genetic constitution. The seeds are best stored at an optimum moisture level of 3-7% at sub-zero temperature as most of the metabolic processes of the seeds slow down. Loss in seed vigour precedes loss in seed germination eventually leading to seed deterioration (Brutting *et al.,* 2013).

Table 5. Correlation coefficient among qualitative and quantitative descriptors between regenerated and conserved accessions of soybean

		FE	DF	DM	PH	NBPP	NPPP	PL	PW	NSPP	HSW	YPP
FE	REG	1										
	CON	1										
DF	REG	0.12	1									
	CON	0.26**	1									
DM	REG	0.11	0.97**	1								
	CON	0.23**	0.98**	1								
PH	REG	0.02	0.11	0.09	1							
	CON	0.17*	0.18*	0.17*	1							
NBPP	REG	-0.11	0.17*	0.17*	0.20**	1						
	CON	0.05	0.1	0.11	0.27**	1						
NPPP	REG	0.09	0.13	0.16*	0.23**	0.48**	1					
	CON	0.16*	0.05	0.06	0.26**	0.46**	1					
PL	REG	-0.19**	-0.20**	-0.18*	-0.07	-0.18*	-0.01	1				
	CON	-0.11	-0.06	-0.06	-0.05	-0.19**	-0.05	1				
PW	REG	-0.08	-0.33**	-0.31**	-0.32**	-0.17*	-0.14	0.40**	1			
	CON	-0.18*	-0.24**	-0.23**	-0.20**	-0.12	-0.13	0.38**	1			
NSPP	REG	-0.19**	-0.21**	-0.18**	0.05	0.06	0.08	0.24**	0.1	1		
	CON	-0.1	-0.15*	-0.11	-0.01	0.07	0.21**	0.05	0.07	1		
HSW	REG	-0.04	-0.31**	-0.27**	0.01	0.09	0.04	0.16*	0.15*	0.03	1	
	CON	0.02	-0.17*	-0.17*	-0.02	0.09	0.06	0.17*	0.19**	-0.08	1	
YPP	REG	-0.02	-0.11	-0.05	0	0.20**	0.32**	0.14*	0.05	-0.02	0.79**	1
	CON	0.11	-0.07	-0.04	-0.06	0.20**	0.34**	0.19**	0.08	-0.02	0.76**	1

FE: field emergence, DF: days to 50% flowering, DM: Days to maturity, PH: Plant height, NBPP: No. of branches/plant, NPPP: No. of pods/plant, PL: Pod length, PW: Pod width, NSPP: No. of seeds/pod, HSW: 100-seed weight. YP: Yield/plant

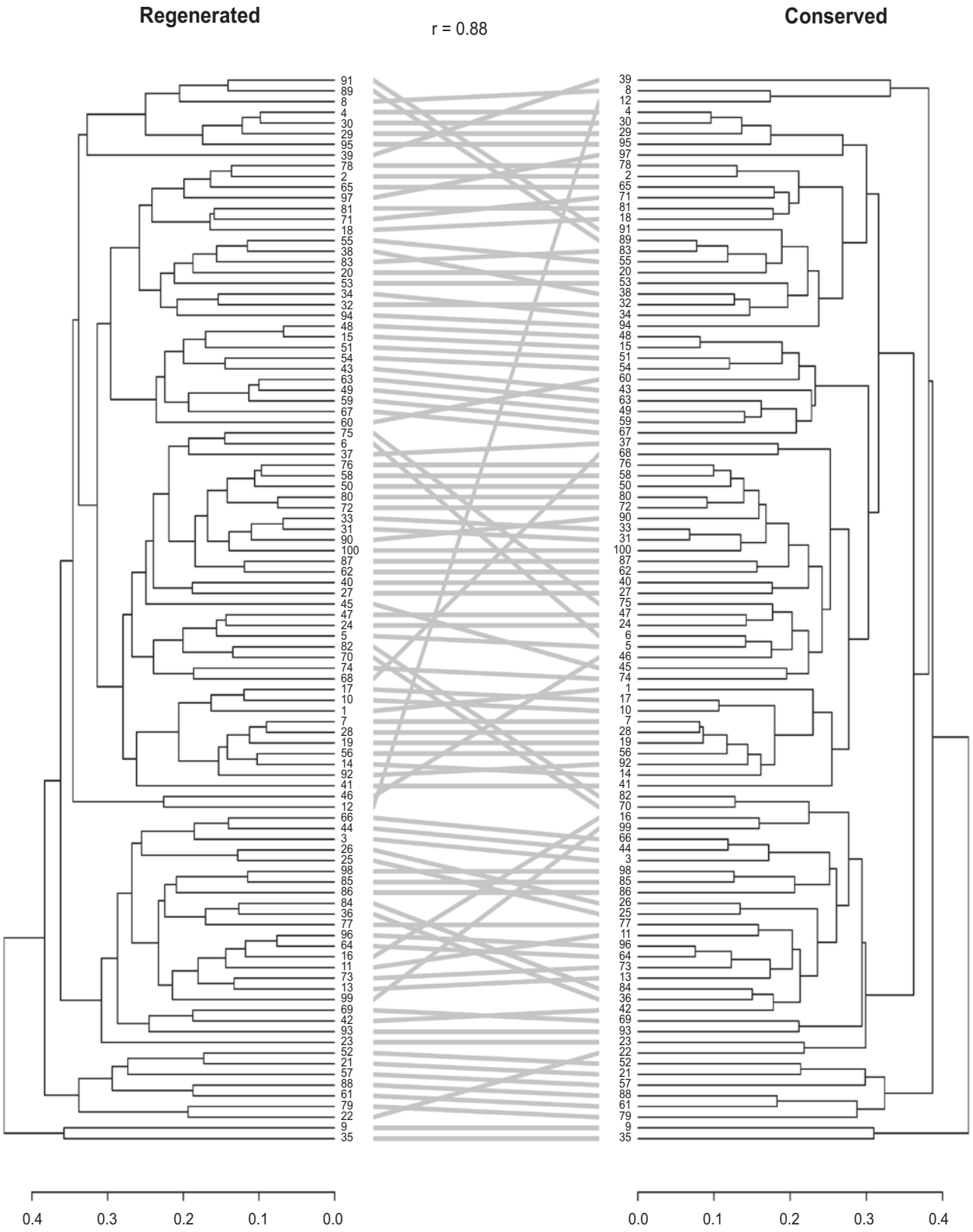

Fig. 1. Cophenetic correlation between the hierarchial clustering of regenerated and conserved accessions in the tanglegram (Numbers are corresponding to the accessions listed in Table 1)

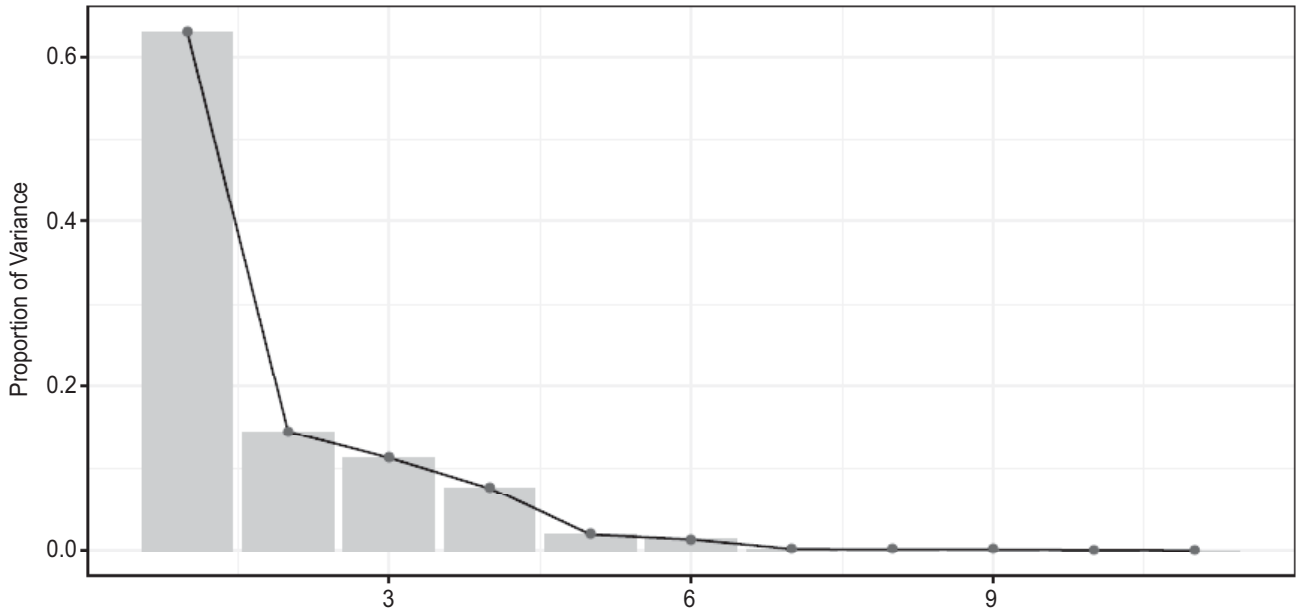

Fig. 2a. Principal components plot of 100 accessions (regenerated and conserved) of soybean

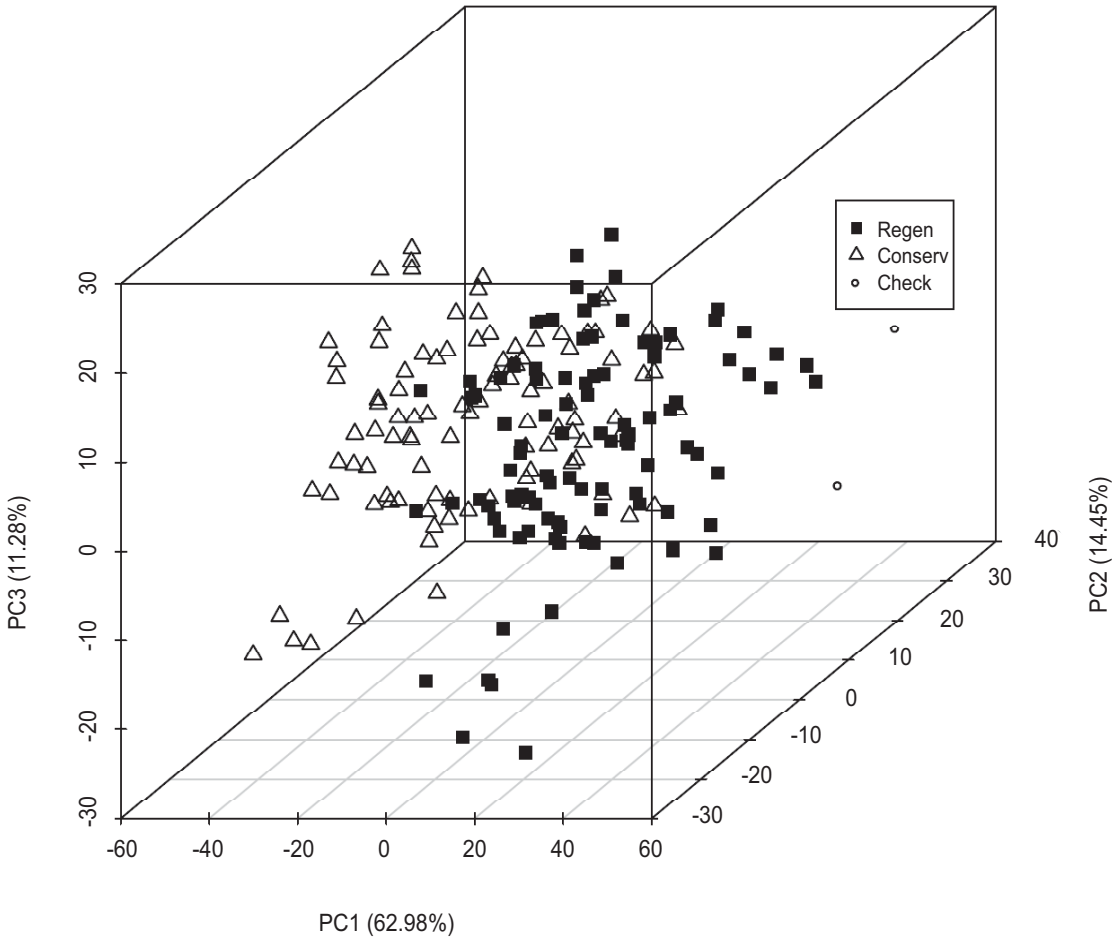

Fig. 2b. Clustering on the basis of three (PC1, PC2, PC3) components revealed overlapping clusters of regenerated and conserved accessions of soybean (Regn: Regenerated; Conserv: Conserved accessions)

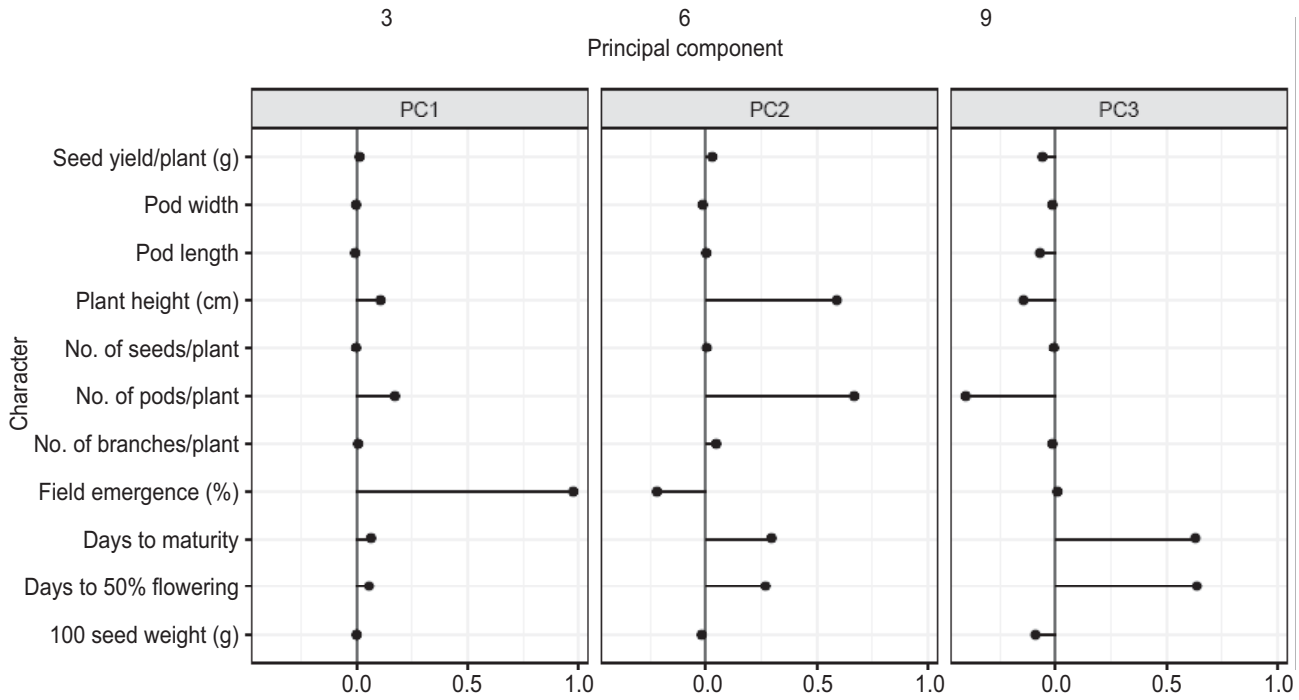

Fig. 2c. Eigen loading values and eigen vectors for first three principal components

The vigour is reduced after long-term storage which varies between crops, among plant species, accession to accession complying with the agreement that the phenotypic expressions are influenced by the genetic constitution of the seed. Monitoring the seed viability and vigour is critical for sustainable conservation of germplasm in genebank (Alyaha, 1995). Monitoring of seed viability and vigour in laboratory conditions is common practice to test the seed quality of accessions conserved under long-term storage conditions. However, the performance of conserved germplasm can be better judged based on their field emergence, good vigour, uniform establishment of seedling and ultimately yield by growing in the field and comparing them with freshly regenerated germplasm. The authors report on the field performance of 100 soybean accessions conserved under long-term storage at -18±2^{o}C temperature for 30 years in genebank. Control set comprised of the regenerated set of same original 100 accessions from NGB which comprised the conserved accessions to compare their performance in the field.

Although, under standard genebank storage conditions, high viability is supposed to be maintained even after many decades, it is observed that seeds of several accessions of soybean could not retain viability after storage (30 years) closer to initial viability and suffered natural ageing leading to decrease in seed quality to various extents. Our studies indicate that seeds when stored with moisture (3-7% mc) and germination (75%) at -18°C, the seed metabolic activities do not cease completely but continues to take place at a very slow pace, the rate of which may not be uniform in all the accessions thereby causing difference in viability pattern of these accessions during ageing in genebank. These differences are only expressed during seed germination of different seed lots of stored germplasm. At low moisture contents under long-term storage (3-7% mc) when most of the water content in the seeds is in the bound form, non-enzymatic lipid auto-oxidation might be the primary cause of seed deterioration (McDonald, 1999; Murthy *et al.* 2003; Smith and Berjak, 1995; Walters *et al.,* 2005).

Similarly, in case of wheat, barley, oat germplasm monitored after 10 years in Spanish Genetic Resources Centre (Ruiz *et al.,* 1999), in rice after 30 years in IRRI (Hay *et al.,* 2013, 2015), in several threatened species up to 26 years in National Botanic Garden in Belgium (Godefroid *et al.,* 2010), soybean after 30 years in Bulgarian National Seed Bank (Desheva *et al.,* 2017), several cultivated and wild taxa after 25 years in Centre for Genetic Resources, The Netherlands (van Treuren *et al.,* 2013) it was observed that germination declined to

various extents tested in laboratory conditions. Studies conducted by Moyo *et al.,* (2015) showed significant difference in the performance of genotypes with respect to vigour and field emergence in sorghum genotypes conserved for 10 years under long-term storage.

To best of our knowledge, there is no information available on the field performance studies of long-term conserved germplasm in genebank in literature, similar to the results obtained in our studies. However, on the basis of studies on short- or medium-term storage germplasm or artificial ageing conditions, conducted by Potts *et al.* (1983), Alvarez *et al.* (1997) Karmakar *et al.* (1999) it was observed that most of the seed characteristics *viz.* seed size, seed coat colour, thickness, oil content were associated with seed quality traits. Therefore, low seed quality due to deterioration is reported to be the cause of poor stand establishment in the field and consequently yield loss in barley (Abdalla and Roberts, 1969), wheat (Ganguli and Sen-Mandi, 1990), oilseed rape (Ghassemi-Golezani *et al.,* 2010), maize (Ghassemi-Golezani *et al.,* 2011).

For other traits studied, no significant differences were observed in yield and yield contributing components between regenerated and conserved accession indicating that no apparent heritable changes occurred in soybean germplasm during the storage period of 30 years in genebank, as these accessions were multiplied for the first time. There is a great variation in the field emergence of conserved and regenerated accessions. One of the reason for this could be attributed due to the differences in seed size, colour, country of origin and variation of the genotype itself which finally led to the varied range of germination (Dassou and Kueneman, 1984; Mugnisjah *et al.,* 1987; Kuchalan, 2006; Zahid, 2013). The other reason might be due to the poor initial viability of soybean accessions not conforming to genebank standards and also due to the seeds during processing were subjected to lower standards of processing conditions in the genebank. The soybean germplasm were packed in aluminium foil pouches non-hermetically sealed. In non-hermetically seed pouches there might have been presence of more oxygen which could have triggered the process of seed deterioration leading to the observed difference in the pattern of germination of conserved seeds *vis-à-vis* regenerated seeds.

It can be concluded that genotypic variation, pre-harvest conditions, initial viability and storage conditions appear to be most influential factors for maintaining the higher seed viability in genebank also and in turn, the germinability in field conditions which was supported by other studies undertaken by Lu *et al.* (2004) and Benkova and Zakova (2009) in various crops conserved for long-term in genebanks.

Acknowledgments

Authors express their sincere thanks to the Director, ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR), New Delhi, for guidance as well as for constant encouragement during the course of study. Thanks are also due to Head, Division of Germplasm Evaluation and Dr TV Prasad, Senior Scientist, ICAR-NBPGR for helping directly or indirectly in various ways for sparing facilities for conducting the experiment.

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Indian J. Plant Genet. Resour. 31(2): 164-168 (2018)
DOI 10.5958/0976-1926.2018.00019.0

Two-leaf Nightshade (*Solanum diphyllum* L.)–An Addition to the Flora of Delhi, India and Weed Risk Assessment of the Species

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(Received: 15 December 2016; Revised: 31 January 2017; Accepted: 13 February 2017)

Solanum diphyllum L. (the two-leaf nightshade) is reported as a new record for flora of Delhi. Study of natural populations of the species and weed risk assessment supported it's tendency of widespread naturalization. Plant characters such as high fruit-bearing and high seed number per fruit, perennating root stock, high germination rate, seed dormancy, no natural predators (animal toxicity) and dispersal through movement of soil were the main parameters favouring fast spread of the species. A detailed description of the species along with distribution, propagation and dispersal has been discussed.

Key Words: Delhi, Flora, *Solanum diphyllum* L., Two-leaf Nightshade, Weed Risk Analysis

Introduction

Two-leaf nightshade or twin leaved nightshade (*Solanum diphyllum* L.), a native species from South Mexico to Costa Rica in Central America, was introduced as a garden plant in different continents across the world. It escaped from cultivation and widely naturalized in many tropical and subtropical regions of the world. This species belongs to the family Solanaceae, subfamily Solanoideae and tribe Solaneae (Zhang *et al.,* 1999). It is widely cultivated as an ornamental plant for clusters of dark green globose berries that turn beautiful bright yellow or reddish yellow when ripe. Most parts of the plant are very poisonous, including the leaves and berries (http://lee.ifas.ufl.edu/Hort/GardenPubsAZ/Two-leaf_Nightshade.pdf). This species has ethnomedicinal value in Tamil Nadu and Kerala (Devi Prasad and Shyma, 2013; Anilkumar *et al.*, 2014; Shalini *et al.*, 2014) and has potential medicinal value against varieties of carcinoma (Fatma *et al.*, 2010). It is also reported for ornamental value in gardens of India (https://groups.google.com/forum/?hl=en#!topic/indiantreepix/5JSz2_GU7a0;www.flowersofindia.net/catalog/slides/Twoleaf%20Nightshade.html).

During an educational trip to Herbal Garden, Punjabi Bagh, New Delhi in the month of September in 2014, the authors came across this species. A detailed examination of the characters has helped in its identification as *Solanum diphyllum* L. Critical examination and perusal of literature revealed that this species was not reported in floristic records of Delhi and adjoining areas. During subsequent visits in year 2015 and 2016, plant populations colonized new areas within garden; were also observed to be escaping beyond the garden boundaries.

In this paper, occurrence of *S. diphyllum* as a new floristic record in Delhi, along with its occasional use as an ornamental species has been reported. Keeping in view it's high weedy potential, fast spreading rate and occurrence reported from diverse states of India (Devi Prasad and Shyma, 2013; Reema Kumari, 2004, 2013; Anilkumar *et al*., 2014; Singh *et al.*, 2014; Singh *et al*., 2014; Singh and Garg, 2015; Singh and Singh, 2015), weed risk analysis was based on observations undertaken during 2014-16 at the reported site using standard method (Singh *et al*., 2013). A detailed description, distribution, propagation and dispersal is included in the present communication to facilitate easy identification and further research pertaining to plant genetic resource management.

Botany

Solanum diphyllum L., Sp. Pl. 184. 1753; D'Arcy in Ann. Missouri Bot. Gard. 61: 845. 1974; Zhi Y. Zhang *et al*. in C.Y. Wu and P.H. Raven, Fl. China 17: 317. 1994; M. Das *et al.,* J. Econ. Taxon. Bot. 21: 158. 1997; T.K. Paul and M.C. Biswas in Bull. Bot. Surv. India 37: 137. 1995. M. Reema Kumari in Rheedea 23(1) 50-51. 2013; *Pseudocapsicum diphyllum* (L.) Medik., Philos. Bot. (Medikus) 1: 122. 1789.

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Fig. 1. *Solanum diphyllum* L. (the two-leaf nightshade) in Herbal Garden, Punjabi Bagh, New Delhi: (top row- from left to right) immature fruits in cluster; mature orange yellow berries; (bottom row- from left to right) new plants growing in waste areas; herbarium specimen deposited in National Herbarium of Cultivated Plants (NHCP) (HS22401; collector: AP, RG-28/2016)

Flowering/ fruiting: March-August; Fruiting: May-November (in Delhi).

Plant is a herbaceous-woody perennial, small shrub of about 1-2.4 m height, smooth stem with brown bark; leaves- leathery, glossy, dark green on the upper surface, light green on the ventral surface, born in pairs from a single bud, one is large and elliptic-obovate, size 6×2 cm, the other smaller and rounded, size 2×1 cm; petiole 2 mm; inflorescences bearing 5-23 tiny drooping white flowers of 1 cm diameter borne in leaf axils. Each flower has five recurved petals of lavender tinge and stamens with large yellow anthers. The fruits are berries, spherical, smooth with a slight division around the middle, especially when unripe, green and hard when young, around 0.9-1 cm, mature into bright yellow to yellow orange. Each fleshy fruit has 30-45 seeds, about 1-1.5 mm, flattened, creamish-light brown, kidney-shaped with undulating surface (Fig.1).

Distribution

The two-leaf nightshade was in cultivation in the 1960's as an ornamental plant in subtropical and tropical parts of the world. Later it was spotted as a "bird-dispersed" volunteer in hedges and pastures in South Florida in the late 1960's, and reported as a "widespread escape" by 1967. Presently it is naturalised in diverse habitats of South and Central Florida (http://lee.ifas.ufl.edu/Hort/GardenPubsAZ/Two-leaf_Nightshade.pdf).

The first report on occurrence of two-leaf nightshade in India was published in the year 1995 with only a few individual plants growing at two locations in Howrah district of West Bengal (Paul and Biswas, 1995) and later reconfirmed (Das *et al*., 1997). In 2006, the species was recorded to be widespread, occupying grasslands and forests edges in Lonavala and Khandala and other wastelands of Pune, Pimpri-Chinchwad and neighbouring towns of Maharashtra. Its occurrence was reported in

various botanical gardens of Pune including the Empress Garden, Mudwa Experimental Garden of Botanical Survey of India (BSI), the associated garden of BSI office campus and other public gardens (Singh *et al*., 2014).

Subsequent reports of occurrence of species as a new record for North-eastern India (Assam) (Nath *et al*., 2010), Maharashtra (Pune and Powai), Karnataka (Bengaluru) and for southern India (Reema Kumari, 2004, 2013; http://indiabiodiversity.org/observation/show/378847), Bihar (Bhagalpur district) (Halder *et al*., 2013; Annual Report, 2013), Tamil Nadu (Shalini *et al*., 2014), Kerala (Wayanad and Periyar districts) (Devi Prasad and Shyma, 2013; Anilkumar *et al*., 2014), Uttar Pradesh (Varanasi, Mirzapur and Sonebhadra districts) (Singh and Singh, 2015) and scattered reports from various parts of India (Rajkumar Singh *et al.*, 2014) indicated its fast spread to different parts of the country. Singh and Garg (2015) have reported its spread to four northern states viz., Bihar [(West Champaran district), Chhattisgarh (Koriya district)], Madhya Pradesh (Anuppur district) and Uttar Pradesh (Allahabad, Gorakhpur and Varanasi districts) and Eastern Ghats (Kalahandi region, Koraput district, Odisha) (Murugan *et al*., 2015; Sahu *et al*., 2015) suggesting the spread mainly through plant nurseries as primary source. Report on widespread occurence of species in the entire Acharya Jagadish Chandra Bose Indian Botanic Garden, BSI, Howrah justified its categorization as a "serious weed" posing a suspected threat to many endemic species (Singh *et al*., 2014). Deliberate introduction of the species in various gardens and pattern of spread to neighbouring areas through dispersal by various means has been evidently reflected through author's observations and various published records.

During the collection of germplasm by the second author to Vishakhapatnam district, Andhra Pradesh in 2015, it was also observed that there were naturalized population of the species (K Pradheep/Babu Abraham-2083; HS22157; 31.10.2015).

The authors noted the occurrence of *S. diphyllum* in Delhi for the first time at the Herbal Garden, Punjabi Bagh (Horticulture Department, Delhi Nagar Nigam) in 2014 along the boundary wall represented by a few individual plants (one year-old plants). These were growing with the grasses, *Coccinia grandis*, *Convolvulus arvense, Abutilon indicum*, *Chenopodium album*, etc. Subsequent observations during September-November in 2015 and 2016 by the authors indicated fast spread of populations from original location towards central side of garden area and also outside the garden premises on road side. Personal communication with garden authorities indicated it's planting as an ornamental long back. During study, the authors observed weedy populations occurring in different parts of the garden (Fig. 1).

The authors observed plants only under self-sown state in different populations in the Herbal Garden, Punjabi Bagh, Delhi. Intermittent occurence of plants with other cultivated species growing in garden and pattern of spread also supported its dispersal through seed movement along with the soil clods. *Solanum diphyllum* is also known by the local name '*pili makoe*' (makoi is common name used in northern India for *S. nigrum*). This species was not recorded from floristic literature of Delhi and adjoining region (Clarke, 1883; Duthie, 1960; Maheshwari, 1963; Mishra, 2015) nor was it recorded for any use (Ambasta *et al*., 1986) except for deliberate introduction as an ornamental plant. However, the species is also reported to be planted in some gardens of Delhi (Green Park area, Herbal Garden, Punjabi Bagh, Delhi and Old Delhi Ridge) (https://sites.google.com/site/efloraofindia/species/m---z/s/solanaceae/solanum/solanum-diphyllum; http://indiabiodiversity.org/biodiv/species/show/231159). Hence, it forms the first floristic record of Flora of Delhi. Flowering and fruiting specimens were collected and seeds were deposited in the National Herbarium of Cultivated Plants (NHCP) (HS22381, 22401, 22405 and 22464; Seed Sample no.3070).

Propagation and Seed Germination

Solanum diphyllum propagates through seeds and 30-45 seeds/berry were recorded. A three-four year old plant produced at least 2000-2500 seeds in a growing season. It was observed that mature plants dry with dormant rootstock in December-January but resprout in March.

Seeds from mature berries were extracted, dried and subjected to seed germination study. Seed germination was upto 100% using GA_3 treatment (using moist filter paper) (pers. comn. Dr J Radhamani, ICAR-NBPGR). Studies have indicated that seed can stay alive in soil even when buried an inch in the soil for up to two years; seeds can sustain drought stress but are sensitive to high salinity induced by NaCl (Mohamed *et al*., 2010). Studied populations showed seed spread/dispersal through mechanical means (movement of

the soil clods, irrigated water) in the garden or man managed areas where there was movement of the dried fruits from one place to another. However, according to reports available in literature, seeds are mainly dispersed by the birds (Galindo-gonzález *et al*., 2000; Singh and Garg, 2015).

Weed Risk Analysis

Many garden plants of exotic origin pose a high risk of naturalization and potential as a weed in the area of cultivation (Groves *et al*., 2005; Pandey *et al*., 2015). Two-leaf nightshade with reports of invasiveness in many countries (http://fnai.org/Invasives/Solanum_diphyllum_FNAI.pdf) coupled with high drought tolerance poses risk of it's escape from gardens and establishment as a naturalized population.

Among many introduced garden plants under cultivation, the two-leaf nightshade is naturalized in parts of India. Keeping this in view, weed risk assessment was done based on standard procedure (Singh *et al*., 2013). This information provides status of evasiveness of the species based on biological and ecological parameters. Species was subjected to weed risk assessment system based on a question-based scoring containing 49 questions. The response to the questions generated a numerical score with positive correlation to the weediness (Annexure 1). The score of '15' out of 49 for this species revealed its potential as "serious weed" in cultivable/ agricultural land. Traits such as high-fruit bearing and high seed number/fruit, perennating rootstock, high germination rate, seed dormancy, no natural predators (animal toxicity) and dispersal through movement of soil were mainly observed to influence fast spread of the species.

Information on new record of this species to flora of Delhi may add new distribution range for the region. Besides, it's spread as a weed in areas of cultivation as studied through visual observations and weed risk analysis, it will add to the knowledge regarding risk of fast naturalization through escaping from cultivation.

Acknowledgements

Authors express their sincere thanks to Director, ICAR-NBPGR, New Delhi for providing need-based support to carry out this study. The authors are also thankful to the Head, Division of Plant Exploration and Germplasm Collection, for valuable guidance.

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Annexure 1

Weed Risk Assessment Questionnaire for *Solanum diphyllum*

Botanical name:			*Solanum diphyllum*	Outcome:	Reject
Common name:			Two leaved night shade	Score:	15
Family name			Solanaceae	Your name: Dr. Anjula Pandey	
History/Biogeography					
A	1	Domestication/	1.01	Is the species highly domesticated. If answer is 'no' go to question 2.01	n
C		Cultivation	1.02	Has the species become naturalized where grown	y
C			1.03	Does the species have weedy races	y?
	2	Climate	2.01	Species suited to Indian climates (0-low; 1-intermediate; 2-high)	1
		Distribution	2.02	Quality of climate match data (0-low; 1-intermediate; 2-high)	1
C			2.03	Broad climate suitability	
C			2.04	Native or naturalized in regions with extended dry periods	n
			2.05	Does the species have a history of repeated introductions outside its natural range	n
C	3	Weed	3.01	Naturalized beyond native range	y
E		elsewhere	3.02	Garden/amenity/disturbance weed	garden
A			3.03	Weed of agriculture/horticulture/forestry	Weed of horticulture ?
E			3.04	Environmental weed	y
			3.05	Congeneric weed	
Biology/ Ecology					
A	4	Undesirable	4.01	Produces spines, thorns or burrs	n
C		traits	4.02	Allelopathic	
C			4.03	Parasitic	
A			4.04	Unpalatable to grazing animals	y
C			4.05	Toxic to animals	y (eaten by birds)
C			4.06	Host for recognised pests and pathogens	?
C			4.07	Causes allergies or is otherwise toxic to humans	y
E			4.08	Creates a fire hazard in natural ecosystems	n
E			4.09	Is a shade tolerant plant at some stage of its life cycle	y
E			4.10	Grows on infertile soils	n
E			4.11	Climbing or smothering growth habit	
E			4.12	Forms dense thickets	
E	5	Plant type	5.01	Aquatic	
C			5.02	Grass	
E			5.03	Nitrogen fixing woody plant	
C			5.04	Geophyte	
C	6	Reproduction	6.01	Evidence of substantial reproductive failure in native habitat	
C			6.02	Produces viable seed	y
C			6.03	Hybridises naturally	
C			6.04	Self-fertilisation	
C			6.05	Requires specialist pollinators	
C			6.06	Reproduction by vegetative propagation	n
C			6.07	Minimum generative time (years)	
A	7	Dispersal	7.01	Propagules likely to be dispersed unintentionally	y
C		mechanisms	7.02	Propagules dispersed intentionally by people	
A			7.03	Propagules likely to disperse as a produce contaminant	y
C			7.04	Propagules adapted to wind dispersal	n
E			7.05	Propagules have dormancy	y
E			7.06	Propagules bird dispersed	y
C			7.07	Propagules dispersed by other animals (externally)	n?
C			7.08	Propagules dispersed by other animals (internally)	?
C	8	Persistence	8.01	Prolific seed production	y
A		attributes	8.02	Evidence that a persistent propagule bank is formed (>1 yr)	y
A			8.03	Well controlled by herbicides	
C			8.04	Tolerates or benefits from mutilation, cultivation or fire	
E			8.05	Effective natural enemies present in India	

A=agricultural, E= environmental, C=combined; Total score - 15

Indian J. Plant Genet. Resour. 31(2): 170-178 (2018)
DOI 10.5958/0976-1926.2018.00020.7

Population Structure and Genetic Diversity of Wheat Landraces from North-western Indian Himalaya

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(Received: 30 November 2016; Revised: 07 December 2017; Accepted: 23 January 2018)

The present study was aimed at analyzing the genetic diversity of ninety six selected landraces from North-western Himalayan region using 21 microsatellite markers. The PIC value of STMS loci ranged from 0.011 (Xgwm383) to 0.746 (Xgwm369) with mean value of 0.34 for all loci. Total 51 alleles were detected with an average of 2.428 alleles per loci. Clustering of wheat landraces using three different algorithms gave the same kind of results. Lower inbreeding coefficient (F_{ST}) indicated low level of sub-population differentiation. Greater heterozygosity and gene flow among individuals were observed among wheat landraces. The partitioning of total molecular variance also showed similar, maximum variation was contributed by among individuals within populations (83.1%) followed by within individuals (15.12%) and the variation at among populations level was negligible (1.78%). It is concluded that observed number of alleles, effective number of alleles and Shannon's information index were greater in landraces of Kumaon division in comparison to other Himalayan region.

Key Words: Genetic Diversity, Landraces, Microsatellite, North-western Himalaya

Introduction

Triticum aestivum L. em Thell., commonly known as bread wheat is an important cereal globally and its cultivation had evolved with the expansion of major civilizations all over the world. Wheat is being traditionally grown in North-western parts of Indian Himalaya since ancient times. The Himalayan highlands are the reservoir for landraces because of the preponderance of locally developed traditional crop varieties owing to high agro-climatic heterogeneity and local socio-cultural diversity (Partap *et al.*, 2001). Though bread wheat is a globally important crop for human food and nutrition, genome wide molecular studies are very limited majorly because of its large genome size (~16×10^9 bp) and allohexapolyploidy comprising of three closely related genomes (A, B and D) with around 80% repetitive DNA (Gupta *et al.*, 1991; Bennett and Leitch, 1995). These genomic complexities also have been substantial barriers to analyze genetic diversity and population parameters in wheat germplasm. Studies on genetic diversity of economically important traits and population structure are important to conserve and enhance the germplasm use for crop improvement and other plant breeding activities (Uddin *et al.*, 2008). In today's scenario most of the rich plant biodiversity, particularly crop landraces, which sustained agriculture for the last 9000 years is on the verge of extinction due to the introduction of modern high yielding varieties (Brown and Brubaker, 2002). Wheat landraces have played a very important role in the local food security and sustainable development of agriculture as well their significance as genetic resources for wheat improvement. Though wheat breeding programs have made significant progress, use of very few elite germplasm lines as parental stock has led to a decrease in genetic diversity and has narrowed down the wheat genetic base (Hoisington *et al.*, 1999). Improved cultivars have such a great impact that wheat landraces from plains are almost vanished and now landrace diversity can be found only in hilly areas where climatic conditions are least suitable for cultivating high yielding varieties. Therefore, this study aimed at analyzing genetic diversity and population structure of important wheat landraces of Himalayan region of Uttarakhand and Himachal Pradesh. Moreover, there are very limited studies on wheat landraces from North-western Himalayan region. In the previous studies, morphological and agronomic traits as well as physiological indices were widely used for assessing genetic diversity, however these traits are influenced by environment. More recently, molecular markers

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have been increasingly exploited for diversity analysis. Therefore, Simple Sequence Repeats (SSRs), which are among the most versatile molecular markers, abundant, highly polymorphic, genome specific, co-dominant in nature and show a fairly even distribution over the genome (Choudhary *et al.*, 2016), have been used in this study to assess molecular diversity and other population genetics parameters in wheat landraces of Indian Himalayan region.

Materials and Methods

Plant Material and DNA Extraction

Wheat landraces were collected from fields of different regions of North-western Himalaya where considerable variability are being maintained. These landraces were further grown in field of ICAR-NBPGR Regional Station, Bhowali, Uttarakhand during 2013-14 *rabi* season. The seeds of these landraces were harvested and grown in greenhouse following standard agronomical practices to raise healthy plants during *rabi* season 2014-2015. A set of ninety six landraces were randomly selected for molecular diversity analysis (Table 1). Leaf samples of one month old plant were collected and stored at -80 °C for DNA extraction. Genomic DNA was extracted using the modified cetyltrimethyl ammonium bromide (CTAB) method (Saghai-Maroof, 1984). The quality and concentration of extracted DNA was estimated by Nano-drop (ND1000, Thermo Scientific, USA). DNA samples concentration was further checked in 0.8% agarose gel electrophoresis using known DNA concentration standards.

Microsatellite Genotyping

Total 21 polymorphic SSR primers were selected from 250 SSRs through polymorphic analysis on eight randomly selected wheat landrace accessions. Primers with multiple bands were also discarded to avoid conflict to decide upon allele whether alternate form is in the same genome or different genome, since wheat genome has multiple copies of similar genomes (A, B, D). These twenty one polymorphic SSR markers (Supplementary Table S1) are randomly distributed across the three genomes. DNA amplification was performed in a 10µl volume containing 20 ng of genomic DNA, 0.2 U DNA polymerase, 1.5 mM Mg, 0.25 mM dNTPs and 0.2 M primer. The PCR cycle consisted of an initial denaturation at 94 °C for 4.5 min, followed by 36 cycles of 94 °C for 55 sec, annealing at 50-60 °C (varied from primer to primer) for 55 sec, 72 °C for 55 sec, and a final extension step of 72 °C for 5 min before cooling at 4 °C. All PCR (polymerase chain reaction) amplifications were carried out in G-Storm thermocycler (Gene Technology Ltd UK). PCR products were electrophoresed in 3.5 % agarose gel.

Statistical Analysis

For estimating different population genetics parameters using PopGene (ver. 1.32 Yeh *et al.*, 2000) wheat landraces were grouped into three sub-populations based on their local niche environment *i.e.* Garhwal and Kumaon divisions of Uttarakhand and Himachal Pradesh. NTSYS-PC (ver. 2.11X; Exeter Software, N.Y., Rohlf, 2000) was used to prepare the dendrogram based on Nei genetic distance obtained from PopGene analysis. NTSYS-PC was also used for hierarchical clustering of wheat landraces based on UPGMA similarity matrix. DARwin version 6.0.10 (Perrier and Jacquemoud-Collet, 2006) was used to create weighted neighbor joining tree based on simple matching (SM) dissimilarity matrix. The Bayesian model-based clustering analysis was used for determining the optimal number of genetic clusters found among wheat landraces using the software STRUCTURE 2.3.4 (Pritchard *et al.,* 2000), which partitions individuals into number of clusters (K) based on the multi-locus genotypic data. The admixture model and correlated allele frequencies were applied for each run with 10,000 iterations and 100,000 Markov Chain Monte Carlo (MCMC) replications. The best K value (genetically distinct clusters) was defined using the Evanno *et al.* (2005) method. The individuals were arranged based on 'Q' (the estimated membership coefficients for each individual, in each cluster). The data set were also subjected to analysis for molecular variation (AMOVA) using Arlequin (ver. 3.5.1.2, Excoffier *et al.*., 2005). Population structure by AMOVA is based on an analysis of variance of gene frequencies, taking into account the number of mutational differences between molecular haplotypes. Fixation indices (Weir and Cockerham, 1984) and population pairwise F_{ST} (pairwise estimates of the correlation of alleles between populations) values were also computed by using the above software. Graphical software R version 3.5.1 (Rcmd function) was embedded with Arlequin version 3.5.1.2 software to generate graphical illustrations of results.

The polymorphism information content (PIC) value described by Botstein *et al.* (1980) and modified by

Table 1. Prominent wheat landraces used in the diversity analysis

S.No.	District	IC Number	Landrace name	S.No.	District	IC Number	Landrace name
Himachal Pradesh				Kumaon, Uttarakhand			
1	Chamba	IC381111	Sherwan	48	Almora	IC260848	Mishrygahun
2		IC381124	Gandham	49		IC260854	Mishrygahun
3		IC381190	Amreek Gandham	50		IC595395	Thanga gehun
4	Hamirpur	IC208899	Unknown	51	Bageshwar	IC260857	Dhudgahun
Garhwal, Uttarakhand				52		IC260858	Dhudia
5	Chamoli	IC260865	Gahun	53		IC266976	Thangi
6		IC260866	Gahun	54		IC266977	Bhati
7		IC260868	Gahun	55		IC266978	Rati
8		IC260869	Gahun	56		IC393109	Gehun
9		IC260871	Gahun	57		IC393110	Gehun
10		IC260877	Gahun	58		IC393116	Gehun
11		IC260890	Chudi Gahun	59		IC393117	Gehun
12		IC383581	Lakha Gainhu	60		IC393118	Gehun
13		IC383592	Wheat	61		IC398292	Lal Gehun
14		IC383593	Lakha Gainhu	62		IC398294	Juinsi Ninsa
15	Dehradun	IC345589	Ghaon	63		IC398296	Setta
16		IC345598	Ghawn	64		IC398297	Tank
17		IC345604	Ghaon	65		IC398298	Sathi
18		IC345620	Ghaun	66		IC398302	Thaenkna
19		IC345671	Gahoun	67		IC398303	Chini
20		IC345673	Gahoun	68		IC398305	Munnar
21		IC345687	Gahoun	69		IC398307	Thull
22		IC345688	Gahoun	70		IC398309	Safed Jhusial
23		IC345690	Gahoun	71	Champawat	IC266764	Gehun
24	Pauri	IC430330	Gahun	72		IC266789	Jhausa
25		IC430369	Donia	73		IC266791	Gerua
26		IC430373	Chudiya	74		IC392578	Guhu
27		IC564090	Mundari	75		IC406688	Mishri Gehun
28		IC564096	Bareek lal	76		IC406690	Jusia Gehun
29		IC564113	Lal mundiya	77		IC406715	Dolat kani
30		IC564114	Safed mundiya	78		IC406724	Safed Gehun
31		IC564159	Safed mundari	79		IC595382	Ratuva gehun
32	Rudraprayag	IC260880	Gahun	80	Nainital	IC260845	Jhusia
33		IC260887	Mundarigaun	81		IC573137	Ryat gaun
34		IC260888	Gahun	82		IC573138	Syat gyan
35		IC260894	Lal Gahun	83		IC573140	Chnosi
36		IC260895	Lal Gahun	84		IC573157	Munda
37		IC260901	Gahun	85	Pithoragarh	IC266831	Munara
38		IC260902	Gahun	86		IC266847	Gerua
39		IC382649	Cheuri	87		IC266852	Dudh Gehun
40		IC382653	Muneri	88		IC266854	Unknown
41		IC382658	Muneri	89		IC266872	Dapati Gehun
42		IC382664	Deshigenhun	90		IC266884	Mota gehun
43		IC393131	Gehun	91		IC266921	Mota Gehun
44	Tehri	IC589303	Baniya gehun	92		IC406697	Mundia
45	Uttarakashi	IC589276	Hasia Gehun	93		IC444217	Daapti Gehun
46		IC589278	Lal mishri	94		IC444226	Raje Gehun
47		IC589300	Lal Mishri Gehun	95		IC444229	Bhotta Gehun
				96		IC444232	Bhotia Gehun

Anderson *et al.* (1993) for self-pollinated species was calculated as follows:

$$PIC_i = 1 - \sum_{j=1}^{n} P_{ij}^2 PIC_i = 1 - \sum_{j=1}^{n} P_{ij}^2$$

where, P*ij* is the frequency of the *j*th allele for the *i*th marker, and summed over n alleles. PIC is also an estimate of the discriminatory power of a SSR marker locus.

Results and Discussion

Landraces are naturally a rich genetic resource evolved along with continuous selection against various adverse biotic and abiotic stresses, and yield parameters over long period of time. These are the major source of trait specific genes for crop improvement and broadening the wheat genetic base. The present study was concentrated on finding out the genetic diversity, allelic richness, allelic abundance and many other important population parameters in randomly selected wheat landraces from Himalayan region. Our findings revealed that significant genetic diversity exists in wheat landraces of the region.

Clustering of Wheat Landraces

Cluster analysis based on different algorithms (Figures 2 & 3) revealed that genetic differentiation in these wheat landraces was not the effect of geographical distance. This may probably be because of isolation by distance is not enough to hinder gene flow among landraces of different regions. This result reveals that sampling distance for wheat landraces can be further increased and tested to distinguish among wheat landraces based on their sampling distance. This information also indicated that by increasing both the number of samples collected from a single sampling site and distance between sampling sites can maximize efficiency of sampling with maximum collected diversity.Wheat landraces were also clustered based on the Bayesian model-based clustering analysis using the software STRUCTURE 2.3.4 (Pritchard *et al.,* 2000). The programme partitions individuals into number of clusters (K) based on the multi-locus genotypic data. Individuals in the sample are probabilistically assigned to populations, or jointly to two or more populations if their genotypes indicate that they are admixed (Figures 3, 4 and 5). All ninety six landraces were grouped into four major clusters (K = 4). Substantial amount of admixture among the wheat landraces at genetic level was observed, which was in accordance with the results obtained by the above clustering methods. Overall, if we look at all the three methods of clustering with different algorithms

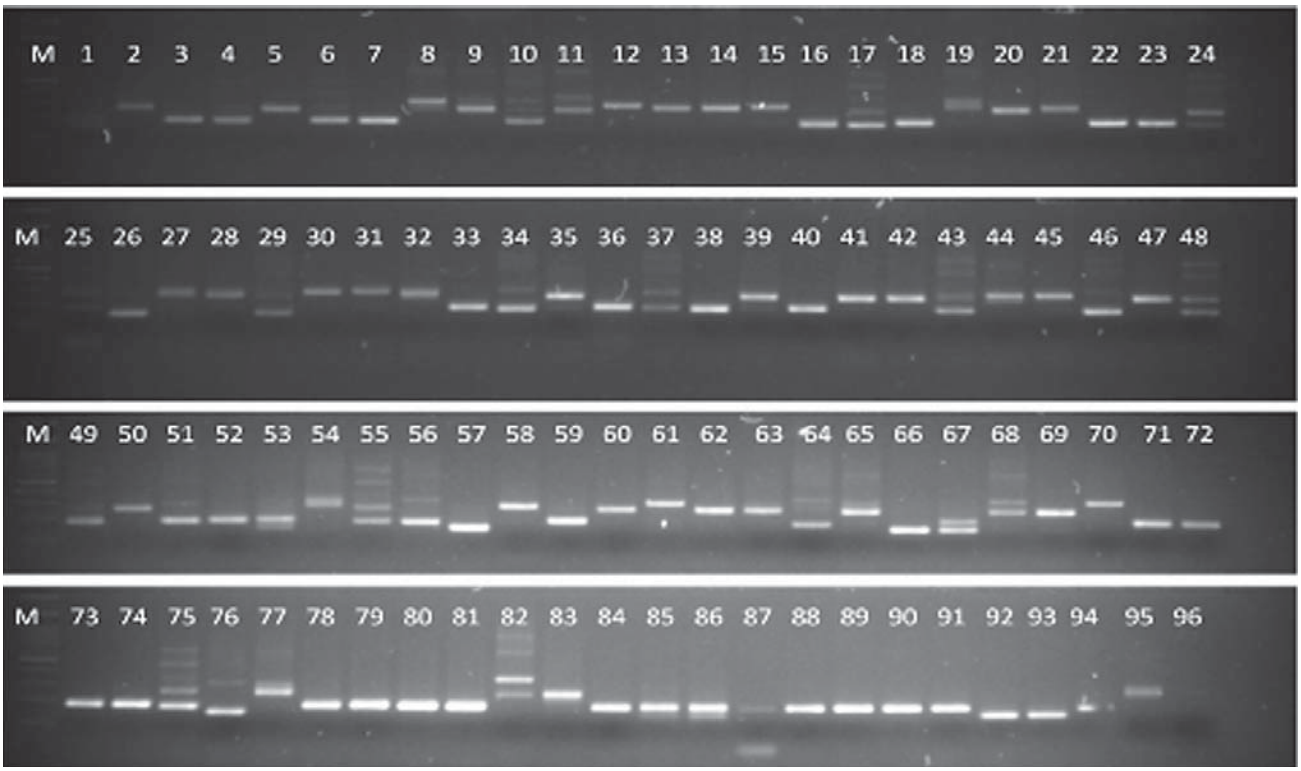

Fig. 1. SSR primer (Xgwm-369) profile of wheat landraces obtained using agarose gel electrophoresis. (M= 50bp marker; wheat landraces are labelled from 1 to 96 as given in the Table No. 1)

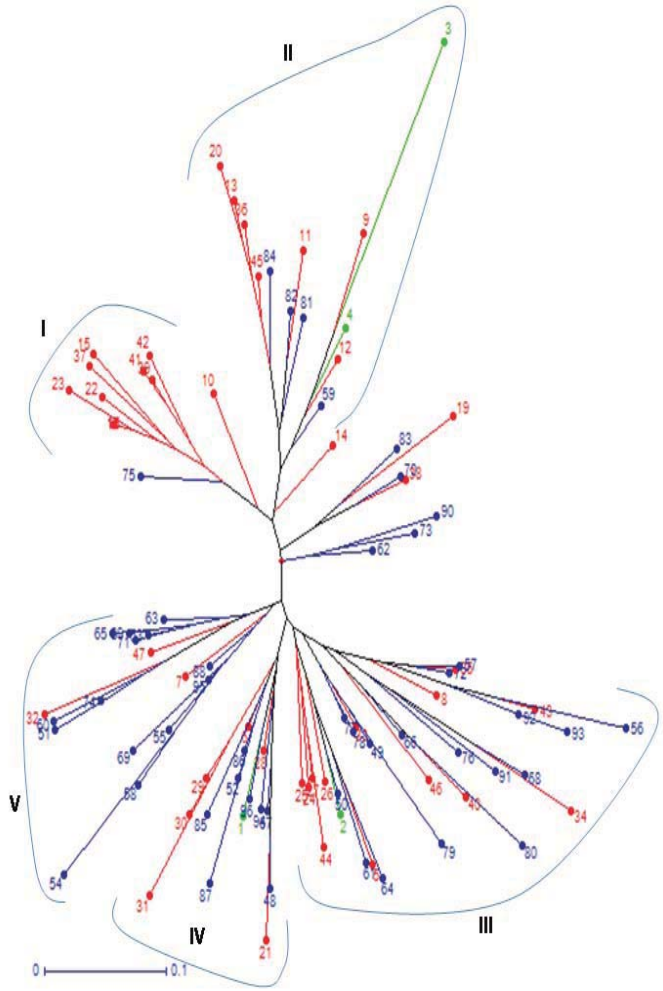

Fig 2. Unrooted neighbour joining tree generated by DARwin v. 6.0.10. Colour indicates geographical location [green: H.P. (1-4); red: Garhwal division (5-47); blue: Kumaon division (48-96)]. The numerical values representing the different landraces are the serial number of the Table 1.

and population genetic parameters, similar pattern was revealed, which strengthen the results. Though different clustering methods from software like STRCUTURE, NTSYSpc and DARwin have grouped the landraces in distinct clusters, but the individual populations were distributed randomly irrespective of their geographical locations. After partitioning the variation in different categories, the AMOVA results also indicated that major source of variation is individual genotypes not populations defined based on their geographical locations (Table 4). The pairwise F_{ST} estimates also suggest the same i.e. divergence at population level is negligible (Table 3).

Allelic Diversity

The microsatellite molecular markers were selected for genotyping of landraces in this study considering their high polymorphism, specificity (Pestova *et al.*, 2000), reproducibility (Stachel *et al.*, 2000) and high variability (Brown *et al.*, 1996). Genotyping and analysis of wheat landraces differentiated the intra and inter-population diversity and confirmed the robustness of microsatellite loci for genetic diversity studies of wheat landraces (Figure 1) reported in earlier studies (Plaschke *et al*., 1995; Eujayl *et al*., 2002; Maccaferri *et al*., 2005; Arora et al 2014). Number of private alleles specific to particular populations was very low (Supplementary Table S2), which indicates that the evolutionary period for population divergence after separation from recent common ancestor of the wheat landraces is very short or genetic divergence in bread wheat might be slow. Rare alleles (frequency < 0.05%) are those alleles which got introduced in to the population in very recent past

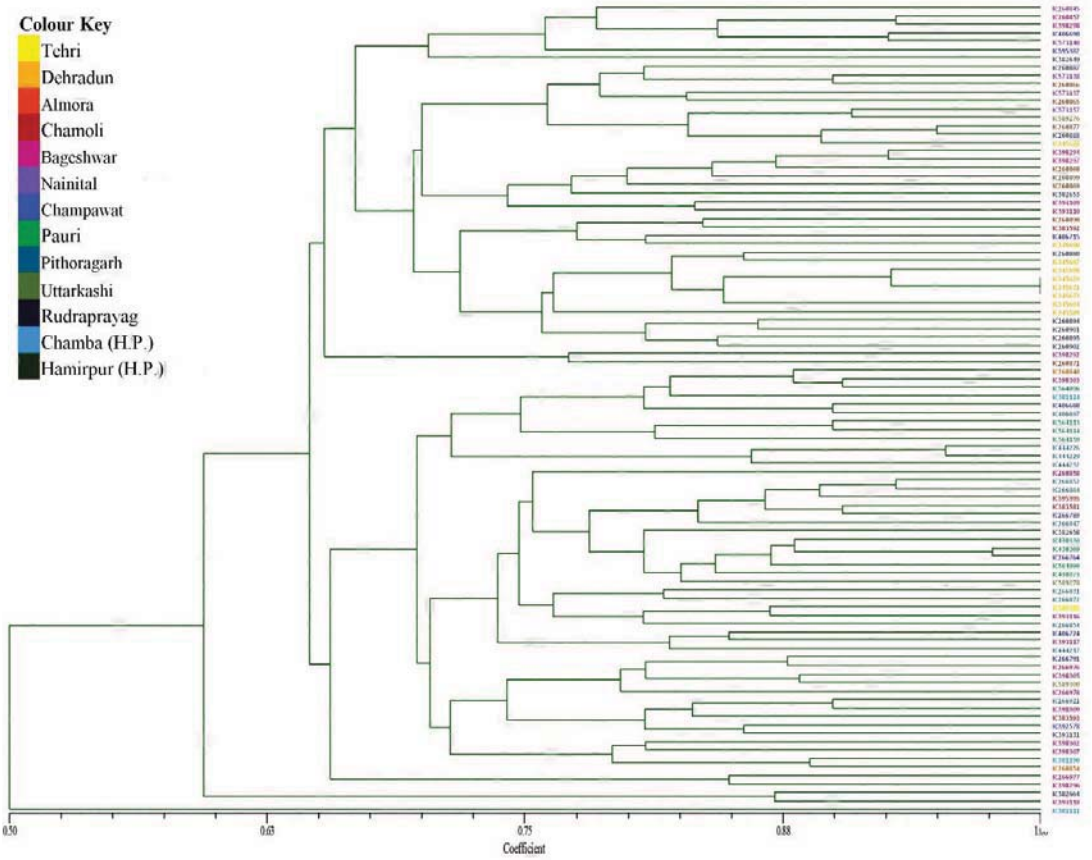

Fig. 3. SAHN dendrogram based on UPGMA clustering method from similarity matrix of DICE coefficient generated by NTSYSpc v. 2.11X

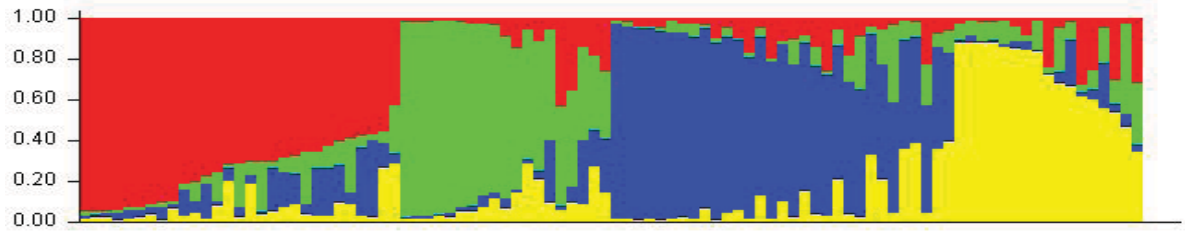

Fig. 4. Population structure of 96 wheat landraces for k=4 in a single line plot using STRUCTURE ver. 2.3.4 software. Four different colours represent four sub-populations of wheat identified with each bar representing the estimated membership (0 - 1) of single genotype in each of the four clusters

(Supplementary Table S2). Rare alleles may get chance to established or lost based on its functionality and role in fitness of the individual. Moderate number of rare alleles (18) has been observed, indicating that the genotypes are dynamically evolving in their local niches with moderate level of selection forces (Supplementary Table S2).

Effective number of alleles (Ne) which measures the number of equally frequent alleles that would take to achieve a given level of gene diversity (expected heterozygosity), allows us to compare populations where the number and distributions of alleles differ drastically, because it is measured locus by locus rather than by mean gene diversity. In simple term, when the heterozygosity is high, effective number of alleles is also high. Therefore, it measures the distribution of allele frequencies among alleles of the same locus, equal distribution leads to highest Ne. From the analysis, lower level of Ne has been observed for wheat landraces from all the regions (Table 2). Shannon's information

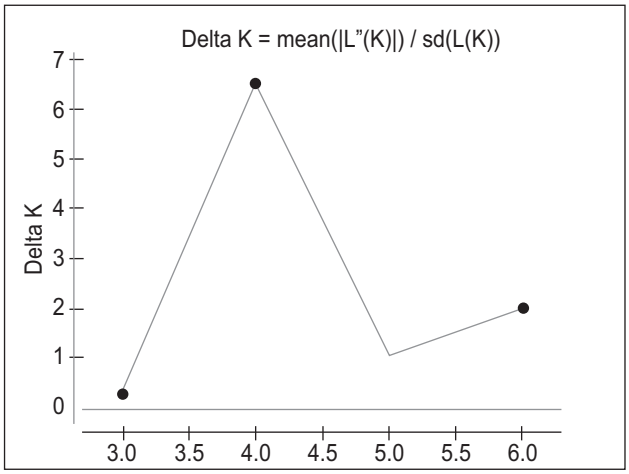

Fig. 5. Best K was determined by Evanno *et al.*, 2005 method. K value is plotted against delta K. K value at highest delta K is selected as best K which is 4.

index (I) is an important measure of diversity similar to Ne. The highest value for 'I' is obtained when each allele is present only once in the entire sample being measured; it penalizes redundancy at the allelic level, with respect to the entire sample. Therefore, this index is important when rare alleles are abundant and are of much importance for selecting a genotype especially in developing core or mini-core. Shannon information index also estimated lower for these landraces (Table 2). Level of Levene's (1949) expected heterozygosity, Nei's (1973) expected heterozygosity and average expected heterozygosity was observed similar for all landraces irrespective of their geographic location which further fit with the above results (Table 2). Comparatively observed number of alleles, effective number of alleles, and Shannon's information index were greater in landraces of Kumaon division in comparison to landraces from H. P. and Garhwal Division, even though Kumaon and Garhwal division populations grouped together and H. P. population grouped separately probably because of heterozygosity level within populations across loci.

Population Differentiation

Lower F_{ST} indicated the very low level of sub-population differentiation and existence of substantial level of heterozygosity reflects the gene flow among individuals might be through inbreeding or admixture among populations. Gene flow (Nm) value lesser than one indicates restricted gene flow among populations as per Wright (1965). Higher level of gene flow (2.36) among populations explains existence of higher variability with low level of sub-population differentiation (Table 2). The same also has been established from previous studies that level of population differentiation is inversely proportional to the magnitude of gene flow among sub-populations (Díaz-Matallana *et al.*, 2009; Wang *et al.*, 2012; Abouzied *et al.*, 2013).The pairwise F_{ST}

Table 2. Overall summary of genetic variation and heterozygosity statistics across the loci

	Allelic variation across the loci			Heterozygosity across the loci		
	Parameters	Mean	St. Dev	Parameters	Mean	St. Dev
Landraces from Himachal Pradesh	na	1.76	0.77	Obs Het	0.02	0.08
	ne	1.56	0.56	Exp_Het	0.32	0.27
	I	0.43	0.38	Nei	0.28	0.24
Landraces from Garhwal Division	na	1.76	0.77	Obs Het	0.06	0.08
	ne	1.56	0.56	Exp_Het	0.34	0.25
	I	0.43	0.38	Nei	0.33	0.24
landraces from Kumaon Division	na	2.38	0.74	Obs Het	0.04	0.06
	ne	1.72	0.78	Exp_Het	0.32	0.26
	I	0.52	0.41	Nei	0.32	0.25
Over all	na	2.43	0.68	Obs Het	0.05	0.06
	ne	1.77	0.78	Exp_Het	0.34	0.25
	I	0.55	0.4	Nei	0.34	0.25
	FST	0.1				
	Nm*	2.36				

na = Observed number of alleles; ne = Effective number of alleles [Kimura and Crow (1964)]; I = Shannon's Information index [Lewontin (1972)], Observed heterozygosity; Expected heterozygosity was computed using Levene (1949); Nei's (1973) expected heterozygosity, $F_{ST=}$ inbreeding coefficient (population structure at subpopulation to total population), Nm = Gene flow estimated from $F_{ST} = 0.25(1 - F_{ST})/F_{ST}$.

Table 3. Population pair wise F_{STs}

	Himachal Pradesh	Garhwal Division	Kumaon Division
Himachal Pradesh	0.0000		
Garhwal Division	0.04955	0.0000	
Kumaon Division	0.05190	0.03113	0.0000

(inbreeding coefficient total to sub-grouped individuals) comparison among populations was done to measure population's divergence. F_{ST} was recorded very low to negligible indicating the no or very less divergence among populations grouped based on the geographical origin of landraces (Table 3).

Analysis of Molecular Variance (AMOVA)

For assessment of source of variation among landraces total molecular variance was partitioned in categories *viz*. among populations, among individuals within populations and within individuals. The results revealed maximum variation was contributed by among individuals within populations (83.1%) followed by within individuals (15.12%). The variation level at among populations was negligible (1.78%) (Table 4). Management of landrace diversity by the farmers has been conferring vitality and viability to traditional agricultural systems. The dominance of only a few wheat varieties in production system poses a major threat to the genetic diversity of the crop. As Himalayan highlands are the reservoir for wheat landraces due to preponderance of locally developed traditional crop varieties owing to high agro-climatic heterogeneity, this study was taken up as a model in Uttarakhand and its adjoining parts of HP. The landraces in the region are being managed by subsistence growers that possess lower risk of failure under marginal production environment and are also key to food and nutritional security of isolated communities. Large extent of variation and low level of differentiation was observed among the landraces studied. These landraces can be a potential resource for trait specific crop improvement and as well in restoring lost wheat genetic base by germplasm enhancement activities.

Acknowledgements

Authors are grateful to ICAR-National Bureau of Plant Genetic Resources, New Delhi for providing the facilities to carry out the present study. We are thankful to the concerned scientists and technical staff involved in collection and maintenance of wheat landraces at ICAR-NBPGR Regional Station, Bhowali, Uttarakhand.

Table 4. Analysis of Molecular Variance (AMOVA)

Source of Variation	d.f.	Sum of squares	variance components	Percent of Variation
Among Populations	2	17.686	0.05761 Va	1.78
Among individuals within populations	93	546.132	2.69140 Vb	83.1
Within individuals	96	47	0.48958 Vc	15.12
Total	191	610.818	3.23859	
Fixation Indices	FIS : 0.84609 FST : 0.01779 FIT : 0.84883			

The senior author is also grateful to the Department of Science and Technology (GOI), New Delhi for award of INSPIRE fellowship.

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Indian J. Plant Genet. Resour. 31(2): 179-185 (2018)
DOI 10.5958/0976-1926.2018.00021.9

Consumer Preference: Crucial in Determining the Grain type-richness in Rice Varieties Grown across Kerala, India

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(Received: 30 November 2016; Revised: 08 May 2017; Accepted: 15 June 2017)

Popularity, preference and acceptability of a variety are dependent on grain qualities among other aspects. With emphasis on development of rice varieties with consumer acceptable grain qualities is gaining momentum, an attempt was made to assess the variability in grain characteristics of traditional as well as high yielding rice varieties grown across Kerala. The analysis of variance revealed existence of highly significant differences among the genotypes for all the grain characteristics studied. Low influence of environment on trait expression was evident from narrow difference observed between phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV). The results of cluster analysis and Principal Component Analysis (PCA) proved that the varieties were diverse in their grain characteristics. The cluster analysis indicated remarkable homogeneity among the traditional rice varieties for the grain characteristics evaluated. The cooking parameters were best expressed by the short-grain high yielding variety Varsha.

Key Words: Amylose content, Cooking quality, Elongation ratio, Grain characteristics

Introduction

Rice (*Oryza sativa* L.) is the most important cereal crop in India and Kerala in particular. The diverse rice agro-ecological niches in Kerala demands cultivation of an array of varieties. Until recently, development of varieties with high yield, pest and disease resistance was of prime importance. In view of these considerations, several high yielding varieties have been developed. However, in recent times a major shift in focus has occurred in rice breeding programmes. Emphasis on quality as well as quantity of rice produce is being stressed. Development of varieties with preferable cooking qualities and consumer acceptance is gaining momentum.

Commercially, grain size, shape, kernel colour and appearance are important physical features that determine both market value and consumer preference of rice. The choice of a variety is highly dependent on its cooking quality, grain colour, aroma and grain yield along with other grain quality attributes. White kernelled fine grained rice is widely consumed throughout India. However, unlike in other parts of India, the preference of rice grain type in Kerala differs significantly. Keralites prefer bold, red kernelled and non-sticky parboiled rice. The grain colour of rice varieties grown in Kerala vary from straw gold to brown tinged to complete brown to black and grain shape varies from round to short bold to long bold to long slender kernelled grains (Leenakumary, 2011). The red rice preferred by Keralites undergoes minimal milling and polishing.

Success in crop improvement generally depends on the magnitude of genetic variability and the extent to which the desirable characters are heritable (Vanaja and Babu, 2006). Heritability and genetic advance are important selection parameters in plant breeding. Heritability estimates along with genetic advance are generally more helpful in predicting the gain under selection than heritability estimates alone (Bisne *et al.,* 2009). Grouping or classification of genotypes based on suitable scale is quite imperative to understand the usable variability existing among them (Shahidullah *et al.,* 2009). Thus cluster analysis that groups the genotypes based on standardized euclidean distance and ordination Principal Component Analysis (PCA) are highly informative. Genetic diversity along with the information on genetic parameters of the traits helps in deciding the selection criteria for generating valuable

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genotypes (Majumder *et al*., 2011). The traditional and high yielding rice varieties grown in the varied agro-ecological niches of Kerala is a rich cafeteria of variability with respect to morphological, physico-chemical and organoleptic qualities. However, the rich diversity of forms available in the rice germplasm of Kerala has been evaluated only on a limited scale. Hence, as a prerequisite towards breeding for improved grain attributes, the present study was undertaken to assess the variability and phylogenetic diversity among varieties grown across Kerala with respect to grain quality and cooking characteristics.

Materials and Methods

A varietal evaluation trial with fifty rice varieties was laid out in a randomized block design with two replications at the Department of Plant Breeding and Genetics, College of Horticulture, Vellanikkara, Kerala Agricultural University (KAU). The varieties included thirtyseven high yielding varieties and thirteen traditional rice varieties grown across Kerala. Variety Jarwa from Andaman and Nicobar Islands was included as a check to elucidate the correctness of grouping of varieties based on distinctness. Good agronomic management of the crop was done as per standard package of practices recommendation (KAU, 2011).

The grain characteristics were recorded on two random samples drawn from a seed lot of ten plants selected at random in each replication of an entry. Observations on grain length (GL), grain breadth (GB), GL/GB, kernel length (KL) and kernel breadth (KB) were measured using grain caliper on ten representative grains from each of the ten selected plants. 1000 grain weight, grain density (GD), elongation ratio (ER), volume expansion after cooking (VE) and amylose content were determined as per standard procedure (Shobha Rani *et al.*, 2004). Freely downloadable OPSTAT Package developed by CCS Haryana Agricultural University, Hisar, India was used for statistical analysis (http://www.hau.ernet.in/opstat.html). Clustering of genotypes and principal component analysis (PCA) were done using software Windostat Version 9.2 from Indostat Services, Hyderabad, India.

Results and Discussion

The analysis of variance revealed the presence of highly significant differences among the genotypes for all the characters studied (Table 1), which might be due to diverse source of materials as well as environmental influence affecting phenotypes. Table 2 Summarises the mean values recorded for the characters studied. Grain length varied between 6.36 mm (Chomala) and 9.56 mm (Sulochana) with a mean value of 7.9 mm while grain breadth ranged from 1.79 (Chomala) to 3.28 mm (Samyuktha) with a mean value of 2.55 mm.

According to DUS descriptor for rice (Shobha Rani *et al.*, 2004), grains can be classified as very short (<6.0 mm), short (6.1-8.5 mm), medium (8.6-10.5 mm), long (10.6-12.5 mm) and very long (>12.5 mm) based on grain length and as very narrow (<2.0 mm), narrow (2.1-2.5 mm), medium (2.6-3.0 mm), broad (3.1-3.5 mm) and very broad (>3.5 mm) as per the grain breadth. In accordance with this classification, 44 genotypes were of short and seven medium grain type while three genotypes were very narrow, 26 narrow, 21 medium and one broad grain type. This point out that during crop improvement efforts undertaken, selection for grain type was by far unidirectional, focussing on bold seeded grain type in tune with the grain type preference of Keralites. Grain length to breadth ratio was maximum for Sulochana (4.05) and minimum for Samyuktha (2.29) with a mean value among varieties being 3.13. The value of kernel length ranged from 2.75 mm (Varsha) to 5.98 mm (Aathira) with a mean value of 4.53 mm. Kernel breadth exhibited a mean value of 2.01 mm with a range of 1.25 mm (White Ponni) to 2.77 mm (Navara 11-2). Based on kernel length and kernel length to breadth ratio, the varieties were classified following Shobha Rani

Table 1. Analysis of variance for grain quality characters of rice varieties

		Mean sum of squares									
Source of Variation	DF	GL (mm)	GB (mm)	GL/GB	GD (g/mm^3)	TGW (g)	KL (mm)	KB (mm)	VER	ER	AC (%)
Replication	1	0.102	0.005	0.052	0.062	0.00003	0.114	0.002	0.004	0.012	0.038
Treatments	50	0.816**	0.156**	0.292**	0.274**	0.225**	0.865**	0.186**	0.880**	0.103**	17.839**
Error	50	0.032	0.003	0.029	0.016	0.00083	0.038	0.009	0.125	0.005	0.329

DF= Degrees of freedom; ** Significant at 1% level

GL= Grain length; GB= Grain breadth; GD= Grain density; KL= Kernel length; KB= Kernel breadth; TGW= 1000 grain weight; AC= Amylose content; VER= Volume expansion ratio; ER= Elongation ratio

Table 2. Mean performance of rice genotypes for grain characteristics

S. No	Varieties	GL (mm)	GB (mm)	GL/ GB	GD (g/mm^3)	KL (mm)	KB (mm)	VER	ER	AC (%)	TGW (gm)
1	Vaisakh	8.87	3.11	2.86	0.78	3.65	2.24	4.00	2.01	16.65	28.40
2	Uma	7.20	2.71	2.66	1.06	3.61	2.26	2.33	2.00	16.14	25.00
3	Jyothy	9.09	2.63	3.45	0.93	5.32	2.02	2.00	1.51	15.53	28.50
4	Aiswarya	8.72	2.57	3.39	1.19	4.61	2.10	1.67	1.64	17.82	28.10
5	Harsha	7.76	2.57	3.02	1.08	3.99	2.17	1.88	2.03	22.50	24.10
6	Annapoorna	8.32	2.44	3.46	0.80	3.98	2.12	2.21	1.79	13.57	24.90
7	Matta Triveni	8.32	2.70	3.09	0.75	4.52	2.06	2.04	1.66	17.68	27.10
8	Swarnaprabha	8.77	2.75	3.19	0.90	4.85	2.09	3.75	1.75	20.87	26.20
9	Samuyktha	7.34	3.28	2.30	0.90	5.04	2.36	4.00	1.76	17.54	23.70
10	Kairali	8.30	2.48	3.34	1.29	4.23	1.85	2.04	1.85	13.29	24.20
11	Jayathi	7.74	2.51	3.08	0.88	4.09	2.14	2.13	1.98	14.60	23.70
12	Kanchana	7.82	2.58	3.03	0.70	4.80	1.78	1.88	1.64	14.92	28.80
13	Triveni	8.98	2.54	3.53	0.67	5.00	1.95	1.93	1.72	15.77	28.30
14	Makom	7.94	2.28	3.48	0.54	4.42	1.67	1.63	1.68	11.51	23.80
15	Varsha	6.76	2.77	2.44	0.99	2.75	2.31	4.08	2.31	16.75	25.90
16	Thekkencheera*	7.72	2.61	2.96	0.54	3.74	2.16	1.92	1.87	14.88	22.20
17	Cheriyaaryan*	8.24	2.62	3.15	0.97	3.93	2.28	2.28	1.84	15.58	27.80
18	Ahalya	7.33	2.49	2.94	0.80	3.47	2.24	1.50	2.07	15.67	24.10
19	Parambuvattan*	7.75	2.37	3.28	1.00	4.25	2.20	1.88	1.71	16.80	26.60
20	Aruna	7.67	2.53	3.04	0.81	4.11	1.93	1.92	1.83	13.94	27.90
21	Onam	7.32	2.33	3.14	0.92	4.94	1.87	1.68	1.51	20.21	25.00
22	Revathi	7.36	2.24	3.29	0.92	5.26	1.62	1.38	1.41	13.85	24.20
23	Jarwa	8.00	2.63	3.04	0.89	5.69	1.94	3.13	1.67	17.59	24.20
24	Ponmani	6.81	2.51	2.72	0.66	4.44	2.12	3.19	1.52	10.71	21.50
25	Mahsoori	8.00	2.00	4.05	0.90	4.80	1.62	1.83	1.70	11.32	16.70
26	MangalaMahsoori	7.55	2.04	3.71	0.85	5.21	1.67	1.84	1.36	22.41	18.80
27	Nila	7.18	3.09	2.33	0.61	4.81	2.64	2.57	1.44	21.33	22.90
28	Aathira	7.81	2.65	2.95	0.86	5.98	2.32	1.63	1.33	18.06	26.20
29	Anaswara	7.70	2.68	2.88	0.66	4.96	2.10	2.13	1.51	22.04	24.70
30	White ponni	7.47	1.95	3.83	0.82	4.57	1.24	1.88	1.54	10.67	16.80
31	Thulasi	7.65	2.99	2.56	0.79	5.53	2.42	2.29	1.33	15.49	24.20
32	Sulochana	9.57	2.36	4.06	1.55	3.47	1.94	2.38	1.23	14.83	24.00
33	KAU-M-87-1	7.21	2.52	2.86	1.45	4.85	1.98	2.00	1.45	13.33	23.40
34	Krishnanjana	8.29	2.42	3.43	0.69	5.23	2.12	1.63	1.50	19.13	24.90
35	M-20	8.44	2.21	3.82	0.82	5.56	1.74	1.54	1.41	13.89	21.70
36	Karthika	7.08	2.37	2.99	1.30	4.79	1.98	1.63	1.43	17.82	20.40
37	Remanika	7.43	2.46	3.02	0.78	5.17	2.09	1.48	1.36	18.06	23.40
38	Prathyasa	7.73	2.67	2.89	1.13	5.37	2.04	1.88	1.50	21.80	26.80
39	Arimodan*	8.23	2.44	3.38	1.06	4.38	1.43	1.88	1.70	14.55	26.60
40	Thottacheera*	8.30	2.74	3.03	0.94	4.22	2.29	1.50	1.66	14.32	27.70
41	Kalladiaryan*	7.91	2.82	2.81	0.89	3.75	2.48	2.14	1.83	17.08	27.30
42	Navara 11-2	8.23	2.77	2.98	1.56	4.27	2.77	1.63	1.71	11.74	29.20
43	Kunjukunju Varna	7.56	2.58	2.93	0.98	3.69	1.80	1.75	1.95	15.91	24.50
44	Paramban Kayama*	8.86	2.80	3.17	1.03	4.54	2.16	1.88	1.74	20.12	31.70
45	Chuvannamodan*	8.69	2.60	3.35	1.01	4.49	1.82	1.97	1.82	16.00	27.70
46	Chomala*	6.36	1.80	3.54	3.04	4.37	1.69	1.63	1.38	18.29	15.20
47	Karuthamodan*	7.79	2.48	3.14	1.08	5.38	1.48	1.67	1.40	18.15	26.90
48	Karanavara*	8.44	2.81	3.01	0.98	4.31	1.77	2.20	1.74	16.94	29.50
49	Karuthadukkan*	7.85	2.68	2.93	0.93	4.30	1.79	1.70	1.70	14.41	27.90
50	Kunjukunju*	7.62	2.35	3.25	0.86	4.69	1.58	2.67	1.71	15.02	26.60

*= Traditional varieties

GL= Grain length; GB= Grain breadth; GD= Grain density; KL= Kernel length; KB= Kernel breadth; TGW= 1000 grain weight; AC= Amylose content; VER= Volume expansion ratio; ER= Elongation ratio

et al. (2004). Six varieties namely Revathy, Mangala Mahsoori, White Ponni, M-20 in addition to traditional rice varieties Arimodan and Karuthamodan possessed short slender grains (having KL<6 mm and KL/KB ratio>3.0). Eleven varieties including traditional rice varieties Chomala and Kunjukunju were medium slender (KL< 6 mm and KL/KB 2.5-3.0) while the remaining 34 varieties (66.67%) were of short bold type (KL< 6 mm and KL/KB< 2.5). Eight out of the 13 traditional varieties in the study possessed short bold kernels. This may be the consequence of selection by the farmers for the bold seeded type of grains.

The range of variation for grain density ranged between 0.54 (Thekkencheera) to 3.04 (Chomala) with a mean value of 0.97. The mean value for test weight of grains (1000 seeds weight) ranged from 15.2 g (Chomala) to 31.65 g (Parambankayama). Based on 1000 grain weight, the genotypes can be classified as very low (<15.0 g), low (15-20 g), medium (21-25 g), high (26-30 g) and very high (>30 g). Hence, there were five genotypes under the class low, 23 under medium class, 21 under high and one genotype under very high class.

During cooking, rice kernels absorb water and increase in volume through increase in length or breadth (Hogan and Plank, 1958). The physical characteristics of kernel recorded after cooking were volume expansion ratio (VER) and elongation ratio (ER). The Volume Expansion Ratio (VER) ranged from 1.38 (Revathy) to 4.08 (Varsha) with a mean value of 2.10. Kernel elongation ratio on cooking is considered as a desirable character in most of the rice consuming areas in the world. The range of variation for ER was 1.33 (Sulochana) to 2.31 (Varsha) with a mean value of 1.66.

Consumer preference for rice around the world, when compared as intact grain, is largely dependent on a desire for its cooked texture to be either firm or non-sticky or soft and sticky (Abeysekera *et al*., 2008). Amylose content is the key determinant of the different cooking, sensory and processing properties of rice (Chen *et al*., 2008). Cooked rice becomes moist and sticky due to low amylose content. Based on the Amylose content, rice can be classified into very low amylose content (3-9%), low amylose content (10-19%), medium amylose content (20-25%) and high amylose content (26-30%) (Shobha Rani *et al*., 2004). In the present investigation, 42 varieties possessed low amylose (10-19%) while remaining eight were medium (20-25%) for amylose content.

The genetic parameters for grain quality characters of 50 rice varieties are enumerated in Table.3. PCV was greater than GCV for all the traits studied while the difference between the two was low pointing to influence of environment on trait expression, though at a lower magnitude. Similar results were reported by Rathi *et al*. (2010), Subbaiah *et al*. (2011) and Pathak *et al*. (2016). According to Johnson *et al.* (1955), a combined interpretation of the estimates of PCV, GCV, heritability and genetic gain prove to be more useful in a breeding programme rather than an individualistic approach. The low per cent of PCV (8.23) and GCV (7.91) coupled with high heritability (92.39) but moderate genetic gain (15.67) registered by grain length suggested that the trait can be improved by intermating superior genotypes of segregating population developed from combination breeding as reported by Samadia (2005).

Similar to grain length, grain breadth had also registered high heritability value (76.16) and moderate genetic gain (18.28 %) but only moderate PCV (11.66) and GCV (10.17). This suggests that these traits are primarily under non-additive genetic control and therefore simple selection based on their phenotypic performance alone will not achieve the desirable improvement in these traits. Hybridization followed by selection in the resulting progenies would prove more useful in this case. In contrast to the study, Rathi *et al.* (2010) had recorded high heritability with low genetic advance for this trait.

Genetic gain is a valuable indication to the possible improvement in a trait that can be expected from a breeding programme. All traits except grain length and breadth registered high genetic gain while these two traits had exhibited moderate genetic gain. The results were in consonance with the findings of Vanaja and Babu (2006) for GL/GB ratio, amylose content and volume expansion ratio. Devi *et al.* (2016) reported high heritability estimates coupled with moderate genetic gain for kernel length and width. In contrast to the above findings Pathak *et al.* (2016) had reported low genetic gain for grain length and breadth.

Assessment of diversity in a population is essential for an attempt for varietal improvement. The grain characteristics of 50 rice genotypes were subjected to multi-variate analysis. Cluster analysis of the eleven

Table 3. Statistical and genetic parameters for grain characteristics of 50 rice varieties under study

Traits	Range		Mean	PCV (%)	GCV (%)	h^2 (%)	Genetic advance	Genetic gain (%)
	Minimum	Maximum						
GL (mm)	6.36	9.57	7.90	8.24	7.92	92.40	1.24	15.68
GB (mm)	1.79	3.28	2.55	11.66	10.17	76.16	0.47	18.29
GL/GB	2.30	4.06	3.13	12.80	11.56	81.55	0.67	21.50
GD (g/mm^3)	0.54	3.04	0.97	39.09	36.88	89.01	0.70	71.68
KL (mm)	2.75	5.98	4.54	14.81	14.17	91.52	1.27	27.92
KB (mm)	1.24	2.77	2.01	15.57	14.77	90.02	0.58	28.88
VER	1.48	4.08	2.10	33.74	29.25	75.17	1.10	52.25
ER	1.23	2.31	1.67	14.00	13.28	89.98	0.43	25.94
AC (%)	10.67	22.50	16.34	18.44	18.11	96.37	5.98	36.62
TGW (g)	15.20	31.65	25.04	13.43	13.38	99.26	0.69	27.45

GL= Grain length; GB= Grain breadth; GD= Grain density; KL= Kernel length; KB= Kernel breadth; TGW= 1000 grain weight; AC= Amylose content; VER= Volume expansion ratio; ER= Elongation ratio

physico-chemical characters (Table 4) based on standardised euclidean distance indicated the presence of five clusters. The analysis permitted separation of red kernelled medium slender grained traditional variety Chomala (Cluster V) from the rest of the genotypes. The cluster II was also a monogenotypic cluster, comprised of variety Jarwa from Andaman and Nicobar Islands. As this variety was from a distinct geographical location, totally separated from Kerala, it was expected that there would not be any introgression of genes between this variety and rice gene pool of Kerala comprising of the wild relatives, landraces, traditional rice varieties and the high yielding varieties available in the state. Hence, the distinctness of this variety as evident from the result confirms the correctness of grouping of varieties based on distinctness. However, several earlier reports of non-correspondence of geographic origin with genetic diversity is also evident (Shanmugasundaram *et al*., 2000; Nayak *et al*., 2004; Banumathy *et al*., 2010). Cluster I comprised of five short bold grained varieties. The fourth cluster comprised of 16 varieties, which included a mix of short bold to short slender and medium slender grains. Karuthamodan with short slender grains was the only traditional variety included in this cluster. Cluster III with twenty seven varieties was the largest cluster among the five. All the traditional varieties except Karuthamodan and Chomala were grouped in this cluster. Similarly, all the traditional varieties except Arimodan included in cluster III, possessed short bold grain indicating remarkable homogeneity for the grain characteristics studied. It was evident that majority of the varieties released from Regional Agricultural Research Station, Pattambi, Kerala were grouped under cluster III. This point out that during crop improvement efforts undertaken at the centre, selection for grain type was by far unidirectional, focussing on bold seeded grain type in tune with the grain type preference of Keralites. Clustering based on grain characteristics were earlier done by Sanni *et al.* (2010) using 434 landraces accessions of *O. sativa* L. germplasm from Cote d'Ivoire.

The result of principal component analysis (PCA) implies the importance and contribution of each component to total variance. The criterion used by Clifford and Stephenson (1975) and corroborated by Guei *et al.* (2005) suggest that the first three principal components are often the most prominent in reflecting the variation pattern among accessions and the characters associated with these are more useful in differentiating

Table 4. Distribution of 50 rice varieties in to different clusters

Cluster numbers	Number of varieties	Varieties included
I	5	Vaisakh, Swarnaprabha, Samyuktha, Nila, Varsha
II	1	Jarwa
III	27	Jyothi, Triveni, Aiswarya, Chuvannamodan, Matta Triveni, Karanavara, ParambanKayama, Annapoorna, Kairali, Parambuvattan, Aruna, Karuthadukkan, Kanchana, Arimodan, Makom, Sulochana, Jayathi, Kunjukunjuvarna, Thekkencheera, Ahalya, Uma, Harsha, Cheriya Aryan, Kalladi Aryan, Thottacheera, Kunjukunju, Navara 11-2
IV	16	Onam, Remanika, Krishnanjana, Anaswara, Prathyasa, Aathira, Thulasi, KAU-M-87-1, Karthika, Ponmani, Revathi, Karuthamodan, M-20, MangalaMahsoori, Mahsoori, White Ponni
V	1	Chomala

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

	1 vector	2 vector	3 vector
Eigen value	3.57	1.81	1.67
% variance	32.41	16.49	15.21
Cumulative variance of total variance	32.41	48.90	64.10
GL (mm)	0.08	0.33	0.85
GB (mm)	0.85	0.38	-0.00
GL/GB	-0.74	-0.15	0.50
TGW (gm)	-0.56	0.37	0.55
GD	-0.28	-0.39	-0.24
KL (mm)	-0.49	0.79	-0.21
KB (mm)	0.31	0.29	-0.27
VER	0.61	0.01	-0.23
ER	0.69	-0.49	0.21
AC (%)	0.16	0.48	-0.35

GL= Grain length; GB= Grain breadth; GD= Grain density; KL= Kernel length; KB= Kernel breadth; TGW= 1000 grain weight; AC= Amylose content; VER= Volume expansion ratio; ER= Elongation ratio

accessions (Sanni *et al.,* 2010). Accordingly, the first three principal components accounted for about 64.1% of total variance observed (Table 5).The variable contributing most positively for the first component was grain breadth. Among the property vectors of PC1 (Principal component), weight of 1000 grains, volume expansion ratio and elongation ratio registered higher values. The second principal component that accounted for 48.89% of total variance is positively correlated by the variable kernel length (mm). Similarly, third PC accounted for 64.10% of total variance and is significantly contributed by grain length (mm). Hence, it can be concluded that grain length and breadth and kernel length are the main contributors of variability in grain quality characteristics. The results from cluster analysis and PCA revealed that the clustering pattern gathered complemented each other with slight inconsistencies.

The results of the study suggest that there was considerable genetic variation among rice varieties grown in Kerala for grain cooking and biochemical qualities. This variation offers broad opportunities in breeding program to develop varieties with locally acceptable cooking quality. Among the varieties studied, cooking parameters were best expressed by the high yielding variety Varsha as evident from the high value for elongation ratio and volume expansion ratios. Most of the varieties fall under the category of short bold seeded type. Boldness of the grains being the primary selection criterion of Kerala farmers is confirmed from the results. The distribution of traditional varieties among farmers through informal seed exchange system and their practice of informal selection for bold grained genotypes in addition to gene flow among these genotypes might have contributed to the prevalence of bold seeded types in Kerala.

The indigenous land races in Kerala are invariably semi-tall to tall, poor tillering, lodging with low yield unlike the high yielding varieties. However, the poor productivity of the landraces was not detrimental to their acceptance and popularity in the region, owing mainly to their bold grain type. Maintaining the diversity of forms in cultivated varieties is important not only to overcome genetic erosion but also to avoid monopoly of forms *i.e*., uniformity of a trait among the cultivars. The narrowing genetic base of cultivars predominantly grown in a location is a reason leading to yield loss due to pest and disease outbreak. However, unlike this, grain quality preference in a geographical location is quiet unique and cannot be altered to large extent to preserve diversity. Local preference will continue to decide the grain characteristics in varieties bred in future. Hence, the assessment of variability and diversity for these traits are crucial in any attempt of crop improvement.

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Indian J. Plant Genet. Resour. 31(2): 186-190 (2018)
DOI 10.5958/0976-1926.2018.00022.0

Morpho-agronomic and Molecular Characterization of Gobindabhog, a Traditional Aromatic Rice of West Bengal, India

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(Received: 19 October 2016; Revised: 12 December 2017; Accepted: 19 December 2017)

The agro-morphological characterization of Gobindabhog, a traditional non-Basmati type aromatic rice of lower gangetic plains and *rahr* (red and laterite) region of West Bengal was done at B.C.K.V., Kalyani, West Bengal, India during *kharif* season of 2011, 2012 and 2013 following DUS test guidelines of Protection of Plant Varieties and Farmers' Rights Authority (PPV&FRA). The variety had long statured (scale 7, 130-140 cm height) plants with no anthocyanin colouration on leaf blade, sheath, nodes and internodes; which had late heading (110-115 days) and late maturity (scale 7, 140-150 days). The flower was bi-sexual including six yellow coloured anthers, and an ovary with white/yellowish-white feathery stigma. The lemma and palea of grain were straw or golden-yellow in colour and the grains were awnless, short in length (6.1 mm) with very low test weight (10.17 g). The kernels were short-bold (length 3.97 mm and width 1.95 mm) in shape with white colour, which had low amylose content (17.9%), medium gelatinization temperature (alkali value 3.3) and medium-strong aroma. 23 simple sequence repeat (SSR) markers were used for DNA amplification profile to develop molecular base-pair length database of Gobindabhog rice against non-aromatic international check variety IR 36 in the study. Among them, two markers (RM 341 and RM 339) made greater genetic distance (95.33 *vs*. 174.97 bp and 180.75 *vs.* 143.09 bp, respectively) between Gobindabhog and IR 36.

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

Introduction

The state of West Bengal has precious wealth of genetic diversity in aromatic rice (Shovarani and Krishnaiah, 2001). Among 35-40 such non-Basmati premium scented rices, Gobindabhog, is a native cultivar of lower gangetic plains and *rahr* (red and laterite) region of Bengal, which is traditionally cultivated for about 400–500 years. The name of Gobindabhog was originated probably due to its common use by the Hindus for preparation of *bhog* offered to their worshipped God 'Lord Gobinda' as a religious tradition in gangetic Bengal region for a long time. The earliest record of Gobindabhog rice cultivation was found in a district gazetteer (Hunter, 1877), and then in a book (Mukherji, 1901). At present, it is cultivated in about 30,000–35,000 ha. land in the districts of Burdwan, Bankura, Hooghly, Nadia, Murshidabad, Birbhum and North 24 Parganas in South Bengal, with an average production of 90,000–1,00,000 tonnes paddy every year. Farmers in native areas cultivate Gobindabhog rice in small portions of their agricultural lands following traditional practices intermixed with a few modern technologies in recent times during *kharif* (wet) season. Although it is very popular in domestic market for preparation of *bhog* (rice intermixed with pulses), *payash* (desert), *pistak* or *pitha* (home-made cake), *chira* (flattened rice), etc. during social functions and religious festivals for a long period, but the milled rice obtained from one year aged paddy is marketed in southern states of the country (*viz*. Karnataka, Tamilnadu, Kerala, etc.) during last three decades mainly for preparation of *polao* and *biryani*. In addition, the Standing Committee on Commerce, Parliament of India recommended the export of Gobindabhog rice during 2011 (Rajya Sabha, 2011) based on a Proposal submitted by the RKVY Project on 'Bengal Aromatic Rice' of Bidhan Chandra Krishi Viswavidyalaya, West Bengal. The short-grained variety having pleasant aroma is much potential for international trade especially in the countries like Bangladesh, U.K., Brazil, etc.

Being a signatory to the general agreement on Trade and Tariffs, Government of India has enacted its *sui generis* system Protection of Plant Varieties and Farmers'

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Rights Act (PPV&FRA), 2001 for providing protection to plant varieties based on distinctiveness, uniformity and stability (DUS) test apart from novelty. Thus, agro-morphological, physico-chemical and molecular characterization of Gobindabhog rice needs to be done as legal evidence of DUS, which may also strengthen the right of the farming community to conserve, cultivate and protect the variety against counterfeit ones and/or aggressiveness of multi-national corporate seed sectors in present-day agricultural system.

Materials and Methods

DUS Testing and Determination of Grain Quality

The seeds of Gobindabhog rice was collected from Rice Research Station, Department of Agriculture, Government of West Bengal, Chinsurah, Hooghly, West Bengal, India. Twenty five days old seedlings of Gobindabhog 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 Bidhan Chandra Krishi Viswavidyalaya, Kalyani, Nadia, West Bengal, India during *kharif* season of 2011, 2012 and 2013. 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 Gobindabhog 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, Bidhan Chandra Krishi Viswavidyalaya Kalyani, Nadia, West Bengal, India.

Molecular Characterization by SSR Markers

The molecular characterization of Gobindabhog rice was done at the Division of Plant Biology, Bose Institute, Kolkata, West Bengal during 2006-2008. Three day old rice seedlings of Gobindabhog along with international non-aromatic check variety (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). The PCR products were resolved by native polyacrylamide gel electrophoresis (PAGE) following the protocol given by Sambrook *et.al*. (1989). The length of the amplified DNA bands (SSR alleles) from two rice genotypes was determined with the reference of 100 bp DNA ladders (SibEnzyme Ltd., Russia) by the Molecular Analyst software (BioRad, USA).

The different alleles amplified from the genomic DNA of Gobindabhog rice along with the check were identified on the basis of their length or base pairs (bp) for making genetic characterization of Gobindabhog rice in the study.

Results and Discussion

Agro-morphological Characteristics and Grain Quality

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

Plant: Gobindabhog rice belonged to long-duration type with late heading (scale 7, 114 days) and late maturity (scale 7, 143 days) (Fig. 1).

Stem: It had long statured plant with average stem length of 124.0 cm excluding panicle. The thickness of stem was medium (scale 5) with mean diameter of 0.48 cm. Anthocyanin colouration was absent on nodes and internodes. The attitude of the culm could be categorised as erect (scale 1) at booting stage.

Leaf: The variety produced long, narrow and green leaves. The colour of basal leaf sheath was green (scale 1), while the intensity of green colour of the leaf was medium (scale 5) without any anthocyanin colouration. The average length and width of leaf blade were noted as 65.7 mm and 9.1 mm, respectively. The split-type (scale 3) ligule 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 Gobindabhog rice was categorized as medium (scale 5, 25.2 cm) with the curvature of the main axis as deflexed (scale 5) (Fig. 2). The plant produced very few (scale 3, mean 9.5) well-exerted panicles in the field. The colour of the lemma and palea was green at anthesis, which turned to golden-yellow at ripening stage.

Table 1. Plant characteristics of Gobindabhog rice following DUS guidelines

S. No.	Characteristics	Scale	Remarks measured values etc.
1	Coleoptile: colour	2	Green
2	Basal leaf sheath colour	1	Green
3	Leaf : Intensityof green colour	5	Medium
4	Leaf : anthocyanin colouration	1	Absent
5	Leaf : distribution of anthocyanin colouration	—	—
6	Leaf sheath : anthocyanin colouratin	1	Absent
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	Green
16	Leaf : length of blade	7	Long (65.7 cm)
17	Leaf : width of blade	3	Narrow (9.1 mm)
18	Culm : attitude (for floating rice only)	—	—
19	Culm : attitude	1	Erect
20	Time of heading (50% of plants with panicles)	7	Late (114 days)
21	Flag leaf attitude of blade (early observation)	3	Semi-erect
22	Spikelet : density of pubescence of lemma	5	Medium
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	5	Medium
27	Spikelet : colour of stigma	1	White
28	Stem: thickness	5	Medium (0.48 cm)
29	Stem: length (excluding panicle)	7	Long (124.0 cm)
30	Stem: anthocyanin coloration of nodes	1	Absent
31	Stem : intensity of anthocyanin colouration of nodes	—	—
32	Stem : anthocyanin colouration of internodes	1	Absent
33	Panicle: length of main axis	5	Medium (25.2 cm)
34	Flag leaf: attitude of blade (late observation)	5	Horizontal
35	Panicle: curvature of main axis	5	Deflexed
36	Panicle: number per plant	3	Few (9.5)
37	Spikelet: colour of tip of lemma	2	Yellowish
38	Lemma & Palea : Colour	1	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
44	Panicle : secondary branches	2	Strong
45	Panicle : attitude of branches	7	Semi-erect to spreading
46	Panicle: exertion	7	Well exerted
47	Time of Maturity	7	Late (143 days)
48	Leaf : senescence	7	Late
49	Sterile lemma: colour	1	Straw
50	Grains: weight of 1000 fully developed grains	1	Very low (10.17 g)
51	Grain : length	1	Very short (6.1 mm)
52	Grain : width	2	Narrow (2.2 mm)
53	Grain : phenol reaction of lemma	—	—
54	Decorticated grain: length	1	Very short (3.97 mm)
55	Decorticated grain: width	1	Very narrow (1.95 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 (17.9 %)
60	Varieties with endosperm of amylose absent only-polishedgrain : exertion of white core	—	—
61	Gelatinization temperature through alkali spreading value	3	Medium (Alkali score 3.3)
62	Decorticated grain : aroma	9	Present (Medium-strong)

Fig. 1. Gobindabhog rice field at dough stage

Fig. 2. Panicle of Gobindabhog rice

Fig. 3. Grains and kernels of Gobindabhog rice

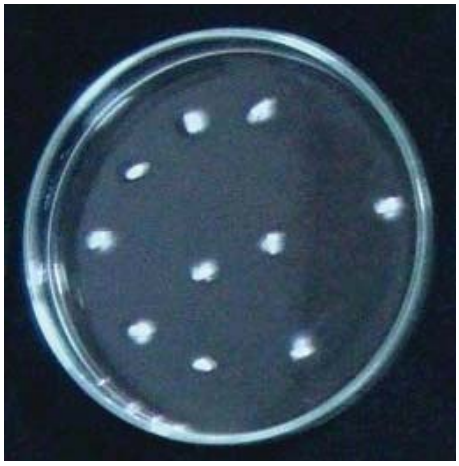

Fig. 4. Alkali digestion test of Gobindabhog rice

Flower: The variety produced bi-sexual flowers including six yellow coloured anthers, and an ovary with white / yellowish-white feathery stigma.

Grain: The grains of Gobindabhog rice were short in size (mean length 6.1 mm and width 2.2 mm) and awnless (Fig. 3). The weight of 1000 fully-developed grains was very low (10.17 g). The colour of lemma and palea was straw (scale 1) or golden yellow, while that of sterile lemma was straw (scale 1).

The kernels were short-bold in shape (length 3.97 mm and width 1.95 mm) and white in colour (Fig. 3), which had low amylose content (17.9%), medium gelatinization temperature (alkali value 3.3) (Fig. 4) and medium-strong aroma.

DNA Amplification Profile and Molecular Weight

23 SSR markers used in the study were selected from chromosome 2, 3, 7 and 8 because two important traits of scented rice, aroma (Ahn *et al*., 1992) and cooked kernel elongation ratio (Ahn *et al*., 1993) 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 Gobindabhog 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, five markers (RM 44, RM 112, RM 152, RM 207 and RM 251) recorded similar molecular weights for both

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

SSR Marker	Motif	Rice Chromosome No.	Annealing temperature (ºC)	Length of base pair (bp)	
				Gobindabhog	IR 36 (International check)
RM 42	(GA)6	8	65	160.88	156.36
RM44	(GA)16	8	55	112.20	112.78
RM72	(TAT)5C(ATT)15)	8	55	161.08	165.65
RM80	(CTT)20	8	65	125.03	121.82
RM112	(GAA)5	2	55	141.02	141.98
RM149	(AT)10	8	59	256.71	246.99
RM152	(GGC)10	8	60	149.87	149.96
RM182	(AT)16	7	59	312.65	296.27
RM207	(GA)25	2	65	129.02	128.48
RM210	(GA)23	8	55	145.32	149.75
RM218	(GA)24	3	55	144.14	155.29
RM223	(GA)25	8	55	154.72	164.34
RM250	(CT)17	2	60	152.71	150.96
RM251	(CT)29	3	55	120.11	119.88
RM282	(GA)15	3	59	128.49	140.39
RM284	(GA)8	8	55	144.96	139.68
RM310	(GT)19	8	55	103.44	107.57
RM337	(CTT)4-19(CTT)8	8	59	158.58	161.29
RM339	(CCT)8(CCT9CCT)5	8	59	180.75	143.09
RM341	(CTT)20	2	55	95.33	174.97
RM505	(CT)12	7	55	117.42	126.25
RM530	(GA)23	2	59	154.64	168.02
RM569	(CT)16	3	59	174.25	167.61

the varieties, while two markers (RM 341 and RM 339) made greater genetic distance (95.33 *vs*. 174.97 bp and 180.75 *vs.* 143.09 bp, respectively) between Gobindabhog and IR 36 varieties in the investigation.

Conclusion

Gobindabhog, a traditional aromatic rice variety of West Bengal, India had late maturity (140-150 days) and the plants were long statured (130-140 cm height) with no anthocyanin colouration on leaf blade, sheath, nodes and internodes. The colour of lemma and palea of grain was straw or golden-yellow and the grains were short in length (6.1 mm) with very low test weight (10.17 g). The kernels were short-bold (length 3.97 mm and width 1.95 mm) in shape and white in colour, which had low amylose content (17.9%), medium gelatinization temperature (alkali value 3.3) and medium-strong aroma. Based on molecular base-pair length database developed by 23 SSR markers, two (RM 341 and RM 339) made greater genetic distance between Gobindabhog and IR 36 in the investigation.

Acknowledgement

We are thankful to Shyamsundar Sister Nivedita Sangha, Burdwan for their efforts toward the conservation-cum-expansion of Gobindabhog rice for a long time. The technical and financial assistance from RKVY Project on 'Bengal Aromatic Rice' sanctioned by Department of Agriculture, Government of West Bengal are acknowledged.

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Indian J. Plant Genet. Resour. 31(2): 191-193 (2018)
DOI 10.5958/0976-1926.2018.00023.2

SHORT COMMUNICATION

DNA Profiling of Pomegranate (*Punica granatum* L.) Field Genebank Semi-feral Collection by Using ISSR Markers

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(Received: 13 February 2016; Revised: 01 September 2016; Accepted: 15 October 2016)

Molecular genetic diversity estimation among 68 semi-feral accessions of pomegranate (*Punica granatum* L.) that were collected from Uttarakhand and Himachal Pradesh was carried out using 43 ISSR markers. All the markers were polymorphic with mean pairwise similarity of 39%. The screening showed that all the 68 accessions were distinct genotypes and the markers grouped them into two major groups and 11 clusters.

Key Words: DNA profile, ISSR, JMP, Pomegranate

Pomegranate (*Punica granatum* L.), a woody perennial shrub or small tree, belongs to the Punicaceae family and is one of the oldest known edible fruits (Damania, 2005). India is the largest producer of pomegranates in the world. Moreover, India produces finest edible quality of pomegranates which are available almost throughout the year. India's target for the year 2025 is to increase pomegranate production by 10 folds and export by 6.97 folds (Chandra *et al.,* 2010). However, pomegranate suffers from bacterial blight, which may cause up to 60-80% yield losses (Ramesh *et al.,* 1991). Searching for novel genetic backgrounds that are resistant to diseases needs broadening of the genetic base of pomegranate germplasm requiring collecting, characterization and genetic diversity analysis of germplasm imperative. Genetic diversity analysis of Indian pomegranate field genebank collections comprising of varieties and germplasm accessions employing morphometric markers and microsatellite based DNA markers has been reported (Archak *et al.,* 2014). The present study was undertaken to obtain a qualitative insight into the extent of genetic diversity present among the semi-feral pomegranate collections from Uttarakhand and Himachal Pradesh using ISSR markers.

Sixty-eight pomegranate accessions (Table 1) mainly belonging to Uttarakhand (35 accessions) and Himachal Pradesh (14 accessions) conserved in the field genebank at ICAR-National Research Centre on Pomegranate, Solapur, Maharashtra (17° 68' N, 75° 91' E, 457m above msl) were used for the study. Young and healthy leaves were excised from a late *hasta-bahar* crop (October-November flowering) from well-established five year old trees and were fixed in liquid nitrogen and stored at –80 °C. DNA extraction, purification and ISSR analyses were carried out as explained by Archak *et al.* (2014). Five ISSR primers — Pome_$(TA)_5(GT)_5$, Pome_DAMD_M13, Pome_$(AT)_5(GT)_5$, Pome_DAMD_HBV, and Pome_$(GACA)_4$ with annealing temperatures (in °C) of 50, 52, 50, 44 and 50 respectively were employed to generate DNA profiles. The DNA profiles were manually scored from gel photographs for the presence (1) or absence (0) of amplicons. Binary data were prepared as rectangular data matrix and pairwise distance (D_{KL}) was computed using Ward's method as:

$$D_{KL} = \frac{\|\bar{X}_K - \bar{X}_L\|^2}{\frac{1}{N_K}+\frac{1}{N_L}} D_{KL} = \frac{\|\bar{X}_K - \bar{X}_L\|^2}{\frac{1}{N_K}+\frac{1}{N_L}}$$

where D_{KL} is the distance or dissimilarity measure between clusters C_K and C_L where C_K is K^{th} cluster, subset of {1,2,3…,n} having N_K number of observations. ‖x‖ is the square root of the sum of the squares of the elements of x (the Euclidean length of the vector x) and $d(x_i, x_j)$ is ‖x‖. Statistical analyses were carried out using JMP Genomics version 5.1 (SAS, 2012).

Five ISSR primers — Pome_$(TA)_5(GT)_5$, Pome_DAMD_M13, Pome_$(AT)_5(GT)_5$, Pome_DAMD_HBV,

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Table 1. List of pomegranate field genebank accessions analysed in the study

S.No.	Genebank ID	Source (District)	S.No.	Genebank ID	Source (District)
1	IC318702	Mandi	35	IC556886	Tehri Garhwal
2	IC318706	Mandi	36	IC556887	Tehri Garhwal
3	IC318707	Mandi	37	IC556888	Tehri Garhwal
4	IC318716	Mandi	38	IC556889	Tehri Garhwal
5	IC318724	Mandi	39	IC556890	Tehri Garhwal
6	IC318733	Mandi	40	IC556891	Tehri Garhwal
7	IC318734	Mandi	41	IC556892	Tehri Garhwal
8	IC318735	Mandi	42	IC556893	Uttarkashi
9	IC318740	Mandi	43	IC556894	Uttarkashi
10	IC318743	Mandi	44	IC556895	Uttarkashi
11	IC318744	Shimla	45	IC556897	Dehradun
12	IC318749	Shimla	46	IC556898	Dehradun
13	IC318766	Solan	47	IC556899	Dehradun
14	IC318793	Solan	48	IC556900	Dehradun
15	IC444198	Narendranagar	49	IC556901	Dehradun
16	IC444200	New Tehri	50	R-18(H2)P-934	
17	IC524026	Nainital	51	R-21(H2)P-1121	
18	IC524030	Almora	52	R-9(H1)P-528	
19	IC524031	Chamoli	53	RC/PB/1181	
20	IC548192	Nainital	54	RC/PB/1261	
21	IC548193	Almora	55	RC/PB/1287	
22	IC548194	Almora	56	RC/PB/1288	
23	IC548195	Bageshwar	57	RC/PB/1290	
24	IC548196	Chamoli	58	RC/PB/1291	
25	IC548197	Chamoli	59	RC/PB/1292	
26	IC548198	Chamoli	60	RC/PB/1294	
27	IC548199	Chamoli	61	RC/PB/1295	
28	IC548200	Chamoli	62	RC/PB/1296	
29	IC548202	Nainital	63	RC/PB/1298	
30	IC548203	Nainital	64	RC/PB/1299	
31	IC556880	Pauri Garhwal	65	H-5	
32	IC556882	Tehri Garhwal	66	H-6	
33	IC556884	Tehri Garhwal	67	H-10	
34	IC556885	Tehri Garhwal	68	H-14	

and Pome_$(GACA)_4$ — amplified 8, 9, 11, 8 and 7 amplicons respectively. All the 43 amplicons were found to be polymorphic. Average number of polymorphic amplicons per primer was 8.6. All the 68 pomegranate genotypes were grouped into two major groups and 11 clusters (Fig 1). The clusters in no way reflected the collection sites. Pomegranate is an introduced fruit to India. Pomegranate breeders often use well-established cultivars for inter-varietal crosses. However, recent focus on the bacterial blight resistance (www.icar.org.in/en/node/8271) meant that genotypes occurring in semi-feral conditions needed to be collected and characterized. Before detailed assay for disease resistance, it was important to ascertain that the genotypes were actually genetically distinct. In the present study, the preliminary screening has shown that all the 68 accessions were distinct genotypes. The results were encouraging to plan detailed morphological, molecular and trait-specific evaluations of the semi-feral genotypes that remain till-date unexplored.

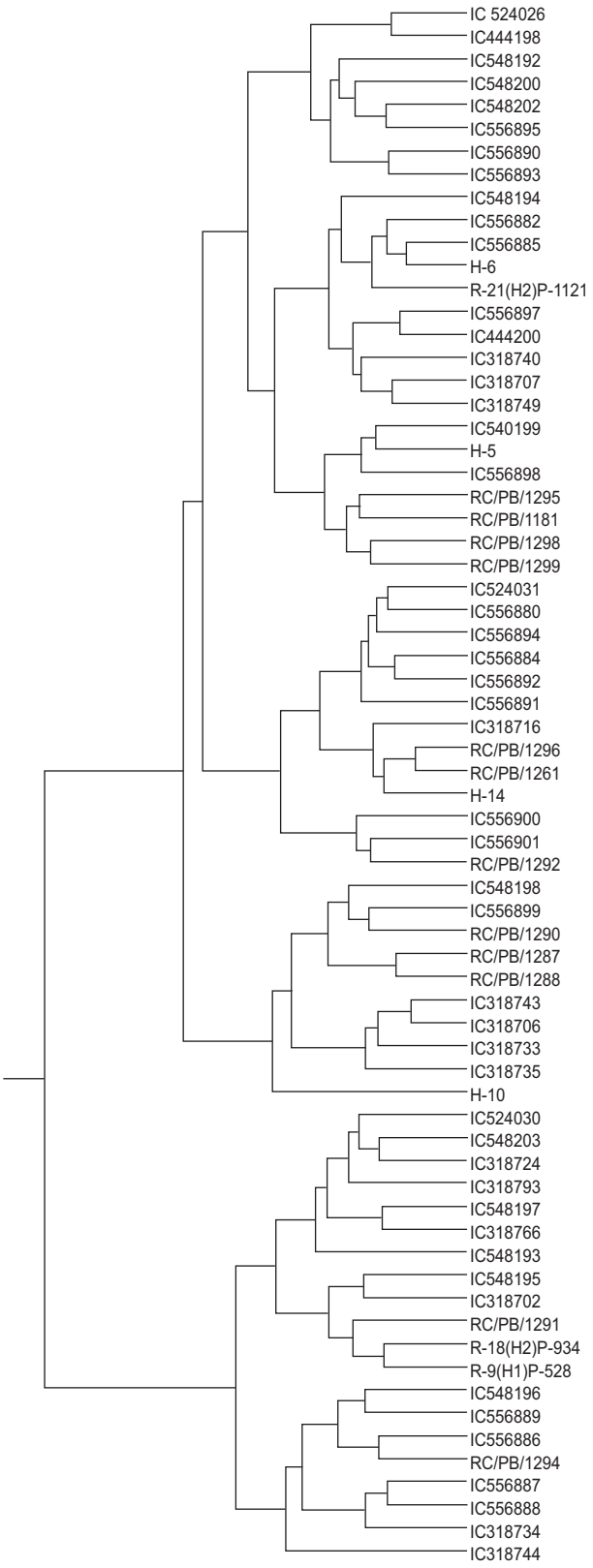

Fig. 1. Genetic clusters of 68 pomegranate germplasm accessions based on 43 ISSR markers

Acknowledgements

This work was carried out as part of M.Sc. (Plant Genetic Resources) thesis work. Badal Singh was supported by ICAR-Junior Research Fellowship. The work was supported by NBPGR's institutional project on "Development of genomic tools for enhanced utilization of horticultural crops". Sunil Archak is supported by ICAR-National Fellowship.

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Indian J. Plant Genet. Resour. 31(2): 194-195 (2018)
DOI 10.5958/0976-1926.2018.00024.4

MEETING REPORT

'Regional Expert Consultation on Underutilized Crops for Food and Nutrition Security in Asia and the Pacific'

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A "Regional Expert Consultation on Underutilized Crops for Food and Nutritional Security in Asia and the Pacific" was organized at Bangkok, Thailand, from November 13-15, 2017, with the following objectives:

- To create awareness on the role and value of underutilized plant resources/ crops/species/plants/ neglected underutilized species/orphan/minor crops (here afterward common abbreviation as UUCs is used in present write-up) which have potential for diversification of food basket to ensure better food and nutritional security in Asia-Pacific region;
- To share experiences and learn lessons to accelerate the use of UUCs as crops for future;
- To assess research and development (R&D) status on priority crops, and policies needed to promote the use of these potential crops for future use in Asia-Pacific region.

The Expert Consultation was organized by Asia-Pacific Association of Agricultural Research Institutions (APAARI), Asia-Pacific Consortium on Agricultural Biotechnology and Bioresorces (APCoAB), Bangkok, Thailand and Council of Agriculture (COA), Taiwan, in collaboration with World Vegetable Centre (WorldVeg),

Participants of the Regional Expert Consultation on Underutilized Crops for Food and Nutrition Security in Asia and the Pacific

Taiwan; International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), India; Crops for Future (CFF), Malaysia; International Centre for Agricultural Research in the Dry Areas (ICARDA), Lebanon; Bioversity International, Italy and Department of Agriculture (DOA), Thailand. In all, 54 participants from 18 countries of the Asia-Pacific region (Bangladesh, Bhutan, Fiji, Germany, India, Iran, Japan, Lao, Malaysia, Nepal, Pakistan, Philippines, Papua New Guinea, Samoa, Sri Lanka, Taiwan, Thailand, Vietnam) deliberated on the issues. The participants comprised Senior Officials, researchers from National Agricultural Research Systems (NARS), Consultative Group (CG) Centers, experts on UUC, representatives of research institutions, donors, private sector, NGOs and a farmer. The meeting was structured to include inaugural, technical, special and plenary sessions. The technical sessions comprised: (i) Thematic Technical Presentations (9 speakers); (ii) Strategies on Underutilized Crops for Food and Nutritional Security (9 speakers); (iii) Country Status Reports on Underutilized Crops (16 countries); (iv) Working Group Discussion and Recommendations (4 groups) and (v) Panel Discussion on Policy Support for UUCs to achieve Sustainable Development Goals (SDG) (8 Panellists).

The meeting provided an excellent platform for sharing experiences and gaining knowledge related to UUC of Asia and the Pacific. These crops have food and nutritional value with the possibilities of their commercial use and eventual benefit to small-holder farmers. The 3-day deliberations led to a collective agreement that R&D and promotion of UUC would address SDG goals on ending hunger, poverty alleviation, good health and well-being, besides attaining sustainable production and consumption, climate change and sustainable use of ecosystems. Consensus also emerged that policy makers should give due attention, generate political will and mobilize funding and subsidies, to promote greater use of these plants. The meeting was beneficial in assessment on the importance of most potential crops, status of their R&D and gap areas which need attention to mainstream these crops into the existing cropping pattern systems. Interaction with participants from various countries provided a platform for exploring the possibilities of establishing a regional network for knowledge sharing (on crops, experts, centres of excellence for capacity building) and research collaborations. It was agreed that a global funding system for scholarships, exchange, projects along with awareness generation programs is urgently required. Important action points that emerged included definition of UUCs at global and national levels, documentation and baseline data development of species and diversity, facilitation of germplasm exchange, along with collecting, characterization and evaluation through bilateral or regional cooperation, capacity building at regional level for conservation and use of UUCs, education and awareness-generation for greater promotion of these crops.

The proceedings and recommendations of the above meeting can be viewed at:

- http://www.apaari.org/web/wp-content/uploads/downloads/2018/Regional%20Expert%20Consultation%20on%20UUC%20-%20Proceedings%20and%20Recommendations%20-%20Final.pdf

The Thematic, Strategic Papers and Country Status Reports of Asia-Pacific Countries have also been compiled and edited which were presented during the Expert Consultation, and can be viewed at:

- http://www.apaari.org/web/wp-content/uploads/2018/06/Regional-Expert-Consultaton-Underutilized-Crops-Papers-and-Country-Reports.pdf

Indian J. Plant Genet. Resour. 31(2): 196-197 (2018)
DOI 10.5958/0976-1926.2018.00025.6

INTERVIEW

New Secretary Outlines the Priorities of ITPGRFA

Dr. Kent Nnadozie has recently taken over as the Secretary of the International Treaty on Plant Genetic Resources (ITPGRFA) during GB7 at Kigali. Dr. Nnadozie has a long experience of working on legal and policy matters and intergovernmental processes, with both the ITPGRFA and FAO's Commission for Genetic Resources for Food and Agriculture. A lawyer by training, Dr. Nnadozie holds master's degree in business administration and his Ph.D. work focusses on international legal systemic issues regarding genetic resources.

Sunil Archak on behalf of IJPGR interviewed Dr. Kent Nnadozie on 22-06-2018 at FAO, Rome.

IJPGR: Congratulations on assuming the responsibility of Secretary of ITPGRFA. The world is looking at you at the moment when the International Treaty is undergoing many changes. What are the three top priorities you have set for yourself?

Kent Nnadozie: Thank you. I am honored that the Governing Body has entrusted me with this responsibility and I look forward to working with India and all Contracting Parties to continue making progress on the conservation and sustainable use of plant genetic resources around the world. The International Treaty is, indeed, at a very exciting stage of its evolution. Our challenge will be to keep up with the changing environment and to continue making a difference in the world. The most challenging issue and, therefore, the top priority for me now is to assist Contracting Parties in reaching agreement in the ongoing negotiations to enhance the functioning of the Multilateral System of the Treaty. This includes agreeing on adequate benefit-sharing arrangements, and revising the scope and coverage of the Multilateral System.

There are, of course, many priorities facing us today, but if I have to pick three, I would say:

1. **Continue supporting smallholder farmers**, who are among the most vulnerable when it comes to coping with the consequences of environmental, climatic, or manmade disasters. We are doing this in a number of ways, such as (a) by supporting projects in developing countries through the Benefit-sharing Fund (BSF), and (b) by providing training and building capacity where it is most needed, included supporting the protection of Farmers' Rights and traditional knowledge. I recently had the opportunity to visit BSF project sites in rural Malawi, Kenya and Tanzania, where we met with the farmers as well as the PGR experts involved in the projects. I was touched and delighted to see how much they are able to achieve with the support provided through the BSF. We must continue to support projects in developing countries.
2. **Establishing a strong financial and operational footing for the International Treaty.** The growth of the Multilateral System of Access and Benefit-sharing (MLS) and the evolution of the Funding Strategy remain important priorities for the International Treaty. We will work closely with the Working Group tasked with these important policy issues. (a) First is the need to provide a more reliable source of financial resources needed to continue key Treaty programs, e.g. projects supporting farmers in developing countries through the International

Treaty's Benefit-sharing Fund. Since inception, the BSF receives many more deserving project proposals than available funds. More regular funds into the BSF will make it more possible to try to meet this growing demand. (b) Hand-in-hand with adequate and predictable benefit-sharing is that the more diverse and inclusive the list of crops available through the MLS (as listed in Annex 1) will be, the greater the chances will be for farmers and breeders to develop the crop varieties they need to adapt to changing climatic conditions and to feed our growing world population. This will also lead to conserving and, indeed, boosting the world's agro-biodiversity, which in turn, will provide a more varied and more nutritious food basket.

3. **Working towards achieving universality by having all countries join the International Treaty.** The International Treaty on Plant Genetic Resources for Food and Agriculture is important for every nation in the world, and it is important to have everybody join our global community. We will all benefit by sharing and working together to conserve the natural agricultural heritage and rich agricultural biodiversity we have been entrusted with for current and future generations. This is also in keeping with the 2030 Agenda for Sustainable Development. To mention just a few of the Sustainable Development Goals (SDGs):
    a. **SDG 2** calls on all of us to end hunger, which we are doing by promoting sustainable agriculture and supporting the most vulnerable segments of society, including smallholder farmers;
    b. **SDG 13** calls on all of us to combat climate change, which we are contributing to through our BSF projects and by building capacity in various Contracting Parties; and
    c. **SDG 15** calls on all of us to halt the loss of crop diversity, which we are working in numerous ways, not the least of which is through our BSF projects and by encouraging sustainable use of PGRFA.

IJPGR: What are the topmost challenges that you are looking at?

Kent Nnadozie: There are a couple of challenges that come to mind:

1. Ensuring reliable and adequate **financial resources** to enable us to continue the valuable programmes and activities of the International Treaty.
2. Effectively **moderating the different views on critical issues**, such as on the enhancement of the MLS.

IJPGR: How do you visualize bringing tech-rich and gene-rich countries together in a consensus-oriented forum like the ITPGRFA?

Kent Nnadozie: I truly believe that bringing together the two groups you mention is a win-win situation. Those you are referring to as "tech-rich" need the "gene-rich" and vice-a-versa. The reality is both need one another. So, the main task is to highlight the mutual advantages of such a coalition, because we all share common objectives under the Treaty, and face common challenges to global food security and climatic challenges to agriculture.

IJPGR: Do you plan to get in more contracting parties, like China?

Kent Nnadozie: Our aim is and should be universality, which means inviting all those who are not yet Contracting Parties to the International Treaty to join our growing community. The larger our global community becomes the better for everyone. Sharing the world's plant genetic resources, sustainably using these precious resources – these are our key objectives. Joining the International Treaty is a win-win situation for everyone.

Indian J. Plant Genet. Resour. 31(2): 198-204 (2018)

Plant Germplasm Registration Notice*

The Plant Germplasm Registration Committee of ICAR in its XXXVth meeting held on September 1st, 2016 at the ICAR-National Bureau of Plant Genetic Resources, New Delhi approved the registration of following 14 germplasm lines out of 40 proposals considered. The information on registered germplasm is published with the purpose to disseminate the information to 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. HI KK1 (NP4+*Lr1*) (IC0620368; INGR16024); HI KK2 (NP4+*Lr2a*) (IC0620369; INGR16025); HI KK3 (NP4+*Lr2c*) (IC0620370; INGR16026); HI KK4 (NP4+*Lr3a*) (IC0620371; INGR16027); HI KK5 (NP4+*Lr9*) (IC0620372; INGR16028); HI KK6 (NP4+*Lr10*) (IC0620373; INGR16029); HI KK7 (NP4+*Lr15*) (IC0620374; INGR16030); HI KK8 (NP4+*Lr17a*) (IC0620375; INGR16031) and HI KK9 (NP4+*Lr20*) (IC0620376; INGR16032) Wheat (*Triticum aestivum*) Lines Locally Adapted to Host Differentials for Indian Pathotypes of Wheat Leaf Rust (*Puccinia triticina*)

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Leaf rust caused by *Puccinia triticina* Eriks. (*Pt*) is most common among three rust diseases of wheat (*Triticum aestivum* L.). Leaf rust differentials being used in India consist of sets 0, A and B (Nayar *et al.*, 2001). Majority of the leaf rust differentials in set 'A' and most varieties in set 'B'are winter types, which require a long photoperiod for flowering. Hence, maintenance of these differentials is difficult, particularly in the plains of India due to their long duration and often there is either seed set failure or production of shriveled grain. Hence, need was felt for developing near-isogenic *Lr* lines in the background of a locally adapted variety. NP 4 was selected as a background parent which is not known to carry any *Lr* gene or suppressor factor (Kaushal *et al.,* 1982) for leaf rust resistance, and being early maturing, slow rusting, lodging-tolerant, non-shattering, heat and drought tolerant and bold seeded variety. Hence, a back cross programme was initiated in 1997-98 for transferring the *Lr1, Lr2a, Lr2c, Lr3a, Lr9, Lr10, Lr15, Lr17a* and *Lr20*genes into NP 4 background. Tc-backcross lines carrying the target *Lr* genes *viz*., RL 6003 (Tc+*Lr1*), RL 6016 (Tc+*Lr2a*), RL 6047(Tc+*Lr*2c), RL 6002 (Tc+*Lr*3a), RL 6010 (Tc+*Lr*9), RL6004 (Tc+*Lr*10), RL 6052 (Tc+*Lr*15), RL 6008(Tc+*Lr*17a) and RL 6092 (Tc+*Lr*20) were used as donors. Six backcrosses were attempted followed by intense selection during each generation for selecting NP 4 plant type (awnless spikes and pubescent glumes) coupled with resistance phenotype of the target gene. Leaf rust pathotype (pt) 12-2 (1R5) was used for selecting resistant plants carrying singly the genes *Lr1*, *Lr2a*, *Lr15*; pt 12-5 (29R45) for *Lr*9, *Lr10*, *Lr20*; pt 17 (61R24) for *Lr2c, Lr3a* and pt 107 (45R3) for *Lr17a*.

Compiled and edited by Anjali Kak and RK Tyagi, Division of Germplasm Conservation, ICAR-National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi-110012.

Table1. Comparison of seedling infection types (at 16-20ºC) to selected avirulent leaf rust pathotypes of the newly developed near-isogenic lines (HI KK 1-9) with the corresponding donor *Lr* lines (RL Nos.) and the recurrent parent NP 4

Wheat genotypes	Seedling infection type[1]	A virulent leaf rust pathotypes used for testing
HI KK 1 (NP4+*Lr1*)	0;	11, 12, 12-2, 12-1, 12-3, 12-5, 63, 106, 12-8, 162-1, 162A
RL 6003 (Tc+*Lr1*)	0;	
NP 4	3+	
HI KK 2 (NP4+*Lr2a*)	0;	2 11, 12, 12-2, 12-3, 12-5, 12-8, 17, 63, 104-2, 106
RL 6016 (Tc+*Lr2a*)	0;2+	
NP 4	3+4	
HI KK 3 (NP4+*Lr2c*)	0;1	16, 16-1, 17, 77-9, 77-10, 77-11
RL 6047 (*Lr2c*)	0;1	
NP 4	3+	
HI KK 4 (NP4+*Lr3a*)	0;	17, 20, 104-1, 106, 107, 108, 108-1
RL 6002 (*Lr3a*)	0;	
NP 4	3+	
HI KK 5 (NP4+*Lr9*)	0;	11, 12-5, 16, 17, 63, 77-5, 77-9, 77-11, 104-2
RL 6010 (*Lr9*)	0;	
NP 4	3+	
HI KK 6 (NP4+*Lr10*)	;2	10, 12-2, 12-5, 63, 77, 107, 107-1, 162-3
RL 6004 (*Lr10*)	;2	
NP 4	33+	
HI KK 7 (NP4+*Lr15*)	0;	10, 11, 20, 63, 104-4, 106, 107, 107-1, 108, 108-1
RL 6052 (*Lr15*)	0;	
NP 4	3+	
HI KK 8 (NP4+*Lr17a*)	0;2	10, 12, 12-1, 12-3, 63, 106, 107, 107-1
RL 6008 (*Lr17a*)	0;2+	
NP 4	33+	
HI KK 9 (NP4+*Lr20*)	;1	10, 12-1, 12-2, 12-3, 77-4, 77-6, 104-2, 104B, 162-2, 162-3
RL 6092 (*Lr20*)	;1	
NP 4	3+	

[1]As described by Roelfs *et al*., (1992)

The lines developed were seedling tested with several a virulent pathotypes for confirming homozygosity for target *Lr* gene (D.R. Knott, Pers. comm.).

Close similarity between seedling infection types of the newly developed backcross lines and the corresponding donor lines confirmed successful transfer of the target *Lr* genes in NP 4 background (Table 1).

Homozygous resistant lines carrying singly nine genes *viz*., *Lr1, Lr2a, Lr2c, Lr3a, Lr9, Lr10, Lr15, Lr17a* and *Lr20* have been developed. These near-isogenic lines having early maturity and other desired agronomic traits of NP4 are easy to maintain under Indian conditions, and hence, should be widely useful for virulence analysis and genetic studies.

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2. TCP129 (IC0615165; INGR16033), a Turmeric (*Curcuma longa*) Genotype, Highly Tolerant to *Colletotrichum* Leaf Spot and *Taphrina* Leaf Blotch Diseases

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Turmeric crop (*Curcuma longa* L.) is affected by two major diseases caused by *Colletotrichum* leaf spot (Reddy *et al.*, 1963) and *Taphrina* leaf blotch (Rao, 1995). The genotype TCP129 (IC0615165; INGR16033) is found to have unique characters having highly tolerant to leaf blotch (*Taphrina maculans* L.) (PDI-12.78) and leaf spot (*Colletotrichum capsici* L.) (PDI-7.26)diseases. TCP 129 (IC0615165) was collected from Gairkata (26.69 ºN and 89.03 ºE, alt. 98 asl) in Jalpaiguri district of West Bengal in the year 2001. Co ordinated Varietal Trials (CVT) under AICRP on Spices (IISR, Calicut) were taken up from 2013 to 2016 for resistance to leaf spot and leaf blotch foliar diseases along with local and national check in this region. Breeding of turmeric was done by clonal selection. The investigation was done in Uttar Banga Krishi Viswavidyalaya, Pundibari, Coochbehar, West Bengal.

Table 1. Average performance of TCP-129 (IC0615165, INGR-16033)

Genotype	Germi nation (%)	Leaf Blotch (PDI)	Reduction Over local check	Reduction Over National check	Leaf Spot (PDI)	Reduction Over local check	Reduction Over National check	Yield (3m2) (kg/plot)	Projected Yield (t/ha)
TCP-129	98.06 (78.69)	12.78 (20.87)	46.48	55.17	7.26 (15.47)	70.03	42.24	14.34	28.91
TCP-2 Local check	95.44 (72.63)	23.88 (29.49)	-	-	24.23 (29.31)	-	-	7.36	14.84
Prativa National check	95.46 (72.66)	28.51 (32.27)	-	-	12.57 (20.76)	-	-	7.56	15.24
SEm(±)	2.37	0.92			1.28			0.77	
C.D.	4.73	1.92			2.57			1.45	
C.V.(%)	15.18	13.85			10.08			15.56	

(Figures in the parenthesis are angular transformation values)

Morpho-agronomic characteristics: It has long plant height (100.93cm.), few number of shoots (2-4), petiole length intermediate, lamina length long, width broad, pseudostem habit open, leaf disposition erect and leaf margin wavy. Mother rhizome was found (1-3), dry recovery (%) high (23.75%), rhizome habit intermediate, rhizome shape straight, tertiary rhizome present. Rhizome yield per plant was 206.28gm. yield per plot (Kg/3m^2) 14.34kg/plot, projected yield 28.91t/ha (Table 1).

Associated characters and Cultivated Practices: Curcumin (3.8%), Oleoresin (9.08%), essential oil content (7%). It was found highly resistant to leaf spot (PDI-7.26) and leaf blotch (PDI-12.78) diseases (Table.1) . Cultivation practices should be done having bed size: 3m ×1 m with spacing 30 (row to row) and 20 cm plant to plant. FYM was given prior to sowing in February- March at the rate of 2-5t/ha. Sowing was done in April-May and area of adaptation of this genotype was found in West Bengal, Uttar Pradesh, Bihar, Kerala, Himachal Pradesh, Telangana, Gujarat and Tamil Nadu.

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3. RILHF-2 (IC0616051; INGR16034), a Pea Germplasm Highly Resistant to Rust (*Uromyces fabae* Pers. de-Bary)

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Pea rust caused by *Uromyces fabae* Pers. de-Bary is a major disease of pea in tropical and sub-tropical regions of the world. Under favorable condition (warm and humid weather) the rust disease causes substantial yield losses and it may result in complete loss in yield under prolonged epidemic conditions.

RILHF-2 was developed from the cross between pea genotypes HUVP 1 (susceptible) and FC 1 (resistant) at the Agriculture Research Farm, Banaras Hindu University, Varanasi, India. Two hundred fifty single F_2 plants were harvested separately in 2000-01, seeds from each of the 250 F_2 plants were planted in bulk in the Rabi season of 2001-02, and the later generations i.e. F_4, F_5 and F_6 generations were raised following single seed descent method in successive years (Singh *et al.*, 2012). Each progeny was planted in a single row of 2.0 m and the spacing was kept at 40 × 10 cm. A highly rust susceptible check i.e., HFP-4 was planted after every 10th row and also at the borders surrounding the experiment as spreader row for uniform disease

expression in the field subsequent to artificial inoculation by spore suspension (Singh *et al.*, 2012). The data were recorded on five randomly chosen plants from each F_6 progeny for disease severity scored at three different dates. Each F_6 plant progeny was separately harvested in bulk to obtain $F_{6:7}$ seeds.

Single plant progenies were evaluated for rust disease in the field during 2005-06, 2006-07 and 2007-08 as $F_{6:7}$, $F_{6:8}$ and $F_{6:9}$ generations. Among progenies, the RILHF-2 was found to be highly resistant to pea rust on the basis of above three years of initial field screening conducted at Banaras Hindu University, Varanasi. Seed of RILHF-2 was sent for multi-location rust screening at hot spots across India during 2008-09 and 2009-10 under AICRP on MULLaRP. On the basis of performance, it was recommended to be included in 'National genetic Stock Nursery' (NGSN). The RILHF-2 is a tendril-less genotype with normal stipule. It matures in 120 days and has the seed weight of 20.10 g/100-seed. The major components of slow rusting are longer latent period, lesser pustule size and lower infection frequency (Singh *et al.*, 2015). RILHF-2 possibly possesses these components of slow rusting thereby imparting higher resistance to rust disease, thus it will serve as a donor parent for rust resistant breeding programme in pea.

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4. UUHF/ACM/Garlic-11-1 (IC0598236 (INGR16035), a Garlic (*Allium ampeloprasum* var. *ampeloprasum* L.) Genotype with Bolder Size of Bulbs, Bears Umbels with Micro-Cloves, Suitable for Cultivation in Frost Prone Hills

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A few plants of great headed garlic were found as admixture in the garlic samples collected by the Department of Vegetable Science, Uttarakhand University of Horticulture and Forestry, Ranichauri Campus, Tehri-Garhwal from different areas of the district. The selected material was clonally multiplied and evaluated for different morpho-agronomic and tuber yield and quality traits over the years. The genetic material IC0598236 was identified as *Allium ampeloprasum* var. *ampeloprasum*L. (great headed garlic, levant garlic or elephant garlic) on the basis of longer, dark green, broad and leathery leaves, bigger size of bulbs weighing 70-75 g composed of 10-12 cloves (6-8 g each) and terminal umbel consisting of a large number (150-175) of micro cloves (Brewster, 1994 & 2008; Mishra, 2016). The geographical distribution of great headed garlic is confined to restricted areas of South-western England and France (Bohanec *et al.*, 2005). Occurrence of this *allium* in India though has not been documented so far was probably because of old introductions from European countries. The umbels consist of sterile stamens and ovaries. The ovaries owing to storage of food materials behave as vegetative propagules in the form of micro-cloves/bulbils. The bulbils or micro-cloves have ability to sprout and develop new plantlets. However, the bulbs produced as a result of planting these plantlets, were not comparable in size to those obtained from the crops raised from commercial cloves and therefore, commercial bulbs could not be obtained from the micro-cloves in the same year. The miniature bulbs (weighing 12-15 g) produced from transplanting the micro-clove grown plantlets also consist of 10-12 tiny cloves (miniature cloves). Raising crop by planting miniature cloves, however, exhibited almost *at par* bulb yield with comparable size and compactness to those produced from commercial cloves (Mishra and Pandey, 2015). By sowing 8-10 kg of micro-cloves/bulbils of great headed garlic in nursery beds, sufficient quantity of plantlets can be produced to plant one hectare area at 10x10 cm spacing and about 54-65 q of miniature bulbs can be harvested. Thus, miniature cloves separated from miniature bulbs could be used as planting material at the

S.No.	Name of the genotypes	Bulb weight (g)	10 Clove weight (g)	Total soluble solids (%)	Dry matter content in cloves (%)	Marketable bulb yield (q/ha)
1.	*A. ampeloprasum* var. *ampeloprasum* L. (IC0598236)	66.8	64.4	41.9	46.8	250.32
2.	*A. sativum* L. (Agrifound Parvati)	30.03	31.03	39.3	46.1	160.05
3.	*A. sativum* L. (Ghansali Local)	34.1	36.13	44.3	56.2	119.71
	CD at 5%	3.8	4.7	2.4	3.2	15.9
	CV (%)	13.6	11.8	5.6	7.9	14.6

rate of 1.5 q/ha for raising commercial crop in against higher seed rate of commercial cloves (8.0 q/ha).

The experimental trials on great headed garlic were conducted by planting the cloves at 20x10 cm spacing during second fortnight of October cloves of the years 2012-13, 2013-14, 2014-15 and 2016-17. The crop was supplemented with manures @ 15 t/ha and NPK @ 100:80:60 kg/ha. For maintaining proper soil moisture, the crop was irrigated by sprinkling water as and when required. Mulching with dried grasses was also done to conserve soil moisture and keep the field free from weeds. The bulbs were harvested during first fortnight of May.

The data averaged over four years (2012-13 to 2015-16) indicated that the marketable bulb yield in IC0598236 was 250.32 q/ha which was considerably higher than that in checks Agrifound Parvati (160.05 q/ha) and *Ghansali Local* (119.71 q/ha), cultivars of *Allium sativum* L. The cloves of IC0598236 have TSS as high as 41.9% in against 39.3% in *Agrifound Parvati* and 44.3% in *Ghansali Local*. Similarly, bulb and clove weight were also found to be much higher in IC0598236 (66.8g and 6.4g, respectively) as compared to Agrifound Parvati (30.03g and 3.1g, respectively) and Ghansali Local (34.1 g and 3.6 g, respectively). The dry matter content of the cloves was found to be 46.8% in IC0598236 at par to *Agrifound Parvati* (46.2%), however, *Ghansali Local* registered quite higher dry matter content in cloves (56.2%) as given in the Table:

The great headed garlic genotype IC0598236 was found to be tolerant to foliar diseases like purple blotch and downy mildew. However, incidence of root rot is realized during prolonged, high soil moisture conditions. There was no conspicuous infestation of common pests in alliums of temperate hills. This species is also tolerant to severe and prolonged frost. About 10-12 cloves are loosely arranged on a hydroscopic basal plate and covered with two layers of papery sheath (Fig.1B). Larger inherent interspaces between cloves and enlargement of basal plate during *Monsoon* further widen the same and this inclusively lower the storability of bulbs beyond the month of October.

The studies indicated that the great headed garlic genotype IC0598236 with Registration No. INGR16035 had almost 36% higher bulb yield as compared to *A. sativum* cultivars. The IC0598236 was also promising in respect of bulb weight, TSS, dry matter content in cloves and tolerance to diseases, insects and frost as compared to commercial cultivars of *A. sativum*. Owing to number beneficial traits, *A. ampeloprasum* var. *ampeloprasum* genotype IC0598236 holds the potential as a commercial crop in western Himalaya.

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5. NAIP (PB)-01 TCR W390 (IC0599974; INGR16036), a Musk Mallow (*Abelmoschus moschatus* subsp. *moschatus* × *A. moschatus* subsp. *tuberosus*) Perennial Germplasm with Bright Red Flower

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Two infra-specific taxa of *Abelmoschus–A. moschatus* subsp. *moschatus* and *A.moschatus* subsp. *tuberosus* were hybridized to produce an ornamental hybrid. The hybrid can be easily produced by hand pollination and F_1 can be propagated through stem cuttings. The hybrid has perennating tap roots which makes it flourish for many years. Year round cultivation and flowering under equatorial climate has been observed. The hybrids came to flowering within 50-55 days after sowing. The progenies were intermediate between the parents for most of the quantitative traits but exceptional for bright red flower colour, big petal size, prolificacy and extended flowering span which makes it a worthy candidate for ornamental horticulture. Compared to parents, long flowering span exceeding 9 months was observed in the F_1 hybrids. On an average, the potted hybrid produced 20 primary branches with 5-6 flowers per day. Flowers open fully by about 8.00 am and remain till sunset. Flowers can also be kept as cut flower for around 8-10 hours in indoor. Rooted cuttings and seedlings can be used to raise beds which offer a contrasting canopy of red and green on borders and back grounds of gardens and parks.

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6. IC0560611 (IC0560611; INGR16037), a Jasmine (*Jasminum malabaricum*. Wight) Germplasm with High Concrete Recovery (0.375%) and Higher Percentage (17%) of Esters Group of Volatiles

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Jasmine is an important traditional flower crop belonging to the family Oleaceae. In, India it is grown for different uses which includes traditionally for loose flowers, gardening purpose, making garlands and for the extraction of concrete and absolute and essential oils, used in high grade perfumery industry. It is the major flower crop of Southern parts and also grown in some of the northern parts of the Country. *Jasminum malabaricum* germplasm (IC0560611)is unique because of its high concrete recovery (0.375%) with significantly higher percentage (17%) of esters group of volatiles in flowers. It was collected from northern parts of Western Ghats and successfully domesticated at IIHR, Bengaluru, Karnataka (13^{o} 58' N Latitude, 78^{o}E Longitude and 890 m above mean sea level) (IIHR Annual Report, 2012-13).

Morpho-agronomic characteristics: It is a large climber and responds well to pruning with opposite membranous leaves, which are broadly ovate in shape. Flowers are white in colour with slightly pinkish corolla and up to 6cm long and 3cm wide at the mouth. Flowers have 6-8 petals of 1.8 to 2.3 cm in size. And corolla is pinkish in colour. Number of flowers per cyme varies from

60-80. Flowers are born in cymes and bloom from February to May and sets seeds which are very rare in the jasmine species.

Associated characters and cultivated practices: *Jasminum malabaricum* germplasm (IC0560611) produces high concrete recovery (0.375%) with significantly higher percentage (17%) of esters group of volatiles in flowers. There are only three species which are commercially exploited for this purpose. New species with high concrete recovery can boost the perfumery industry. Therefore *Jasminum malabaricum* has a potential for commercial flower production and can be used in future breeding programmes. *Jasminum malabaricum* grows well in well drained rich sandy loam soils. The ideal conditions for successful cultivation are warm summer with ample water supply and sunny days.

Table 1. Evidence of Esters present in *Jasminum malabaricum*

Name of the Compounds	K.I.*	*J.malabaricum*
Esters		
2-Hexenyl propanoate	1111	8.299
Benzyl ethanoate	1168	0.150
Hexyl butanoate	1190	0.082
Methyl salicylate	1198	0.695
Hexyl 2-methylbutanoate	1237	0.897
Ethyl salicylate	1270	0.007
Sabinyl acetate	1298	0.156
(Z)-3-Hexenyl pentanoate	1308	0.082
Isobutyl benzoate	1322	0.140
cis 3-Hexenyl tiglate	1324	3.880
Hexyl tiglate	1351	0.627
Butyl benzoate	1352	0.288
Isoamyl benzoate	1422	0.472
cis-3-Hexenyl Benzoate	1570	0.545
Phenyl benzoate	1668	0.382
5-Hydroxypentyl benzoate	1708	0.463
Methyl hexadecanoate	1926	0.116
Ethyl hexadecanoate	1975	0.015
		17.182

*Kovat index

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GUIDELINES TO AUTHORS

GENERAL

Indian Journal of Plant Genetic Resources (IJPGR) is the official publication of the Indian Society of Plant Genetic Resources. Aim of the Journal is to disseminate knowledge on plant genetic resources (PGR) research and application. Being the only journal in the area of PGR, the journal aims to provide a forum for discussion and debate on current issues of PGR. For publication in the journal, the authors must be a member of the Society. IJPGR publishes full-length papers or short communications of original scientific research in the field of plant genetic resources. Review articles (with prior consent or invitation only) summarizing the existing state of knowledge in topics related to plant genetic resources will also be published.

Contributions should be as concise as possible. The maximum length of the review article, full-length papers and short communications is usually restricted to 12, 6, 3 printed pages including illustrations and tables, respectively.

SCOPE

- Basic research on biosystematics, genetics and genomics related to PGR
- Applied research on field and laboratory evaluation of PGR
- Supportive research in conservation and quarantine of PGR
- Policy research on access and benefit sharing; IPRs
- Status reports on ecology, conservation, traditional knowledge and use of PGR

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ORGANIZATION OF THE MANUSCRIPT

Full-length papers

Title Page: The title page of the manuscript should be the first page and should include the title, names and addresses of the authors, abstract and keywords.

Title: Keep the title brief, specific and informative and amendable to indexing. It should be typed in running text with first letter of word as capital and latin names in italics.

Name and Address: The name of authors and the address of the institution where the work was carried out should be mentioned below the title. Present address of correspondence, if different, should be given as footnote indicating by asterisk (*) the author to whom the correspondence and reprint requests are to be made. E-mail addresses should also be indicated, if any.

Abstract: The abstract should clearly state the rationale, objectives, methods, and important conclusions of the study. It should not exceed 150 words.

Key Words: The abstract should be followed by not more than five key words indicating the contents of the paper and useful for abstracting purposes.

Main Text: The main text of the paper should start from the second page which should contain the title of the paper followed by the text divided into following main headings which are to be typed in running text and flushed with the margin: Introduction, Materials and Methods, Results, Discussion, Acknowledgements, References. Wherever appropriate, results and discussion can be combined and acknowledgements be omitted.

Introduction should be brief and limited to the statement of the problem and aim of the experiment.

Materials and Methods should include relevant details on the nature of material, experimental design, the techniques employed and the statistical method used. For well-known methods, citation of reference will suffice.

Results and Discussion should be clear to readers in different disciplines. Units of measurement should be SI.

Tables should be typed on separate sheets, each with a heading stating its contents clearly and concisely. Numerical data and calculations should be thoroughly checked.

Figures of only good quality that are essential to a clear understanding of the paper shall be accepted. Legends to the illustrations should be typed on separate paper. Information in the legend should not be repeated in the text and similarly, the same data should not be represented in both graph and table form. All figures, whether photographs, maps, graphs or line drawings should be numbered consecutively. Illustration number and title of the article with authors' name should be given at the back of the plates in soft pencil.

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Acknowledgements should mention only guidance or assistance received in real terms, and financial grant provided by an agency. Acknowledgements for inspiration, typing etc., need not be mentioned.

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Withers LA and F Engelmann (1998) In vitro conservation of plant genetic resources. In: A Altman (ed.) *Agricultural Biotechnology.* Marcell Dekker Inc., New York, pp 57-58.

WOI (1985) *The Wealth of India - Raw Materials. A Dictionary of Indian Raw Materials and Industrial Products – Raw Material* Vol 1: A (Revised). Publications and Information Directorate, Council of Scientific and Industrial Research, New Delhi, 513 p.

Engels, JMM and V Ramanatha Rao (eds) (1988) *Regeneration of seed crop and their wild relatives.* Proceedings of a Consultation Meeting, 4-7 December 1995. ICRISAT, Hyderabad, India and IPGRI, Rome, Italy, 167 p.

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E-mail:*ispgr2015@gmail.com*

Publisher website: *www.nbpgr.ernet.in/ispgr*

Journal hosting and online submission of manuscript: *www.indianjournals.com*

Print ISSN: 0971-8184
Online ISSN: 0976-1926

Indian Journal of Plant Genetic Resources

Vol. 31 No. 2 2018

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Published by the *General Secretary*, Indian Society of Plant Genetic Resources, NBPGR Campus, New Delhi-110012 and printed at *Angkor Publishers (P) Ltd.*, B-66, Sector 6, NOIDA. Mobile: 9910161199, E-mail: angkor@rediffmail.com.