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RESEARCH ARTICLE

On-farm Conservation of Rainfed Rice Landrace Diversity in Chhattisgarh, India

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India is considered as one of the centers of origin and diversity for Asian rice, where a large number of landraces are still under cultivation. Though, there has been continuous loss of genetic diversity but concerns have been addressed with increased pace in recent years. Keeping this in mind, the present study was aimed to collect, conserve (ex-situ) and identify the determinants of on-farm landrace diversity in the rainfed ecosystem of Chhattisgarh. The landrace diversity of rainfed rice and knowledge about their socio-economic and cultural importance among the local farming communities of six districts of Chhattisgarh was documented during this study. The farmers are presently maintaining various drought tolerant rice landraces on on-farm (in-situ) through rainfed cropping system. Hence, considering the significance of genetic resources of rice particularly for drought tolerance under changing climate locally adapted cultivars of rice growing in rainfed conditions were collected during *kharif* 2015. Based on the morphological and quantitative characters as observed in farmer's field, 48 diverse samples of rice landraces were assembled along with associated indigenous traditional knowledge. Based on quantitative characters, landraces were grouped into two major clusters each of which further grouped into two sub-clusters keeping in view the similarity in traits. Besides agro-morphological characters, various observations pertaining to agro-ecological habitats, traditional processing methods, socio-economic and cultural development, diversity available, factors responsible for erosion/replacement of diversity, visual values and specialized food quality etc. have been discussed.

Key Words: Diversity, Genetic resources, Indigenous traditional knowledge, Nomenclature, Rainfed rice landraces

Introduction

Rice (*Oryza sativa* L.) is one of the major staple food crops. Asia is the largest producer of rice contributing around 91% of total world rice production, while India contributes about 26% (Verma, 2017). India is the center of origin of Indica rice (*O. sativa*), wherein Odisha and adjoining parts of Chhattisgarh are considered the cradle of rice (Chauhan *et al.*, 2000). It is cultivated in about 45% rainfed area of the world, of which, 41% area alone falls in South and South East Asia (Maclean *et al.*, 2002; Pandey and Bhandari, 2008). Rainfed rice growing areas are considered as highly prone to drought, high temperature and submergence because of uneven rainfall distribution and topography. Most of the landraces and farmers varieties are evolved in rainfed areas and still being maintained by poor and marginal farmers. In India, most of the traditional landraces of rice have been collected from such environments (Chauhan *et al.*, 2000; Shukla *et al.*, 1996; Sarawagi and Rastogi, 2000).

Among different stresses, drought is the major constraint which can reduce rice yield in rainfed areas (approximately over 23 million ha) of South and Southeast Asia (Huke and Huke, 1997). According to Chauhan *et al.* (2000) about 74% cropped area under rainfed ecosystems is occupied by high yielding varieties (HYV) and that could be even higher in irrigated ecosystems.

In Chhattisgarh, rice is mainly grown in rainfed ecosystem that covers about 74% cropped area under central plain, 97% under Bastar plateau and 95% under northern hill zones. The central plains of Chhattisgarh are known as rice bowl of central India (Patel and Srivastava, 2015). In Eastern India, problem of drought is comparatively severe because more than 10 million ha of drought-prone fields have been noticed with 40% yield loss mainly in Jharkhand, Odisha and Chhattisgarh. According to Richharia (1979), significant variation in climate, topography, soil and hydrology coupled with

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varying cultural heritage have resulted into evolution of high genetic diversity in rice germplasm in Chhattisgarh. So far, limited success could be achieved in development of draught tolerant rice as compared to other traits. According to Haque *et al.* (2013), several donors are available but use of unstable donors with high drought tolerance particularly at the reproductive stage of the crop is very crucial to get optimum yield under drought conditions. Hence, considering the impact of climate change and associated factors, identification of suitable genotype for use in improvement of rice for drought tolerance is need of the hour.

Materials and Methods

Study Area

To conduct the present study, an exploration was undertaken in six districts (Rajnandgaon, Balod, Raigarh, Kanker, Naryanpur and Kondagaon) of Chhattisgarh for collection of rainfed rice germplasm during October-November, 2015. The study area lies between 17º46'N to 24º5'N latitude and 80º15'E to 84º20'E longitude with an altitudinal range between 313 to 660 m asl. The study area is bounded by Madhya Pradesh in northwest, Maharashtra in southwest, Telangana in south, Odisha in east and Jharkhand in northeast (Fig. 1).

Various tribal communities (Lodhi, Marar, Thanwar, Suri, Markam, Vattee, Mandvi, Netam, Bhaskar, Uike, Anchla, Kaloo, Vaddey, Muria, Sarda, Gonds and Rathia) reside in the remote localities of study area. Due to undulating topography and backwardness of the area, rainfed agriculture is the major livelihood source for them. Old and experienced farmers were contacted to record the information about the traditional rice landraces grown in the past and their status at present in the area. Coordinates (longitude & latitude) of collection sites were recorded using Global Positioning System (GPS). Important characters of landraces and other related information were recorded using standard formats/ unstructured questionnaire. For sampling of germplasm, random/bulk population method was adopted (Marshall and Brown, 1975; Hawkes, 1976, 1980) while passport data book was used to record information on each accession. Data on eight quantitative and qualitative traits were recorded on-site (Table 1). Test weight of 100 grains was recorded in gr; awn length, kernel length and breadth in mm; while length/breadth ratio was also calculated. Various qualitative traits including tolerance/ resistance to biotic and abiotic stresses were also recorded. For recording the kernel colour, The Royal Horticultural Society (RHS) colour chart was used (Royal Horticultural Society, 2001). The seed coat characters were recorded as per descriptors developed by ICAR-National Bureau of Plant Genetic Resources (Bansal *et al.*, 2006). Data was analyzed using statistical software (MINITAB version 17.0: minitab.com). Cluster analysis of landraces was done keeping in view the similarity in quantitative traits (grain weight, grain length, grain breadth and grain l/b ratio). A matrix of similarity was generated based on the simple Euclidean distance across all accessions of different landraces, which was used in a hierarchical clustering technique of Ward's Minimum variance method (Ward, 1963; Kaufman and Rousseeuw, 1990).

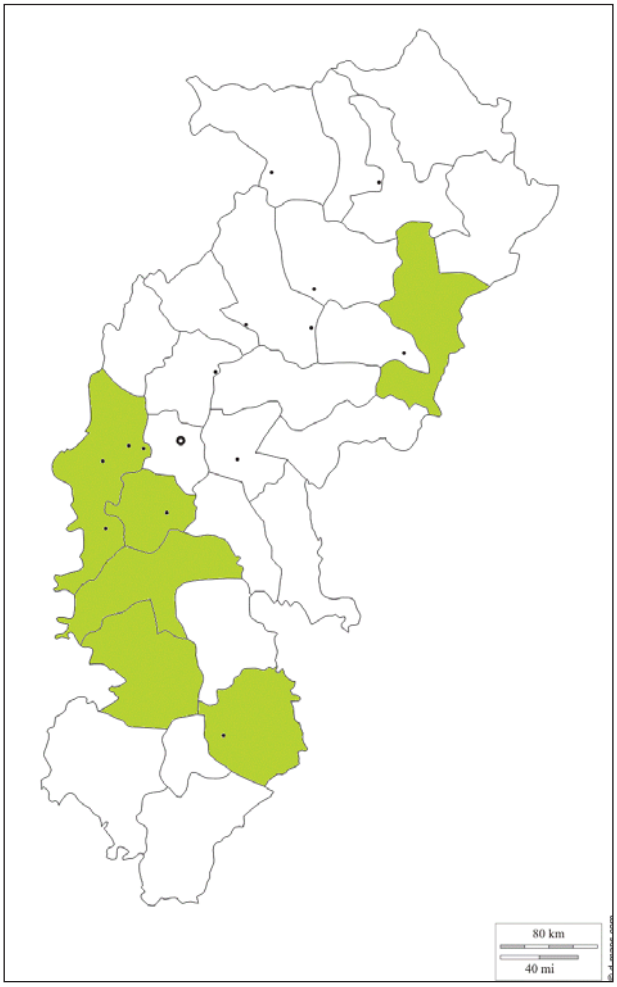

Fig 1. Rice germplasm collected from Raigad (1), Rajnandgoan (2), Balod (3), Kanker (4), Narayanpur (5) and Kondagoan (6) districts of Chhattisgarh.

Table 1. Characteristics of rice landraces collected from Chhattisgarh

S. No.	IC / Collection Nos.	Traditional landraces	Source*	Frequency#	100 grain wt. (g)	Grain length (mm)	Grain breadth (mm)	Grain colour	Kernel colour	Important characteristics and traditional uses
1	IC617726	Ampo dhan	TY	R	2.65	5.0	1.1	Light yellow	White	Grain medium sized, plant height 70-75 cm, drought tolerant, non-shattering and early type
2	IC 622694	Paras mani	FS	R	1.93	3.5	1.2	Yellowish	White	Grain fine, small, sweet in taste, plant height 70-75 cm and yield-12-15 q / acre.
3	RSR/SKY-4	Deshi sapri	FS	R	2.30	5.2	1.1	Yellowish	White	Medium sized grain, plant height 135-150 cm and yield-10-12 q / acre.
4	RSR/SKY-5	Dabaar dhan	FS	R	2.33	3.8	1.0	Reddish brown	Red	Grain bold, awned, plant height 70-75 cm, yield -10 - 12 q / acre. Rice is used for general debilities and diabetes.
5	IC 617727	Lal dhan	TY	R	1.87	4.3	1.0	Brown	White	Grain fine, husk hardy, plant height 70-80 cm and require little irrigation at the time of sowing.
6	IC-623311	Shri dhan	TY	R	2.41	3.0	1.0	Straw	White	Scented, plant height 105-120 cm and yield-10-12 q / acre, tolerant to insect pests. Rice is used for general debility and also offered to local deities during worship.
7	IC-623312	Sathia	FS	R	2.52	3.7	2.0	Blackish	Reddish	Drought tolerant, matures in 60-70 days, grain bold, awned. Grains are supposed to be highly nutritive hence cooked mainly on the occasion of Dushera Puja.
8	IC 617728	Dabaar dhan	FS	R	2.80	4.0	1.3	Reddish brown	Red	Grain awned, tolerant to insect/ disease and drought, plant height 90-100 cm and yield 10 -15 q / acre.
9	IC 622695	Jandra dhan	FS	R	2.19	4.7	1.2	Yellowish	Red	Grain medium sized, extra early, drought tolerant, plant height 65-75 cm.
10	IC 617729	Dabaar dhan	FS	R	2.70	4.2	2.0	Black	Red	Grain awned, plant height up to 90 cm. Rice water is used as medicine in general weaknesses.
11	IC 617730	Turia dhan	FS	R	1.59	4.2	1.1	Straw	White	Scented type with very fine grain, awned. very early, tall, plant height up to 75 cm.
12	IC 622696	Bayo dhan	FS	R	1.84	4.5	1.1	Brownish yellow	Red	Grain fine, small, awned, plant height up to 75 cm. Cooked rice is used for general weaknesses.
13	IC 617731	Turia dhan	TY	R	1.70	4.3	1.7	Golden yellow	White	Grain very fine, scented, panicle in cluster, drought tolerant, plant height up to 120 cm. Grains sweet in taste, used in general weaknesses.
14	IC 622697	Kolia punchhi	TY	R	1.12	4.0	1.0	Reddish brown	White	Grain scented, panicle is like ear head of foxtail-millet, plant height up to 90cm, drought tolerant. Cooked grains are sweet.
15	IC 622698	Dokra munchha	FF	R	1.96	3.7	1.0	Light yellow	White	Awn slight curved like bent body of old age man having moustaches, grain small, bold, plant height 110-120 cm, very early, drought tolerant, matures in September.

Contd.

S. No.	IC / Collection Nos.	Traditional landraces	Source*	Frequency#	100 grain wt. (g)	Grain length (mm)	Grain breadth (mm)	Grain colour	Kernel colour	Important characteristics and traditional uses
16	IC-623313	Dabaar dhan	TY	R	2.63	4.5	2.3	Brownish black	Red	Grain bold, awned, plant height 70-80 cm, yield-10-12 q / acre, drought tolerant. Rice is used for general weaknesses and diabetes.
17	IC 622699	Dabaar dhan	FF	R	2.47	4.0	1.3	Brownish black	Red	Grain bold, awnless, plant height 75-90 cm, yield - 12-14 q / acre, drought tolerant. Rice is used for general weaknesses and diabetes.
18	IC 617732	Para dhan	TY	R	2.79	4.2	1.3	Light yellow	Red	Grain small, awned, plant height 60-70 cm, early, matures during September, highly drought tolerant.
19	IC 617733	Kalapara	FS	R	2.42	3.5	1.0	Black	White	Very early type; matures in August (60-75 days), plant height 70 cm, highly drought tolerant. Kernel white but after cooking its colour is changed to red; sweet and best for Chapati making and for preparation of local beverage.
20	IC-623314	Koliya punchha	FS	R	2.27	6.1	1.2	Dark brown	White	Grain fine and scented, panicle resembles with tail of Fox, plant height 90-105 cm, drought tolerant and matures during October.
21	RSR/SKY-28	Lalichudi	FS	R	1.75	4.2	1.0	Light brown	Reddish	Grain fine, scented. It requires little irrigation at the time of sowing.
22	IC 622700	Bans bhida	FS	R	1.27	4.1	1.0	Light yellow	White	It has high number of tillers like bamboo, grain fine, small, plant height 60-70 cm, drought tolerant, early type and yield 10-12 q/acre. This has been cultivated for the past 100 years.
23	IC 622701	Luchai	TY	R	1.56	4.1	1.0	Light yellow	Red	Grain fine, small, awnless, plant height 60-70 cm, drought tolerant and yields 10 q/acre. Tasty and the kernel increases in size when cooked.
24	IC 622702	Dhan	TY	R	1.68	5.0	1.2	Light yellow	White	Grain fine, medium, plant height up to 60 cm and drought tolerant.
25	IC 622703	Kalinga	FS	R	1.89	5.0	1.3	Light yellow	White	Fine grained, plant height up to 60 cm and drought tolerant.
26	IC 617734	Chuhka dhan	FS	R	1.33	3.1	1.1	Brown with yellow patches	White	Fine grain & scented, plant height 90 cm, number of tillers high, drought tolerant and yields 10- 15 q/acre. Rice good in taste, preferred in feast during Pujas and also has good market price.
27	IC-623315	Dumar phool	TY	R	1.64	3.5	1.0	Brown	White	Panicle is compact like fig (Gular), grain fine, small, scented, plant height 75-90 cm, drought tolerant, non shattering type and yield 12-15 q/acre.
28	IC 617735	Chirai nakhi	TY	R	1.22	4.0	0.9	Light brown	Red	Grain very fine like beak of sparrow, small, scented, awned, plant height 90 cm, yield 12-14 q/acre, drought tolerant and very good taste.

Contd.

S. No.	IC / Collection Nos.	Traditional landraces	Source*	Frequency#	100 grain wt. (g)	Grain length (mm)	Grain breadth (mm)	Grain colour	Kernel colour	Important characteristics and traditional uses
29	RSR/SKY-37	Gada khunta	FS	R	3.03	4.1	1.5	Light Yellow	White	Plant is strong like wooden/iron rod used to hold the animals. Grain bold. Plant height. 160 - 180 cm, drought tolerant, late maturing with strong stems and yields 12-15 q / acre.
30	IC 622704	Dhania	FF	R	2.38	4.0	1.2	Brown	White	Grain bold , stem strong, long panicle and plant height 100 -120 cm.
31	IC 617736	Sathia	FS	R	2.16	4.0	1.1	Light Yellow	Red	Very early, matures in 60 days in August, tall up to 105 cm and highly drought tolerant.
32	RSR/SKY-40	Barangi	FS	R	2.57	4.5	1.5	Light Yellow	White	Grain bold, matures in 100 days, tall up to 80 cm and sweet.
33	RSR/SKY-41	Gurmutia	FS	R	2.38	4.0	1.1	Golden	White	Grain bold, plant height 90 cm, stem and leaf colour blackish green, stem strong, late in maturity (140 days), drought tolerant, yield 15 q / acre and cultivated in compact soils.
34	IC 617737	Bhata sapri	TY	R	2.25	5.3	1.1	Golden	White	Grain medium sized, very dwarf, 45 - 50 cm in height, stem strong, drought tolerant and matures in 60-70 days. Cooked rice is used in general weaknesses.
35	IC 617738	Culture	FS	R	1.83	4.0	1.0	Light yellow	White	Grain fine, plant tall up to 90 cm, stem strong, drought tolerant and matures in 60 - 70 days.
36	IC 617739	Bhata sapri	FS	O	2.10	4.8	1.2	Light yellow	White	Grain medium sized, plant very dwarf, 45 - 60 cm in height, stem strong, mature in 60 -70 days, drought tolerant and good taste.
37	IC 617740	Bhurshi	FS	R	1.63	3.5	1.3	Whitish yellow	Red	Grain small, awned, plant height 60 -75 cm, matures in 70-80 days and drought tolerant. Cooked rice water taste like milk and is preferred for food.
38	IC 622705	Khut bodi	FF	R	2.55	4.2	1.1	Brown with yellow spots	White	Grain small, awned, plant tall up to 75 cm, matures in mid October, grown in rainfed condition and drought tolerant.
39	IC 617741	Barangi	FF	O	3.03	4.5	1.8	Mottled yellow	White	Grain small, rounded, plant height up to 90 cm, brown spots on leaves and stem strong. This landrace gives better yield under zero inputs and highly drought tolerant.
40	IC 622706	Barangi	FF	O	2.68	5.0	1.5	Mottled	White	Grain small, round in shape, plant height up to 90 cm, stem strong and with brown spots on leaves. It gives better yield under zero input and highly drought tolerant.
41	IC 622707	Ashan churi	FS	R	2. 13	5.1	1.0	Dark yellow	White	Grain fine, plant height 90 to 100 cm and drought tolerant.
42	IC 622708	Mandria	FS	R	2.54	4.2	1.8	Golden	White	Grain bold, plant height 90 cm and drought tolerant.
43	IC 622709	Haldi ghanthi	FS	R	2.50	4.4	1.2	Yellowish brown	White	Grain very fine, plant height up to 90 cm and drought tolerant.

Contd.

S. No.	IC / Collection Nos.	Traditional landraces	Source*	Frequency#	100 grain wt. (g)	Grain length (mm)	Grain breadth (mm)	Grain colour	Kernel colour	Important characteristics and traditional uses
44	IC 617742	Kolia dhan	TY	R	2.40	3.5	1.0	Black	Blackish	Grain medium sized, plant height up to 70 - 80 cm, drought tolerant and matures in 90 -100 days.
45	RSR/SKY-57	Bhata sona	TY	R	1.54	4.2	1.0	Black	White	Grain small, very fine, plant height up to 60 cm, matures in 90 -105 days, highly drought tolerant and good taste
46	IC 617743	Anjan dhan	TY	O	2.56	3.5	1.0	Yellowish brown	White	Grain bold, medium sized, plant height up to 60 cm, matures in 90 -105 days and sweet.
47	IC 617744	Culture	TY	O	2.27	6.1	1.5	Light yellowish brown	White	Grain very fine, plant height up to 60 cm and matures in 70 - 80 days. Rice quality is good and very tasty. It is being cultivated since over 70 years.
48	IC 622710	Dabaar dhan	TY	R	2.54	4.0	1.3	Black	Blackish	Grain medium sized, bold, awned, tall up to 60-70 cm and matures during September. Rice is very good in taste and rice water is sweet.

*FF: Farmers field, FS: Farm store, TY: Threshing yard, #R: Rare, O: Occasional

Results and Discussion

During the survey, a total of 48 diverse landraces were collected from 18 villages of six districts of Chhattisgarh. It was observed that the old age (70-80 years) farmers were very much fond of memorizing the local names of most of the landraces grown in the area. The folk nomenclature of local landraces was done by observing their important characters, distinctiveness as well as continuous experimentation with them in their original habitat (Lando and Mark, 1994). The names of landraces were slightly different in some of the villages because of change of dialect of tribal communities. The farmers often assign name to the local landraces based on certain characteristics. Naming of landrace is quite scientific as it possesses certain traits which generally reflect in its name; hence a landrace could be identified easily, if a particular name is assigned to it. Appearance of seed and kernel, crop plants, taste, maturity, plant size, growing conditions are major factors which form the basis of naming a landrace. In Chhattisgarh, the nomenclature of traditional landraces/cultivars was also done on the basis of their use/characteristics (Table 1 and 2).

Literature indicates that over 20,000 rice cultivars/ landraces including some of the landraces viz., Bans bhira, Bhata sapri, Asan chudi, Chirai nakhi, Dhania, Dabar dhan, Gurmatia, Lal dhan, Lochai and Satia have been maintained by traditional farmers in Chhattisgarh (Singh, 2013). According to Pandravada *et al.* (2004), Barangi chudi, Bhatta dhan and Chudi dhan are commonly cultivated landraces of this region. The commonly grown landraces collected from Chhattisgarh were Desi-sapri, Dabaar dhan, Lal dhan, Shri dhan, Sathia, Turia dhan, Bayo dhan, Kolia dhan, Lali chaudi dhan, Kalinga, Chuhka dhan, Dumar phool, Gadha khunta, Barangi, Gurmatia dhan, Bhata sapri, Khutbodi, Ashan churi, Haldi ghanthi and Bhata sona. The landraces containing similar names at different localities have also showed variation in morphological traits. Similar observations have also been reported by Ahmed *et al.* (2016). In Chhattisgarh, the landraces are grown through direct seed sowing method for better yield. Majority of landraces are grown by the poor and marginal farmers under stress prone rainfed land locally known as dand/ bhata/tand/tikra (upland sites).

Variability in Traits

Rice landraces showed high variability with respect to quantitative and qualitative traits and adaptability to local environments. Significant variability was mainly observed for plant height, days to maturity, seed colour, seed-size and shape and awn length (Table 1 and Fig. 2). Variability observed in quantitative traits viz., grain weight, grain length, grain breadth and grain l/b ratio was also analysed. Maximum grain length (6.1 mm) was recorded in Kolia punchha and Culture while minimum (3.0 mm) in Sridhan. Similarly, maximum grain breadth (2.3 mm) was recorded in Dabaar dhan and minimum (0.9 mm) in Chirai nakhi. Rice landraces

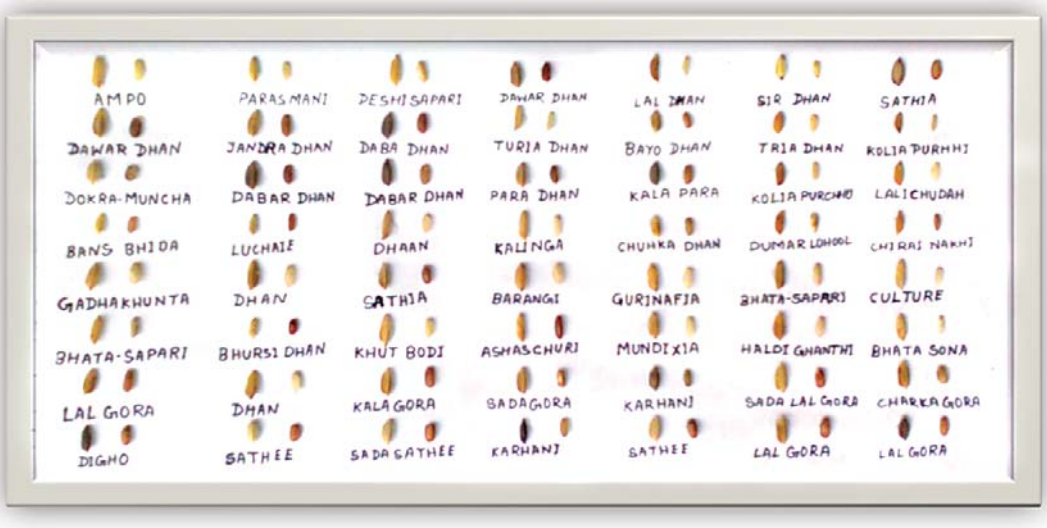

Fig 2. Grain and kernel variability in rice landraces

Table 2. Folk nomenclature and traits associated with rice landraces

Landraces	Meaning of landraces	Associated traits
Dokra mucha	Awn similar to mustaches of an old age man (Dokra-old man; mucha-mustaches)	Awn
Turia dhan	Sharp awn (Turia-sharp awn)	
Kolia punchha	Black coloured awns like tail of animal (Kolia-black; punchha-tail)	
Bans bhida	Dense tillers like many bamboo stems arising from single rootstock (bans-bamboo; bhida-dense/more)	Tiller
Dumar phool	Containing many panicles like fig contains many flowers inside fruit (Dumar-fig; phool-flowers)	Panicle
Chirai nakhi	Grain sharp as like bird's claw of toe (Chirari-bird; nakhi-claw)	Grain shape
Sathia	Matures in 60 days (Sathi-60 days)	Maturity
Gurmati	Suitable to grow in compact soils like jaggery (Gur- jaggery; mati-soil)	Soil type
Haldi ganthi	Husk colour is yellow like turmeric (Haldi-turmeric; ganthi-rhizome)	Grain husk colour
Lal dhan	Kernel colour is red (Lal- red; dhan-rice)	
Dabaar Dhan	Rice used as medicine (Daba/Dabaar) for stomach troubles.	Medicinal rice
Gada khunta	Stem strong as wooden pole embedded as a peg in soil (Gada-embedded; khunta-peg)	Lodging resistant
Bhata sona and Bhata sapri	Easily survive/grow in gravel soil and can withstand in draught conditions and gave better yield (Bhata-gravel soil and Sona-like gold)	Draught resistant
Kala para and Para dhan	Teeds arranged from the other side of Kala river (Kala-river or stream; para- other side of river)	Seed procurement source
Chuhka dhan	Rice supposed to be pious for offering in feast during worship (Chukha-pious; dhan-rice)	Religious importance

showed considerable variability for quantitative traits as indicated by coefficients of variation (CV). Among the traits studied, the highest variability (CV %) was observed for grain breadth (25.13 mm) followed by 100 grain weight (22.54 g) and ratio of grain length and breadth (21.83 mm) (Table 3).

Traits-specific Landraces

The unique traits and general characteristics recorded in 48 landraces are presented in Table 1. Among these, six landraces (Chiral nakhi, Chuhka dhan, Dumar phool, Lali chudi, Shri dhan and Turia dhan) were recorded as scented with fine grains; four landraces (Bhata sapri, Dabaar dhan, Shri dhan, Turia dhan and Bayo dhan) are considered good for general weaknesses and diabetes by Gonds; two landraces (Ampo dhan and Dumar phool) were identified as non-shattering types; Barangi is considered as one of the best landrace because of its highest grain yield under highly drought conditions; Dabaar dhan is cultivated mainly in the upland areas due to its early maturity, sweet taste and medicinal properties. Some of the promising collections identified for various other traits were IC622697, IC617741 and RSR/SKY-37 for 100 grain weight; IC-623311, IC-623314 and IC617744 for long grain and IC617735 and IC-623313 for grain breadth.

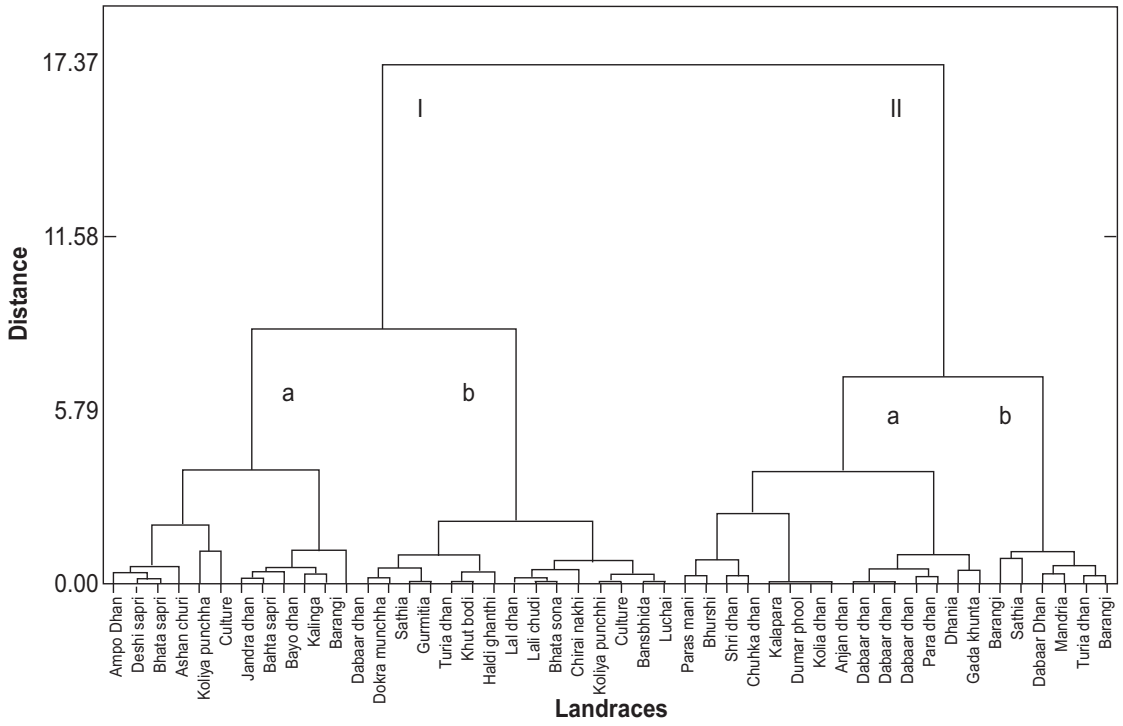

Fig 3. Clusters based on grain characteristics of landraces

Grouping of Landraces

To understand the association ship between the landraces, three quantitative traits (100 grain weight, grain length and grain breadth) were analyzed for grouping the similar genotypes into clusters and accordingly five clusters in 100 grain weight and four each in grain length and breadth were made. The maximum grain length (5.0 mm) in Barangi and grain breadth (2.3 mm) in Dabaar dhan had shown the close association with high 100 grain weight (Table 1). Results of similarity matrix based on Euclidean distance and hierarchical clustering technique are depicted in Fig. 3. The analysis has grouped all the 48 landraces in to two major clusters, consisting 27 landraces in cluster-I and 21 landraces in cluster-II. While cluster-I was further sub-divided into two sub-clusters (Ia and Ib), based on similarity in quantitative characters (grain weight, grain length and breadth, and l/b ratio). Sub-cluster (a) contains Ampo dhan, Deshi sapri, Bhata sona, Ashan churi, Koli punchha, Culture, Jandra, Bhata sapri, Bayo dhan, Dhan, Kalinga and Barangi while sub-cluster (b) contains Dabaar dhan, Dokra munchha, Sathia, Gurmatia, Turia dhan, Khutbodi, Haldi ganthi, Lal dhan, Lalichudi, Bhata sona,Chirai nakhi, Kolia punchhi, Culture and Luchai. Cluster-II was also further sub-divided into two sub-cluster (IIa and IIb). The landraces (Parasmani, Bhurshi dhan, Shri dhan, Chuhka dhan, Kalapara, Dumar phool, Kolia dhan, Anjan dhan, Dabaar dhan, Para dhan, Dhania dhan, Gada khunta and Barangi belong to sub-cluster IIa and Sathia, Dabaar dhan, Mandria, Turia dhan and Barangi to sub-cluster (IIb) (Fig. 3).

Table 3. Quantitative analysis of grain characters in rice landraces collected from Chhattisgarh

Variables	Minimum	Maximum	Mean	SE	CV
100 grain wt. (g)	1.12	3.03	2.16	0.07	22.54
Grain length (mm)	3.00	6.10	4.25	0.09	15.45
Grain breadth (mm)	0.90	2.30	1.24	0.04	25.13
l/b ratio	1.85	5.10	3.57	0.11	21.83

Determinants of On-farm Diversity

Poor economic conditions, remoteness and backwardness are the major reasons which force the poor and marginal farmers for cultivation of landraces under rainfed conditions. In addition, lack of irrigation and marketing facilities, difficulties in access to seed companies and adoption of new technologies are some other factors which are also equally responsible to compel the farmers for cultivating traditional crop diversity in these areas. Despite of all these factors, poor and marginal farmers play a major role in on-farm conservation of landrace

diversity and in providing ecosystem services to the society, which is very much essential for adaptation of crops against climate change. Moreover, the marginal farmers are not capable to continue on-farm conservation of crop diversity and also cannot sustain their livelihood for longer period with presently grown landraces. The society should also not expect maintenance of crop diversity by them for longer period at the cost of short-term gains. If society values resilient agriculture and sustainable food systems, a fruitful intervention to support the poor and marginal farmers particularly in tribal areas and sustenance of on-farm crop diversity needs to be thought off. During the last five years, several projects in support of on-farm conservation in India have also been executed by ICAR and other organizations. According to Gowdy *et al.* (2009); De Boef *et al.* (2013) and use of local crops and varieties (landraces and underutilized traditional crops) to sustain the food security of marginal farmers is very urgent in order to provide a wider range of options for livelihood diversification and economic growth, thereby promoting resilience and enhancing farmers' capacity to adopt against climate change.

The landraces collected from study area are grown traditionally from ancient time by the tribes of Chhattisgarh, indicates that on-farm conserved landrace diversity holds a special position in the livelihood and traditional culture of people of the study area. Xu *et al.* (2012) have also opined that the patterns of variation in on-farm varieties are directly associated with traditional culture and custom of different ethnic groups. His findings confirm the role of on-farm conservation in management of plant genetic resources, as it provides baseline information for betterment of on-farm conservation and sustained utilization of plant genetic resources.

Unfortunately for many reasons, the genetic diversity in rice landraces and other crops is eroding fast in different parts of the world. This fact has also been supported by Chauhan *et al.* (2000) and Hammer and Teklu (2008) by citing high genetic erosion in rice landraces due to replacement of traditional varieties through introduction of HYV. Hence, efforts should be made to provide irrigation facilities and seeds of important landraces with early maturity to the farmers in order to mining the shortage of food in the region and to upgrade socio-economic conditions. Landraces which are being grown under rainfed conditions should also be conserved *ex-situ* for utilizing in crop improvement against drought faced by the farmers in different parts of the country. Genetic diversity in folk varieties offers the basis for evolution of cultivated crops while distinctiveness expressed by various cultivars provides opportunity to adapt them in different environmental conditions. In South Asia, more than 100,000 folk varieties of the Indica group of rice are grown under rainfed conditions where they co-evolved with crop pathogens, insect pests and their predators (Richharia and Govindasamy, 1990; Morishima and Oka, 1995).

Conclusions

Landraces are still being conserved on-farm by ethnic communities and marginal/poor farmers in various parts of the country because these are most suitable for diverse agro-ecological conditions, deeply associated with cultural diversity and are capable to fulfill cultural and food requirements of the people. At present because of weakening of cultural traditions, declining of economic viability due to low yield, changing climate, the enthusiasm of farming/tribal communities to conserve traditional landraces on-farm for maintaining varietal richness is gradually decreasing. These facts necessitate efforts for collection and conservation of crop genetic resources from the areas where still primitive landraces/ cultivars are grown by rural/poor/marginal farming communities (Ishikawa *et al.*, 2002). It is a well known fact that the landraces which possess the desired characteristics such as drought tolerance, early maturity, stickiness, medicinal properties, aroma, kernel colour, etc. can become important resources for breeding and broadening the genetic base of rice. Thus, collecting such trait-specific germplasm would help in strengthening the food security effort in changing climatic condition and also to exploit their potential for biotic and abiotic stresses, yield and other quality traits. From the present study, it can be concluded that the landrace diversity in the study area is quite high. The most motivating factors for landraces diversification in the area are land heterogeneity, risk considerations and weekly market participation. Hence, to promote sustainable on-farm conservation of crop genetic resources and traditional cultural practices of ethnic communities in the study area, incentives to the farmers need to be provided.

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RESEARCH ARTICLE

Morphological Diversity of Buckwheat (*Fagopyrum* spp.) Landraces from Northeast India

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Buckwheat (*Fagopyrum* spp.) is an important crop in the high altitude regions of Northeast Indian Himalayas. The agro-climatic heterogeneity of this region offers a great deal of diversity in agro-morphology of buckwheat species. In the current study, a total of 97 accessions of *Fagopyrum esculentum* and *F. tataricum* were characterized for 16 morphological descriptors. Significant variations were observed for agronomically important traits including days to 50% flowering, days to 80% maturity, primary branches plant^{-1}, plant height and 100-seed weight. Wide range of variation was noted for most of the traits in both species. Characterization of buckwheat accessions from Northeast will facilitate in selecting morphologically diverse parents for breeding programmes particularly adapted to Northeast cropping system.

Key Words: Characterization and conservation, *Fagopyrum esculentum*, *Fagopyrum tataricum*, Northeast India

Introduction

Buckwheat (*Fagopyrum* spp.) is grown as a subsistence crop in the mountainous areas of Asia. The tartary buckwheat (*F. tataricum*), because of its frost tolerance, is generally grown at the higher altitudes whereas common buckwheat (*F. esculentum*) is grown at the lower altitudes. The crop is a pseudocereal. The seeds (strictly achenes) are usually classified among the cereal grains because of their similar uses (Campbell, 1997). The grains are generally used as food and feed. It can also be used as a green vegetable, a green manuring crop and as a smother crop. The grains are highly nutritive and the grain proteins are balanced in essential amino acid content, particularly lysine. Flowers and green leaves are the sources of the glycoside Rutin which has medicinal properties (McGregor and McKillican, 1952). Buckwheat protein is presumed to reduce serum cholesterol, suppress gallstones and tumours, and inhibit the angiotensin I-converting enzyme (Koyama *et al.*, 2013). The buckwheat grains are recommended for patients having typhoid and liver ailments. The flavones of buckwheat flower and leaves are found to be effective in improving insulin resistance in type II diabetes (Han *et al.*, 2009).

Buckwheat is widely grown up to an altitude of 4500 m in Northwest (NW) and Northeast (NE) Indian Himalayan regions (Joshi and Paroda, 1991; Joshi, 1999). In NE India, buckwheat is distributed throughout the states of Sikkim and Arunachal Pradesh. It is also grown in small scales in Manipur, Nagaland, Meghalaya and Assam (Hore and Rathi, 2002). In the high altitude areas of NE India buckwheat is grown as a staple food, where cereal crops cannot be grown due to low temperature. The buckwheat is consumed in various ways. The Monpa tribe of Dirang, Arunachal Pradesh prepares noodles from buckwheat flour. Tribal communities of Arunachal Pradesh also prepare local beer from buckwheat grains. In NE region, buckwheat is locally called by different names such as Paphar (meetha Paphar: *F. esculentum* and teeta Paphar: *F. tataricum*) and Khuster in Sikkim; Jheem, Kyap, Brasma, Chikaw, Bherem, Jamu, Dunchung and Grunching in Arunachal Pradesh; Phapar and Demsi in Assam.

Buckwheat germplasm collected from NE India have been conserved in the National Genebank of India. Earlier, a total of 40 buckwheat accessions collected during the period of 1987-2001 from NE India have been characterized (Hore and Rathi, 2002). However, during

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recent years many new accessions have been collected and there is a need for characterizing these germplasm. Therefore, the current study was undertaken to report the distribution of *Fagopyrum* spp. in the NE states of India and to assess the agro-morphological variability in the existing germplasm collections.

Materials and Methods

Plant Materials

A total of 97 buckwheat landraces (66 accessions of *F. esculentum* and 31 accessions of *F. tataricum*) collected in various exploration trips from different buckwheat growing regions of NE India have been included in this study. The collection sites of the accessions are presented in Fig.1. The indigenous collection (IC) numbers of the accessions are given in Fig. 2.

Morphological Characterization

The field experiments were conducted at ICAR-NBPGR Regional Station, Umiam, Meghalaya (25.6 ºN latitude, 91.9 ºE longitude and 1000 m altitude), during $2011^{-1}2$ and $2012^{-1}3$, under rain-fed conditions. The trials were laid out in augmented block design and the accessions were grown in plots of 5 rows × 1.2 m, with a spacing of 30cm (row-to-row) and 20 cm (plant-to-plant). Standard crop management practices were followed to raise a healthy crop. Data were recorded for 16 morphological descriptors, 8 quantitative and 8 qualitative (Table 1) on five plants randomly chosen from the two middle rows of each plot.

Data Analysis

The data on 8 quantitative traits were subjected to analysis of variance (ANOVA) and the descriptive statistics (mean, standard deviation, coefficient of variation and range) were worked out. The frequency distribution of buckwheat accessions for the qualitative traits was computed. Analyses were performed using IBM SPSS Statistics version 20.0 for Windows (IBM Corp. 2011; Armonk, NY: IBM Corp.). Cluster analysis was performed in NTSYS-pc version 2.11f (Rohlf, 1993). The data were first standardized using STAND function and then converted into a similarity matrix, using the simple matching coefficient (Sneath and Sokal, 1973), with SIMQUAL function. A dendogram was generated from this similarity matrix by the unweighted pair group method using arithmetic averages (UPGMA) (Sokal and Michener, 1958) with the SHAN function. The cophenetic correlation coefficient was calculated with Mantel test (Mantel, 1967) by comparing the matrix of cophenetic values with the similarity matrix, to determine how well the dendogram represents its corresponding pair wise

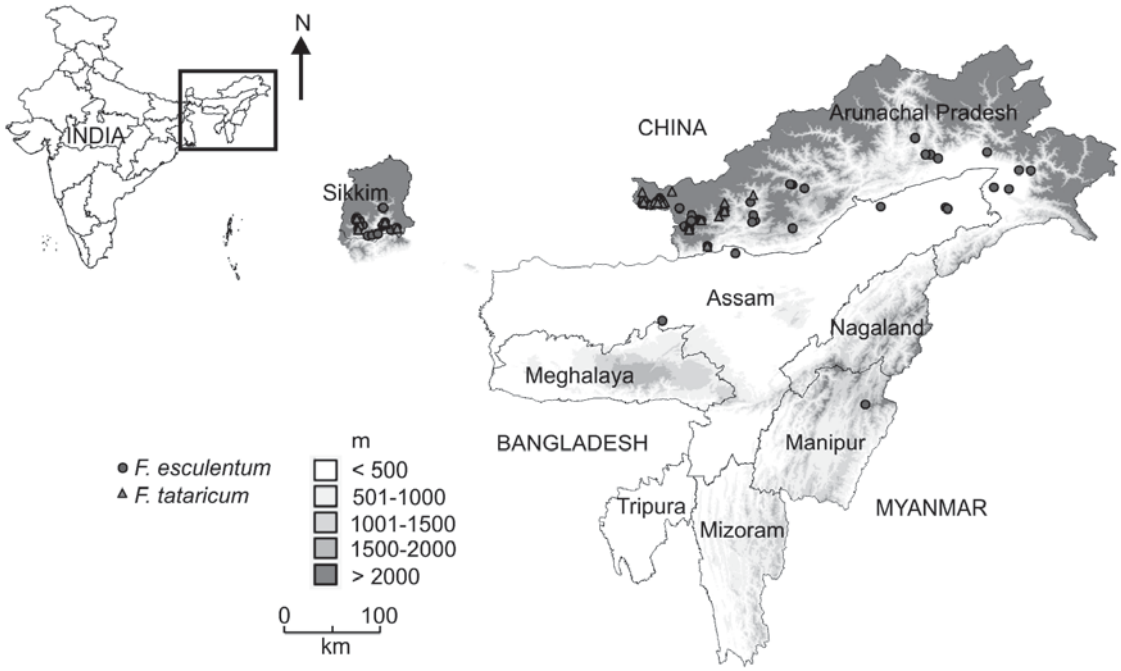

Fig. 1. Collection sites of 97 buckwheat (*Fagopyrum* spp.) landraces from NE India. *F. esculentum* and *F. tataricum* accessions showed separately. Altitude ranges are indicated by different grey shadings.

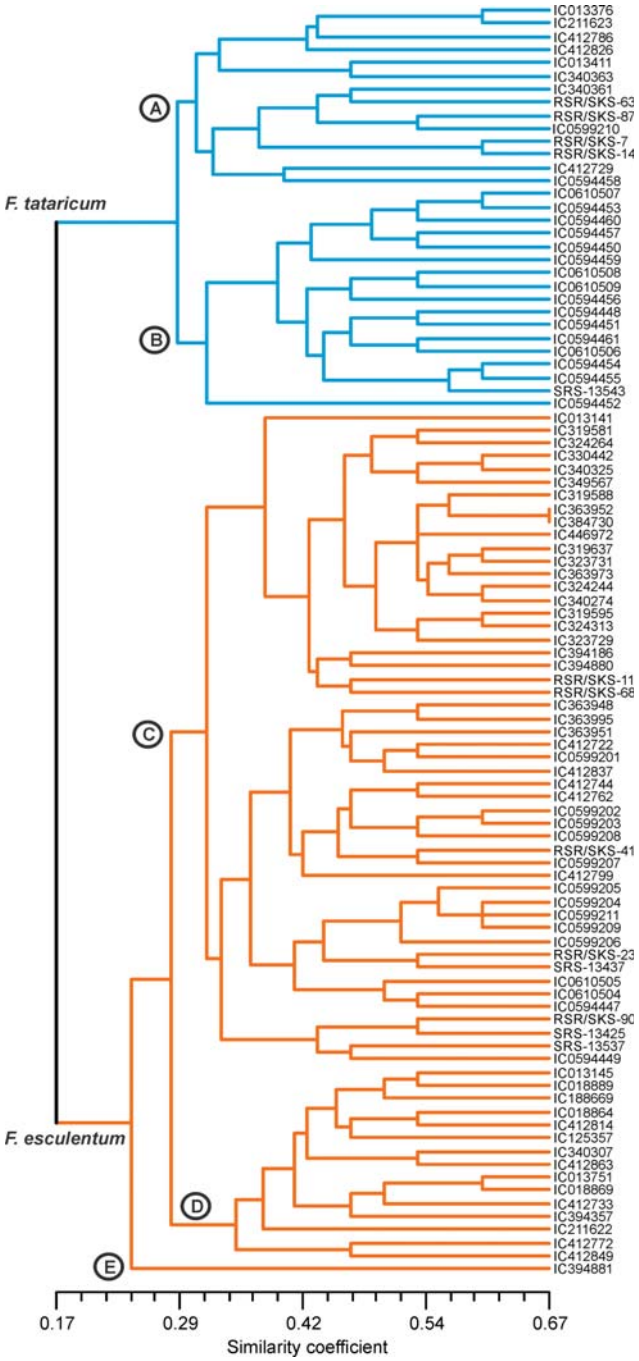

Fig. 2. The UPGMA dendrogram based on morphological data of 97 buckwheat accessions. Similarity values were calculated with the simple matching coefficient. Mantel test r = 0.79854, p = 1.0

distance matrix. This was performed with the COPH and MXCOMP modules. Principal component analysis was done using SPSS.

Results and Discussion

Buckwheat is widely grown in the Indian Himalayas, ranging from Jammu and Kashmir in the NW to Arunachal Pradesh in the NE India. About 340 accessions of these two species (*F. esculentum*: 294 and *F. tataricum*: 46) have been collected from the NE India by ICAR-National Bureau of Plant Genetic Resources, with the highest number of collections from Arunachal Pradesh. The passport data of past collections showed that only *F. esculentum* and *F. tataricum* germplasms are prevailing in NE Indian Himalayas. The buckwheat germplasm used in the present study fairly represent the entire buckwheat growing agro-climatic regions in NE India (Fig. 1). The collections have been made from an altitude range of 103 m (Kamrup, Assam) to 2971 m (Tawang, Arunachal Pradesh). The common buckwheat landraces are grown up to 2782 m altitude, while tartary buckwheat landraces are cultivated up to 2971 m (as recorded during exploration trips).

Table 1. List of descriptors used for characterization of buckwheat germplasm. For qualitative features, each descriptor is followed by a numerical code in parentheses

Serial number	Descriptor	Units/ States
Quantitative descriptors		
1	Days to 50% flowering	Days
2	Days to 80% maturity	Days
3	Leaf length	cm
4	Leaf width	cm
5	Primary branches plant^{-1}	-
6	Plant height	cm
7	100-seed weight	g
8	Seed yield plant^{-1}	g
Qualitative descriptors		
1	Plant growth habit	Erect (3); Semi-erect (5); Spreading (7)
2	Leaf colour	Green (3); Pink (5); Red (7)
3	Leaf margin colour	Green (3); Pink (5); Red (7)
4	Stem colour	Green (3); Pink (5); Red (7)
5	Leaf blade shape	Ovate (1); Hastate (2); Sagittate (3); Cordate (4)
6	Flower colour	White (1); Greenish yellow (3); Pink (5); Red (7)
7	Seed coat colour	Grey (3); Brown (5); Black (7); Mottled (9)
8	Seed shape	Triangular (1); Ovate (2); Conidial (3)

Morphological Characteristics

Descriptive statistics for the traits are given in Table 2. Among the buckwheat accessions, highly significant

Table 2. Comparative performance of different quantitative traits species-wise of buckwheat landraces

Descriptor	Species	Mean±SD	Range	Variance
Days to 50% flowering	All	30.8±3.72	25.0-45.0	13.8
	F. esculentum	30.5±3.34	25.0-39.5	19.3
	F. tataricum	31.5±4.40	26.0-45.0	11.2
Days to 80% maturity	All	78.3±9.98	61.0-99.0	103.7
	F. esculentum	73.8±8.41	61.0-93.0	88.7
	F. tataricum	85.1±5.44	76.3-99.0	70.8
Leaf length	All	5.6±0.86	3.3-8.0	0.7
	F. esculentum	5.7±0.93	3.4-8.0	0.3
	F. tataricum	5.3±0.59	3.3-6.1	0.9
Leaf width	All	4.9±0.62	3.0-6.4	0.4
	F. esculentum	4.8±0.68	3.0-6.4	0.2
	F. tataricum	5.0±0.42	4.0-5.8	0.5
Primary branches plant^{-1}	All	9.7±3.96	2.8^{-1}8.2	16.7
	F. esculentum	8.5±4.04	2.8^{-1}6.0	16.4
	F. tataricum	12.4±1.98	8.2^{-1}8.2	16.3
Plant height	All	68.4±15.44	34.9-99.7	235.5
	F. esculentum	75.6±12.09	36.5-99.7	234.9
	F. tataricum	53.0±9.32	34.9-70.5	146.3
100-seed weight	All	2.4±0.58	1.2-3.1	0.3
	F. esculentum	2.6±0.41	1.2-3.1	0.3
	F. tataricum	1.8±0.54	1.2-2.9	0.2
Seed yield plant^{-1}	All	26.5±10.90	3.6-54.6	124.4
	F. esculentum	27.9±12.71	3.6-54.6	40.4
	F. tataricum	23.6±4.21	16.4-32.6	161.5

variations were recorded for days to 50% flowering, days to 80% maturity, number of primary branches, plant height and 100-seed weight. Variation in the rest of the traits such as leaf length, leaf width and seed yield plant^{-1} was non-significant.The average days to maturity was higher in *F. tataricum* accessions (85.1 days) than that of *F. esculentum* germplasm (73.8 days). Wide variance for days to 80% maturity was noted in both species. Number of primary branches plant^{-1} varied from 2.8 to 16.0 in *F. esculentum* germplasm and from 8.2 to 18.2 in *F. tataricum* germplasm. Large variance was recorded for plant height in both species, while the tartary buckwheat accessions recorded the lowest average plant height. Both species exhibited wide variation for 100-seed weight. Tartary buckwheat accession with smaller seeds recorded the lowest average 100-seed weight (1.8 g). Although the difference for seed yield plant^{-1} among the buckwheat accessions was statistically non-significant, wide range of variation was noted in the common buckwheat germplasm (3.6-54.6 g). Frequency distributions of 66 *F. esculentum* and 31 *F. tataricum* accessions for eight qualitative variables showed considerable variations for plant growth habit, leaf blade shape, stem colour, flower colour, seed shape and seed coat colour both within and among the species (data not shown).

The characterization results revealed wide variation for days to 50% flowering, days to 80% maturity, primary branches plant^{-1}, plant height and 100-seed weight. Similar range of variation was also recorded in the NW Indian Himalayan buckwheat germplasm for primary branches plant^{-1}, plant height and seed yield plant^{-1} (Senthilkumaran *et al.*, 2008). The *F. esculentum* accessions used in the current study were mostly semi-erect type, while the accessions of *F. tataricum* were both semi-erect and spreading types. Among the qualitative characters, considerable variations between *F. esculentum* and *F. tataricum* accessions were noted for leaf blade shape, flower colour, seed shape and seed colour. While, in NW Indian buckwheat landraces major variations were recorded for seed shape and seed colour (Senthilkumaran *et al.*, 2008).

Grouping of Buckwheat Accessions

The UPGMA dendogram based on morphological similarity values (simple matching coefficients) is presented in Fig. 2. The two buckwheat species formed two distinct clusters at 17% similarity level. Within

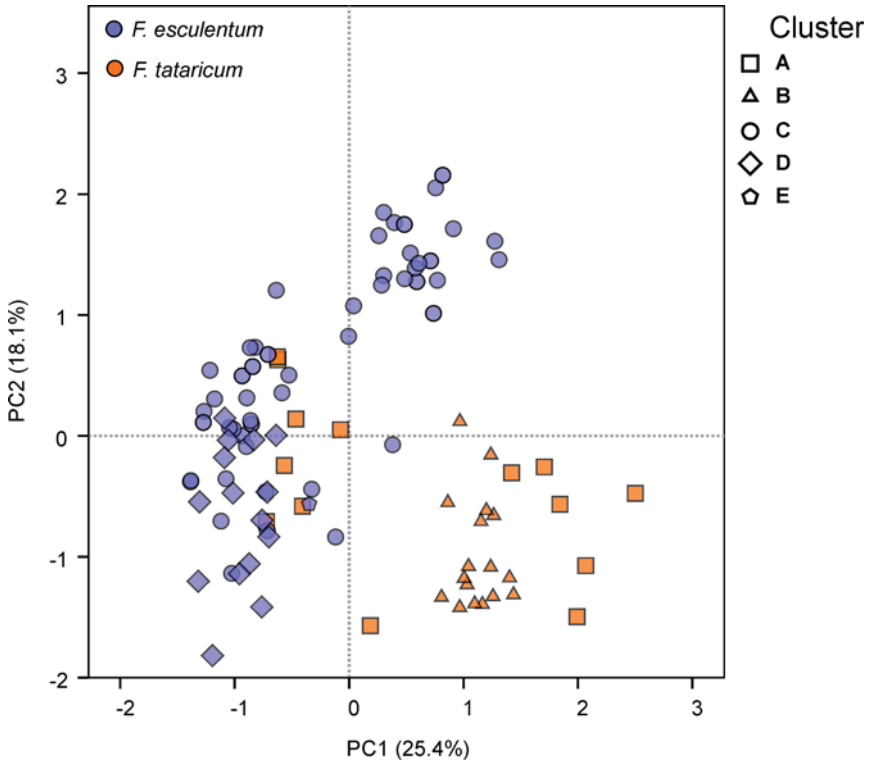

Fig. 3. PCA biplot of 97 buckwheat accessions. Accessions belonging to different sub-clusters (A-E), as in clusters analysis (Fig. 2), are indicated with different shapes.

the tartary buckwheat accessions, two sub-clusters can be discerned. Cluster A contains 14 accessions (seven each from Arunachal Pradesh and Sikkim), which was characterized by spreading plant architecture and triangular to ovate seeds. The accessions of this sub-cluster recorded the highest values for days to 50% flowering (32.6 days) and moderately high maturity duration (80.7 days). Rest 17 tartary buckwheat accessions with the highest average values for days to 80% maturity (88.8 days), number of primary branches (12.6) and the lowest average values for plant height (55.7 cm), 100-seed weight (1.6 g) and seed yield plant^{-1} (23.6 g) formed sub-cluster B. The accessions of this sub-cluster were characterized by semi-erect plants and triangular seeds. Sixty six *F. esculentum* accessions clearly separated into three sub-clusters at approximately 30% morphological similarity. Cluster C grouped 50 common buckwheat accessions collected from Arunachal Pradesh, Sikkim, Assam and Manipur. This sub-cluster possessed accessions with the highest 100-seed weight and low seed yield plant^{-1}. Cluster D grouped 15 accessions having the highest average values for seed yield plant^{-1} (37.8 g), 100-seed weight (2.6 g) and plant height (81.1 cm), and the lowest average values for days to 50% flowering (29.4 days) and days to 80% maturity (68.0 days). A single *F. esculentum* accession having spreading growth habit, less number of primary branches plant^{-1} (3.2) and the smallest leaves formed cluster E.

PCA extracted five principal components (PCs) which accounted for 70% of the total morphological variation. The first principal component (PC1) explained 25.4% of the total variance. The variables days to maturity, flower colour and leaf width had high positive loadings. In contrast, plant height, flower colour, seed weight and seed yield plant^{-1} had high negative loadings. The PC2 explained an additional 18.1 % of the total variance. Variables such as leaf length, stem colour, leaf blade shape, seed shape and seed coat colour had high positive loadings. However, primary branches plant^{-1} and seed yield plant^{-1} had high negative loadings. PC3 explained 11.5% of the total variance and had high positive loadings for primary branches plant^{-1}, seed yield plant^{-1}, leaf length and leaf width. PC4 and PC5 contributed around 8% and 7% of the total variance, respectively. The first two PCs were plotted to observe relationship between the clusters (Fig. 3). Although two buckwheat species showed obvious separation on

the biplot, the distinction among the five clusters was not clear. Majority of the tartary buckwheat accessions belonging to cluster A and B, had high positive coefficients of PC1 but negative coefficients of PC2, and thus occupied bottom right corner of the biplot. Majority of common buckwheat accessions belonging to cluster C had positive coefficients of PC2 and negative coefficients of PC1.

In this current study, both cluster and PCA revealed useful information about the morphological variability present in the buckwheat germplasm of NE Indian Himalayas. Both these analyses have separated the germplasm according to the species with equal effectiveness. The sub-clusters which were obtained in cluster analysis were also detected to some extent in PCA. Overall, form the current results it appeared that considerable variations in the morphological traits exist among and within the buckwheat species which contributed to the grouping of germplasm into two species-wise clusters and then grouping into sub-clusters. Significant differences were obtained for days to maturity, number of primary branches, plant height and 100-seed weight among the two buckwheat species. These analyses generated a classification of buckwheat accessions based on variations in morphological traits and this will be useful for selecting diverse parents in breeding programmes. Senthilkumaran *et al.* (2008) grouped NW Indian buckwheat germplasm through cluster analysis and principal component analysis on a set of 46 buckwheat populations using quantitative traits. Similar study was conducted by Cepková *et al.* (2009) with a set of 77 accessions. Both the studies have analysed *F. esculentum* and *F. tataricum* accessions separately.

In NE India buckwheat is acclimatized to monoculture production and it is the only crop which can be grown successfully in higher Himalayan ranges (above 2,500 m). *F. tataricum* in particular because of its frost tolerance is preferred over *F. esculentum* in many marginal areas of production. Evaluation of tartary buckwheat accessions for frost tolerance would be an important objective in future characterization programmes. Due to the invasion of other commercial crops, the cultivated area of buckwheat is gradually declining and there is need for continuous collection of accessions and devising strategies for ex situ and on farm conservation of the buckwheat landraces of this region. During recent exploration trips, it has been observed that the many farmers in East Kameng district and the Monpa and Sherdukpen tribes of West Kameng district of Arunachal Pradesh are growing tomato in place of buckwheat. Considering the diversity in the NE Indian buckwheat germplasm, systematic efforts need to be initiated to promote in situ (on-farm) conservation of the landraces with specific adaptations from diversity rich areas. This on-farm conservation could complement crop improvement in the region, especially in the case of F. tataricum (Senthilkumaran *et al.*, 2008). Characterization and evaluation of the germplasm along with high yielding and adaptive check varieties would result in identification of superior genotypes for wide-scale cultivation in NE region. In conclusion, the current study provides an understanding on the distribution of common and tartary buckwheat germplasm in NE Indian states and the level of agro-morphological variability among and within two buckwheat species.

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RESEARCH ARTICLE

Nutritional Diversity of Elite Rice Landraces from Subsistence-oriented Farming Systems

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Twenty one prominent rice landraces belonging to different subsistence farming systems in India were evaluated. Manually de-husked rice samples were used for studying sixteen important nutritional parameters. Range of useful nutritional variability was observed among rice landraces and each landrace was found superior for at least one nutritional parameter. Landraces from North-eastern Himalaya in comparison to landraces from other niches were found superior for nutritional traits like protein (12.22-14.32%), phosphorus (0.363-0.740 g/100g), phytate (1.35-2.55 g/100g) and Cu (1.090-2.141 mg/100g) contents. However, clustering analysis revealed no linkage among landraces with respect to their specific traits or geographical origin. The red rice landraces Chuhartu (7.29%) and Katheri (6.63%) from North-western Himalaya registered higher value for crude fat than the check (3.05%). Protein, total phosphorus, antioxidant activity (CUPRAC mg/g GAE) and total anthocyanin content was remarkably higher in black rice (Chakhao amubi and Chakhao poireiton). Significant amount of correlation was observed among different nutritional parameters. Anti-oxidant activity was significantly correlated with phytate, anthocyanin, total phenol and total phosphorus content. 42.17% of nutritional variability was explained by Principal component I which was majorly contributed by total phosphorus, anthocyanin, phytate, CUPRAC and Cu content. The present study indicates the role of local communities in development and enrichment of nutritional diversity of rice.

Key Words: Anthocyanin, Aromatic rice, Black rice, Coloured rice, Nutrient composition, Red rice, Rice for health

Introduction

Rice is the most popular staple food, feeding almost half of the world population, providing 27% of global human per capita dietary energy, 20% of per capita dietary protein and 3% of per capita dietary fat. In Indian scenario, it provides 30.9% per capita dietary energy, 24.1% per capita protein and 3.6% per capita dietary fat (FAO, 2002). Rice, besides its energy and protein content, also serves as source of different minerals, vitamins, antioxidant compounds and dietary fiber. Unlike high yielding rice varieties, landraces are diverse having broad genetic base as being a product of repeated mass selection. They tend to be rich in one or more of these macro- and micro-nutrients. Much of the studies on rice nutrient composition are done on high yielding rice varieties and only limited information on nutritional profile of rice landraces is available. Cultivation of these landraces is restricted to marginal areas, in rainfed upland environments and they are fast depleting as evident from study on Jeypore tract of Odisha state, India where in the late 1950s around 1700 rice varieties were cultivated, by 1996 the number had dwindled down to slightly more than 300 varieties (Patra and Dua, 2003). These days particularly the pigmented rice (black, dark purple and red rice) is gaining importance in developing health conscious human society. These are used as natural food colorant for bread, liquor, functional food and many other local and highly valued dishes. Coloured rice is being consumed as traditional medicine in China, Japan, Korea, Thailand and India for treating anemia, diabetes, strengthening kidney function, promoting blood circulation, removing blood stasis, treating and ameliorating eye sight, etc (Wang *et al.*, 2007; Sompong *et al.*, 2011; Dipti *et al.*, 2012). On

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the other hand, aromatic rice always has been a higher priced commodity among local community and in global market. Aromatic rice landraces are even more valuable and rare food as they have evolved with local taste, aroma and cooking qualities preferred by local people. These traditional rice landraces are invaluable genetic resource and are much sought after by plant breeders and genetic engineers as a source of genes for biotic/abiotic stress tolerance and nutritional traits. Therefore, in this study the nutritional profile of rice landraces collected from diverse agro-ecologies has been investigated.

Materials and Methods

Landraces' Seed Collection and Multiplication

A total of 21 most popular rice landraces collected from diverse ethnic communities of North-western Himalayas (Jammu & Kashmir and Himachal Pradesh), North-eastern Himalayas (Assam and Manipur) and two landraces from Southern India (Andhra Pradesh) (Table 1). These were grown in the experimental farms of Division of Genetics, Indian Agricultural Research Institute, New Delhi (latitude: 28° 38′ 7.11′′ N and

Table 1. Passport information and special characteristics of some important rice landraces from different parts of India

S.No.	Landrace	District	State	Latitude/Longitude	Special remarks
1	Chuhartu	Shimla	Himachal Pradesh	31° 12' N; 77° 45' E	Red aleurone
2	Karad	Chamba	Himachal Pradesh	32° 33' N; 76° 7' E	Red aleurone, field resistant to disease and insects, drought tolerant, karad means very hardy and luxuriant, white awn
3	Mirzag	Budgam	Jammu and Kashmir	33° 54' N; 74° 39' E	Red aleurone, early maturing, high yield, better milling recovery, tolerant to lodging
4	Sukara	Chamba	Himachal Pradesh	32° 33' N; 76° 7' E	Red aleurone
5	Katheri	Kangra	Himachal Pradesh	32° 11' N; 76° 12' E	Red aleurone, aromatic, sweet, sticky rice, given to women after delivery as it is considered to restore certain internal reproductive body parts to normalcy, semi-spreading plant type, purple hull, pink awn
6	Mushk Budji	Pulwama	Jammu &Kashmir	33° 52' N; 74° 53' E	Red aleurone, bold and fine grains, cooks very good, the most popular scented rice of Kashmir valley, very costly, cooked in feasts only by rich people
7	Begumi	Kangra	Himachal Pradesh	32° 2' N; 76° 23' E	Red aleurone, aromatic, long, fine grains
8	Kalafulpatash	Mandi	Himachal Pradesh	31° 42' N; 76° 55' E	Aromatic, sweet grains, high swelling upon cooking, field resistant to diseases, glumes tip black, blackish purple stem
9	Lalbasmati	Kangra	Himachal Pradesh	32° 6' N; 76° 16' E	Aromatic, slightly red, long grains, high yield, orange-red hull,
10	Chittimuthyalu	Adilabad	Andhra Pradesh	19° 40' N; 78° 31' E	Aromatic, sweet, small and non-glutinous, white grains (chitti = tiny, muthyalu = pearl), landrace is in verge of extinction
11	Kalajoha	Dheemaj	Assam	27° 28' N; 94° 32' E	Aromatic, light blackish aleurone, non-glutinous, sweet rice, black hull
12	Chakhao amubi	Bishnupur	Manipur	24° 37' N; 93° 45' E	Aromatic, black aleurone, sweet, long, bold grains, glutinous rice, black hull
13	Chakhao poireiton	Bishnupur	Manipur	24° 37' N; 93° 45' E	Black aleurone, sweet, long, bold grains, glutinous rice, black hull
14	Lipya goal	Jaintia Hills	Meghalaya	25°11' N; 92°1'E	Bold white, chalky grains, glutinous rice
15	Suhagmani	Sonitpur	Assam	26°40' N; 92°51' E	Bold grains, glutinous rice, Semi-spreading plant type, good for flood affected areas
16	Himtu	Kangra	Himachal Pradesh	32° 6' N; 76° 16' E	Sweet, slender fine grains, very early maturing, dwarf, lodging resistant
17	Bohana dhan	Chamba	Himachal Pradesh	32° 33' N; 76° 7' E	Bold grains, high yield
18	Jhini	Kangra	Himachal Pradesh	32° 2' N; 76° 23' E	Sweet, bold, brownish grains
19	Ramjawain	Hamirpur	Himachal Pradesh	31° 36' N; 76° 30' E	Sweet, long and fine grains
20	Rangdi	Mandi	Himachal Pradesh	31° 38' N; 76° 43' E	Cold tolerant, small and fine grains, non-sticky, good yield
21	Ragalvanji	Adilabad	Andhra Pradesh	19° 40' N; 78° 31' E	Non glutinous rice especially used in religious purpose, landrace is in verge of extinction
22	HPR 2143	Pedigree: HPR 9020-2-2-2-1-1-1; Cross: Phulpatas 72 × HPU 741			Check variety

longitude: 77° 13′ 29.86′′ E). Standard field sowing practices for rice were followed. Freshly harvested seeds were used for nutritional analysis. An improved variety HPR-2143 from NEH region was also included in the study for comparison, as control.

Sample Preparation

Paddy grains were dehusked by hand without disturbing aleurone layer and homogenized using Foss Tecator cyclotech grinding mill. Homogenized flour was vacuum seal packed and stored at -20° C until used for analysis.

Estimation of Proximate Components

The proximate composition of homogenized samples was done using the official methods (AOAC 2005) with slight modifications in some cases.

Moisture content was estimated by weighing fresh (FW) weight of dehusked rice seed samples and then samples were dried at 60° C in hot-air oven for 72 h until constant dry weight (DW) was achieved. Then moisture content was calculated as 100 × [(FW-DW)/ FW)] and expressed in per cent.

Ash content was estimated using 5 g of dried ground sample in silica crucible, which was first charred at 250° C for 1 h and then temperature was gradually raised to 450° C and maintained for 2 h. Ash was moistened with double distilled water and 2–3 drops of concentrated HNO3 (69% GR Grade, Merck KGaA, Darmstadt, Germany) was added. Crucibles were again kept in muffle furnace at 450° C for 30 min for complete ashing. Ash content was expressed as g/100 g of fresh weight.

Crude fat was estimated as per AOAC official method 920.39 using petroleum ether at 40-60° C as solvent. Samples were extracted for 24 hours and dried overnight before and after extraction. To ascertain recovery food reference material AS-FRM 14 and in-house QC samples were used and recovery of 97.2 ± 2.2 was obtained.

Total protein was estimated as per AOAC official method 976.05 with some modifications in digestion of samples. The dried sample (100 mg) was homogenized and then digested with digestion mixture made of sulphuric acid, anhydrous sodium sulphate, selenium and hydrogen peroxide in glass digestion tubes at 350° C for 45 min as per the method of Forster (1995). Nitrogen percentage in digest was estimated by Kjeltech (FOSS Tecator) nitrogen autoanalyser. To ascertain recovery in-house QC samples and food reference material AS-FRM 14 were used as control. Recovery percentage of 99.8 ± 1.6 for AS-FRM 14 and 102.7 ± 1.2 for QC samples was obtained.

Dietary fibre was estimated by enzymatic-gravimetric method as per AOAC Official Method 985.29. Total dietary fibre assay kit (K-TDFR) and total dietary fibre control kit (K-TDFC) obtained from Megazyme, Ireland were used in analysis. AS-FRM 14 (Rice-2 from PT-8) obtained from Institute of Nutrition, Mahidol University, Thailand was used as control sample and we obtained 3.39±0.28 g/100 g dietary fibre which is acceptable limit of suggested value (3.48±0.36) for AS-FRM 14.

Sample Preparation for Sugars, Total Phenol, Antioxidants and Starch Estimation

Homogenized samples (100 mg) were extracted with 5 ml of 80% (v/v) ethanol in amber centrifuge tube on tube rotator for 30 min at 40 rpm. This was followed by sonication at 60° C for 20 min in bath sonicator and centrifugation at 5000 g for 15 min. The supernatant was collected and the residue was re-extracted twice. The supernatants were pooled and lyophilized to complete dryness and stored at -20° C until further analysis. Lyophilized extract was dissolved in 5 ml water just before use to determine the total sugar, total phenol and antioxidant capacity using appropriate methods. The residue left over was used for starch estimation.

Determination of Total Soluble Sugar, Total Phenolics Content, Antioxidant Potential and Starch Content

Total soluble sugar content in the extract was determined using phenol-sulphuric acid method by Dubois *et al.* (1956). Determination of TPC in the extract was done using Folin-Ciocalteu (FC) assay as described by Singleton *et al.* (1999) with slight modifications. Antioxidant activity was measured as cupric ion reducing antioxidant capacity (CUPRAC) of the extract and determined according to the method of Apak *et al.* (2004). Gallic acid was used as a positive control and results were expressed in mg GAE (Gallic acid equivalent)/g dry weight. Starch content was estimated as per AOAC 996.11.

Estimation of Phytate

Phytate and free phosphorous content were measured by enzymatic procedure using phytase and alkaline phosphatase enzymes. Phytic acid /total phosphorous assay kit (K-PHYT) from Megazyme, Ireland was

used. Recovery of (95.2% ± 1.4) for control oat flour was obtained and results were expresses as g/100 g sample.

Estimation of Total Monomeric Anthocyanins

Anthocyanins were estimated as per AOAC official Method 2005.02. For each treatment two 10ml dilutions with pH 1.0 buffer and pH4.5 buffer were made and absorbance was taken at both 520 and 700 nm. Diluted test samples were read against the blank sample filled with distilled water. Anthocyanins content was calculated and expressed as Cyanidin-3-Glucoside equivalents, as follows:

$$\text{Anthocyanin pigment (cyanidin-3-glucoside equivalents, mg/l)} = \frac{A \times MW \times DF \times 10^3}{\varepsilon \times 1}$$

where A = (A520nm – A700nm)pH 1.0 – (A520nm – A700nm)pH 4.5; MW (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside (cyd-3-glu); DF = dilution factor established; l = path length in cm; ε = 26 900 molar extinction coefficient (L × mol^{-1} × cm–1) for cyd-3-glu; and 103 = factor for conversion from g to mg.

Mineral Analysis

Mineral content was determined in accordance with the official analytical methods (AOAC, 2005) using with atomic absorption spectrometer (AAS, model-Varian Spectra AA 220 FS, Varian Australia Pty Ltd., Australia) equipped with a D2 lamp background correction system using an air-acetylene flame. Appropriately diluted acid digested samples were analyzed using AAS calibrated with related minerals in different concentrations for different macro (sodium, potassium and magnesium) and micro-minerals (copper, iron and zinc).

ASFRM-6 (fish meal), ASFRM-14 (Rice flour) food reference standards obtained from Institute of Nutrition, Mahidol University, Thailand were used to ascertain recovery and validate analytical methods.

Statistical Analysis

Statistical analysis was performed for analysis of variance, correlation coefficient, duncan's multiple range test and principal component analysis to understand trait relationship and factors responsible for maximum variability using SAS version 9.3 (2011).

Results

The range of variation for sixteen important nutritional quality traits is presented in Table 2. Wide variations among rice landraces were recorded particularly in anthocyanin content, phytate, total phosphorus, Cu, total phenol content and CUPRAC activity. Landrace were grouped based on their specific traits (aleurone colour, aroma) or original habitat (NEH, NEW) and nutritional compositions were compared between contrasting groups (Table 4). These groups were also demarcated on the basis of significant differences for mean values based on DMRT (Duncan's Multiple Range Test) with α value of 0.05. These comparisons gave some significant differences in terms of their nutritional composition like rice landraces from NEH region in comparison to landraces from NWH region exhibited substantially higher amount of total protein, total phosphorus, phytic acid and Cu content (Table 3, Table 4 and Supplementary Table 2). But rice land races from NWH region were found superior than NEH for crude fat content. In comparison to white rice black rice were found to have higher amount of moisture, protein, total phosphorus, phytate, anthocyanins, total phenol and CUPRAC. No linkage was observed between aromatic and non-aromatic rices. Various nutritional parameters like ash, dietary fiber, total carbohydrates, sugar and starch are randomly distributed with respect to their quantity among landraces irrespective of their geographical origin or known special characteristics (Table 3 and 4). The same is also observed in clustering analysis done using nutritional variables (Supplementary Figure 1).

Each individual landrace was found to be superior for one or more nutritional traits (Table 3a and 3b). Most important and useful result obtained in this study was that black rice (Chakhao amubi and Chakhao poireiton) used in this study recorded significantly higher protein content (14.3 and 14.1% respectively) than the check variety (8.1%) and reported protein range (6-9%) in rice (Tan *et al.*, 2001). Ash content was observed significantly higher in Mirzag (4.1%), Himtu (3.9%) and Rangdi (3.8%). The landrace Chakhao poireiton recorded highest value for total phosphorus, phytic acid and CUPRAC activity. The lowest value for phytic acid was observed in Rangdi and Chittimuthyalu (0.11% both). The phyatate content in the check variety HPR -2143 was 0.068%. Kalajoha an aromatic popular landrace from Assam (NEH) recorded highest Cu (2.1 mg/100g) and Zn content (8.3 mg/100g) than any other landrace. Chittimuthyalu from Southern India was found to have highest Mg content (145.1 mg/100g).

The total phenol, anthocyanins and CUPRAC which are considered as measurement parameters for

Table 2. Range of variation for different nutritional traits in rice landraces

Biochemical traits	Max	Min	Mean	SD	CV	SE
Moisture (%)	10.00	6.45	7.85	1.09	13.93	0.24
Ash (%)	4.11	0.77	2.67	0.89	33.41	0.20
Protein (%)	14.32	6.70	10.63	1.94	18.22	0.42
Crude Fat (%)	7.29	2.71	4.20	1.43	34.04	0.31
Dietary Fibre (%)	9.79	5.17	7.24	1.48	20.44	0.32
CHOAVL (%)	72.94	61.24	67.32	3.49	5.18	0.76
Sugar (%)	6.56	3.59	4.94	0.91	18.39	0.20
Starch (%)	66.85	56.90	62.15	3.17	5.11	0.69
Total Phosphorus (g/100g)	0.74	0.04	0.25	0.18	71.76	0.04
Phytate (g/100g)	2.56	0.11	0.79	0.62	78.08	0.14
Total Phenol g/100g	0.33	0.03	0.15	0.10	67.03	0.02
CUPRAC (mgGAE/g)	10.00	0.83	3.52	2.31	65.58	0.50
Anthocyanin (mg/g)	15.89	0.12	3.06	4.07	133.08	0.87
Cu (mg/100g)	2.14	0.22	0.73	0.50	68.84	0.11
Zn (mg/100g)	8.26	2.56	5.37	1.65	30.71	0.36
Fe (mg/100g)	6.78	1.02	3.93	1.43	36.35	0.31
Mg (mg/100g)	145.11	66.84	109.28	17.00	15.56	3.71

the antioxidant activity were significantly higher in the pigmented rice landraces than the non-pigmented rice landraces. As expected the antioxidant activity (CUPRAC mg GAE/g) observed highest in black seeded landraces i.e. Chakhao amubi and Chakhao poireiton (8.1 and 10 respectively) followed by red rices. The CUPRAC value for red rice landraces varied from 2.0 in Mushkbudji to 5.2 in Mirzag. Similarly total phenol content was also high in black rice (0.29 g/100 g) followed by red rice (0.19 g/100 g) and very less in white rice (0.1) (Table 3 and 4). Begumi, a red rice registered highest value (0.33g/100g) for total phenol content followed by black rice Chakhao amubi (0.31 g/100 g) and Chakhao poireiton (0.27 g/100 g). Anthocyanins in both the black rices was

Table 3a. Variations for different nutritional traits in rice landraces from diverse agro-ecologies

Landrace Name	Moisture (%)	Ash (%)	Protein (%)	CF (%)	DF (%)	CHOAVL (%)	Sugar (%)	Starch (%)	T Phos (g/100g)
Chuhartu	6.9±0.3	2.7±0.4	10.0 ±0.8	7.3±0.3	8.6±0.5	66.2	4.8±0.2	60.7±4.7	0.17 ± 0.02
Karad	7.3±0.4	1.7±0.4	10.5±1.4	4.5±0.3	6.4±0.4	67.9	4.4±0.2	64.5±3.7	0.19 ± 0.01
Mirzag	6.6±0.4	4.1±0.4	10.1±0.9	4.2±0.3	8.5±0.5	66.5	6.0 ±0.1	59.6±4.6	0.26 ± 0.01
Sukara	9.3±0.6	3.1±0.2	8.3±0.8	2.7±0.6	5.5±0.9	71.0	5.2±0.7	66.0 ±4.1	0.26 ± 0.06
Katheri	6.9±0.4	3.3±0.1	10.9±1.1	6.6±0.4	9.0 ±0.5	62.5	5.7±0.4	58.1±3.5	0.15 ± 0.01
Mushk budji	7.0 ±0.4	3.4±0.5	10.4±0.8	5.9±0.4	5.3±0.3	66.5	6.6±0.4	60.8±5.1	0.22 ± 0.01
Begumi	8.2±0.7	2.2±0.3	9.4±0.7	2.8±0.8	6.6±1.0	70.7	6.3±0.5	64.6±4.9	0.27 ± 0.04
Kalafulpatash	7.5±0.3	3.2±0.3	9.4±0.6	6.6±0.3	6.9±0.4	68.1	3.9±0.2	63.7±3.5	0.12 ± 0.01
Lalbasmati	7.4±0.4	3.3±0.2	9.9±0.9	6.0 ±0.3	8.3±0.5	66.6	3.6±0.3	62.2±3.2	0.13 ± 0.02
Chittimuthyalu	6.4±0.5	1.5±0.3	10.4±1.3	2.7±0.6	6.0 ±0.6	72.9	6.3±0.7	64.4±4.5	0.04 ± 0.02
Kalajoha	9.2±0.7	2.6±0.3	14.0 ±1.5	3.2±0.4	8.9±0.5	62.1	4.2±0.2	56.9±5.5	0.36 ± 0.02
Chakhao amubi	10.0 ±0.5	2.6±0.2	14.3±2.1	3.6±0.6	6.4±0.4	63.4	6.2±0.3	58.1±6.6	0.56 ± 0.02
Chakhao poireiton	9.1±0.4	2.2±0.4	14.1±2.0	3.6±0.6	9.3±0.5	61.2	5.5±0.3	57.0 ±6.1	0.74 ± 0.03
Lipya goal	9.3±0.5	2.4±0.2	12.7±1.1	3.3±0.5	5.2±0.3	66.7	4.5±0.2	62.9±6.6	0.49 ± 0.01
Suhagmani	9.1±0.5	2.9±0.3	12.2±1.5	3.4±0.5	6.1±0.4	65.9	4.5±0.2	60.7±6.2	0.40 ± 0.02
Himtu	7.7±0.4	3.9±0.2	11.7±1.4	3.5±0.2	9.1±0.6	64.0	4.4±0.3	58.6±4.1	0.20 ± 0.02
Bohana dhan	8.1±0.9	1.0 ±0.2	6.7±0.8	3.1±0.6	9.8±0.9	71.2	4.7±0.5	64.6±4.5	0.06 ± 0.01
Jhini	7.1±0.3	2.7±0.4	9.6±1.2	4.5±0.4	6.0 ±0.4	69.8	4.4±0.1	66.3±4.1	0.19 ± 0.02
Ramjawain	6.7±0.5	2.8±0.2	9.5±1.3	4.3±0.5	6.1±0.4	71.9	4.5±0.1	66.9±4.9	0.17 ± 0.01
Rangdi	6.8±0.4	3.8±0.2	9.9±1.1	3.6±0.3	7.5±0.5	66.2	3.7±0.3	63.8±3.4	0.04 ± 0.01
Ragalvanji	8.2±0.8	0.8±0.1	9.3±0.9	2.9±0.5	6.7±1.0	72.1	4.4±0.7	64.9±6.5	0.16 ± 0.02
HPR 2143	8.3±0.7	1.2±0.1	8.1±1.0	3.1±0.8	9.5±1.0	69.9	4.2±0.3	64.7±5.7	0.03 ± 0.00

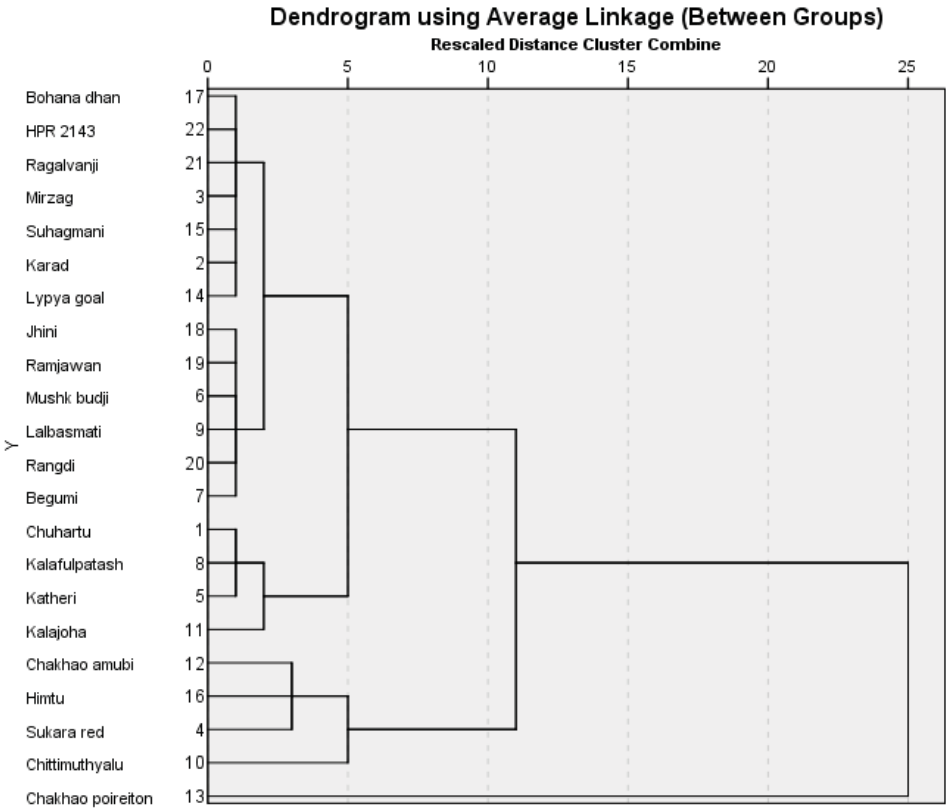

Fig. S1. Dendrogram based on the biochemical values

15.89 and 13.32 mg/g respectively. In comparison to black and red seed rice landraces, white rice landraces and improved check variety HPR-2143 recorded very low CUPRAC (2.2 and 0.95 mg GAE/g) and total phenol content (0.1 and 0.13 g/100g respectively). The dietary fibre content also has shown considerable variation among the landraces studied. This parameter has not shown any correlation with geographic origin or phenotypic characteristics of landrace. The lowest dietary fibre content was recorded in chalky grained Lipya goal (5.2%) and highest in Bohana dhan landrace (9.8%). Ash percent also has shown a great range of variation with minimum 0.77 % in Ragalvanji to maximum being 4.11 % in Mirzag. Crude fat ranged from 2.71 in Sukara to 7.29 in Chuhartu. Overall landraces performed better for all nutritional parameters than the check variety HPR-2143.

Some of the important correlations coefficients among different nutritional traits are given in Table 5. Total protein content was positively correlated with total phosphorus, phytate, anthocyanins, CUPRAC, Cu and Zn content and negatively correlated with total available carbohydrate (CHOAVL) and starch content. Phytate, anthocyanins and total phenol together are positively correlated with CUPRAC activity, protein content and total phosphorus. Zn is positively correlated with Cu and Fe.

Out of twenty one rice landraces studied, eleven rice landraces found superior for one or multiple biochemical nutritional traits (Table 6). But as listed (special remarks) in the Table 1, each and every landrace has its own unique characteristics and local importance. Other important traits (not related to nutrition) possessed by these landraces are seed/grain's physical characteristics like hull colour, aleurone colour, cooking qualities like glutinous/non-glutinous, aroma, adaptation traits like awns, plant height, early maturity, biotic and abiotic stresses tolerance as well as ethnic values.

Discussion

The local rice landraces in subsistence production systems are still popular despite their low yield compared to the high yielding modern varieties. Many of these landraces are under continuous cultivation because of their special taste, aroma, medicinal and nutritional qualities. The popularity of pigmented rice like red rice,

Table 3b. Variations for different nutritional traits in rice landraces from diverse agro-ecologies

Landrace Name	Phytate (g/100g)	Anthocyanin (mg/g)	Total Phenol (g/100g)	CUPRAC (mg GAE/g)	Cu (mg/100g)	Zn (mg/100g)	Fe (mg/100g)	Mg (mg/100g)
Chuhartu	0.49±0.07	4.67±0.02	0.19±0.01	4.5±0.24	0.4±0.06	5.4±0.59	4.5±0.56	91.5±8.5
Karad	0.58±0.06	5.17±0.33	0.16±0.01	5.0±0.11	0.5±0.10	7.8±0.46	4.3±0.67	114.1±6.9
Mirzag	0.85±0.07	3.7±0.11	0.24±0.01	5.2±0.25	0.4±0.11	5.7±0.49	4.7±0.60	115.5±9.5
Sukara	0.86±0.06	3.72±0.27	0.26±0.04	4.3±0.60	0.2±0.03	3.3±0.31	1.8±0.33	125.4±8.0
Katheri	0.38±0.06	3.12±0.9	0.13±0.01	3.6±0.21	0.4±0.06	5.4±1.25	3.4±0.51	90.3±12.7
Mushk budji	0.63±0.09	3.64±0.22	0.03±0.01	2.0 ±0.15	0.8±0.11	5.7±0.78	4.2±0.79	106.3±9.5
Begumi	0.88±0.10	3.44±0.11	0.33±0.02	5.0 ±0.64	0.9±0.08	3.5±0.39	3.5±0.42	99.7±9.3
Kalafulpatash	0.32±0.05	0.62±0.06	0.06±0.01	2.4±0.19	0.7±0.06	6.1±0.38	3.4±0.54	94.2±10.5
Lalbasmati	0.36±0.06	1.91±1.5	0.05±0.01	2.0 ±0.11	0.6±0.07	6.6±0.21	3.9±0.54	101.3±13.2
Chittimuthyalu	0.11±0.03	0.12±0.06	0.15±0.04	1.4±0.41	0.4±0.08	2.6±0.40	3.7±0.32	145.1±18.5
Kalajoha	1.20±0.09	0.88±0.1	0.10 ±0.01	3.1±0.11	2.1±0.08	8.3±0.45	5.5±0.57	100.2±10.3
Chakhao amubi	1.83±0.10	15.89±0.04	0.31±0.01	8.1±0.34	1.4±0.07	7.3±0.55	2.9±0.76	126.9±8.6
Chakhao poireiton	2.56±0.19	13.32±1.37	0.27±0.02	10±0.56	1.4±0.10	4.6±0.87	2.3±0.32	66.8±8.9
Lipya goal	1.65±0.02	1.07±0.08	0.08±0.00	3.3±0.15	1.1±0.12	6.5±0.56	3.9±0.75	112.0 ±9.7
Suhagmani	1.36±0.09	1.86±0.04	0.18±0.01	3.8±0.10	1.5±0.07	6.4±0.77	3.8±0.55	116.7±10.9
Himtu	0.65±0.08	0.12±0.1	0.03±0.00	1.1±0.11	0.4±0.05	4.5±0.18	5.0 ±0.77	136.5±8.6
Bohanadhan	0.18±0.05	0.83±0.04	0.10 ±0.01	0.8±0.04	0.2±0.06	2.6±0.38	2.2±0.33	115.0 ±6.6
Jhini	0.61±0.06	0.27±0.04	0.06±0.01	2.3±0.19	0.6±0.09	6.5±0.08	6.4±0.55	106.4±5.9
Ramjawain	0.54±0.06	0.62±0.02	0.09±0.01	3.1±0.09	0.4±0.08	4.7±0.31	5.2±1.34	109.4±13.1
Rangdi	0.11±0.03	1.34±0.22	0.03±0.01	1.9±0.16	0.5±0.09	6.2±0.07	6.8±0.45	105.4±8.4
Ragalvanji	0.52±0.06	0.49±0.15	0.30 ±0.03	1.0 ±0.27	0.4±0.07	3.2±0.26	1.0 ±0.28	116.4±6.1
HPR 2143	0.07±0.01	0.5±0.01	0.13±0.03	1.0 ±0.19	0.3±0.03	3.3±0.23	3.0 ±0.29	114.0 ±9.1

Table 4. Pattern of variation for nutritional traits among different groups of rice landraces

Landrace group	Moisture	Ash	Protein	CF	DF	CHOAVL	Sugar	Starch	T. Phos	Phytate	Antho-cyanins	Total Phenol	CUPRAC	Cu	Zn	Fe	Mg
Red rice	7.5a	2.9	9.9a	4.8	7.4	67.3b	5.5ab	62b	0.21a	0.6a	3.9ab	0.19ab	4.2ab	0.5a	5.1a	3.7a	106a
Black rice	9.5b	2.4	14.2c	3.5	7.9	62.3a	5.8b	57a	0.65b	2.2b	14.6c	0.29b	9.0c	1.4b	5.2	2.6a	97a
White rice	7.8a	2.5	10.4a	3.9	7.1	68.1b	4.4a	63b	0.19a	0.6a	0.8a	0.10a	2.1a	0.7a	5.9a	4.2a	113a
Aromatic	8.1a	2.6	11.5ab	4.4	6.6	66.6b	4.8ab	61ab	0.24a	0.7a	3.9ab	0.13a	3.4ab	1.0ab	5.3a	3.9a	113a
Non-aromatic	7.7a	2.6	10.3a	4.1	7.5	67.5b	4.9ab	62b	0.24a	0.8a	3.0ab	0.15a	3.5ab	0.6a	6.1a	3.9a	108a
NEH	9.3b	2.5	13.4bc	3.3	7.3	63.8ab	4.9ab	59ab	0.51b	1.7b	6.6b	0.18ab	5.6b	1.5b	6.6a	3.6a	104a
NWH	7.4a	3.0	9.8a	5.2	7.1	67.3b	5.1ab	62b	0.19a	0.6a	3.3ab	0.16a	3.7ab	0.5a	5.5a	3.7a	104a
Overall	7.8	2.7	10.6	4.2	7.2	67.3	4.9	62.2	0.25	0.79	3.2	0.15	3.5	0.73	5.4	3.9	109.3
HPR 2143	8.3	1.2	8.1	3.1	9.5	69.9	4.2	64.7	0.03	0.07	0.5	0.13	1	0.3	3.3	3	114
Normal value*	12.6	1.3	7.6	2.3	3.8	72.4	–	–	0.25	–	–	–	–	0.36	1.9	2.8	52

NWH: North-western Himalayas, NEH: North-eastern Himalayas, CF: crude fat, DF: dietary fat, CHOAVL: total carbohydrate available by difference, T Phos: total phosphorus content; CUPRAC: Cupric Reducing Antioxidant Capacity expressed as gallic acid equivalent (GAE). Superscripts a, b & c demarcate the groups on the basis of significant differences for mean values based on DMRT (Duncan's Multiple Range Test) with α value of 0.05. Values with no superscripts (like for CF%, DF%, Sugar % and Fe) have no differences for the grouping pattern of the rice landraces.

* Source (values for rice, brown, parboiled, home-pounded, raw): Food composition table for Bangladesh (2013) Institute of Nutrition and Food Science, University of Dhaka.

black rice and deep purple rice is partially due to the distinct compositions of phytochemicals, which gives them colour, local taste, and medicinal value and helps in the prevention of different kind of diseases especially associated with oxidative stress (Gunaratne *et al.*, 2013; Hao *et al.*, 2015). The other brown or white local rice landraces are especially grown for their aroma intensity, starch quality and local environmental adaptation like drought, low or high temperature, and better yield performance in low input. Rice landraces in this study are found superior for different nutritional parameters as discussed below.

A significant correlation between rice landrace's habitat and crude fat content was observed, which

possibly may be due to the influence of local food taste of geographically isolated local communities and their selection pressure applied on the crop. But further study is required to assess the impact of local food taste on the nutritional diversity of crop plants. This trait can have commercial importance as rice fat is known to have high level of essential poly unsaturated fatty acids particularly oleic acid and linoleic acid (Yasumatsu and Moritaka, 1964). Rice fat also has been reported to help in lowering blood cholesterol (Rege *et al.*, 2014; Chithra *et al.*, 2015), acting as antioxidant (Bopitiya and Madhujith, 2014). The fat is also rich source of gamma-oryzanol and vitamin E (tocopherol and tocotrienols) which are good antioxidants (Min *et al.*, 2011). Similarly another significant correlation was observed between protein content and landrace's geographical origin. NEH originated rice landraces were higher in protein content than the rice landraces from NWH ascertained that not only climatic conditions, but the local traditions and food taste preferences plays an important role on existing pattern of genetic diversity of local landraces. Further it indicates that the existence of landrace diversity depends on various types of interactions between climatic conditions and human interventions like type of local farming practices, local food taste, local ritual and ethnic activities, medicinal use awareness, seed storage and sharing practices, etc. Another significant and useful correlation was observed between aleurone colour and protein content; rice with black aleurone colour found to have higher protein content than prevalent aleurone colored i.e. whitish-brown aleurone coloured rice landraces. This correlation as well as these genotypes (with >14% protein content) can be very useful in breeding rice varieties with enhanced protein content. Literature shows that the average protein content in the HYVs of rice is around six to nine percent; however some biochemical studies shows that the rice landraces have up to fourteen percent of protein content (Frei and Becker, 2004). This trait is of special significance as rice is a second major human food and therefore it can play vital role in nutritional quality improvement of human society. Rice protein have favorable amino acid composition with a high proportion of lysine and a high protein digestibility thereby increasing the biological value of the rice protein comparative to proteins of other cereals and pulses (Jolliet *et al.*,1998). The black rice landraces were also found superior for other important nutritional compounds like total phosphorus, anthocyanins, anti-oxidant activity and phytate. The phytic acid which was once considered as anti-nutritional compound due to its strong binding affinity for minerals such as calcium, magnesium, iron, copper, and zinc, making them unavailable for absorption in the intestines, is now a beneficial compound as it reduces starch digestibility which is good for diabetic patients. A study indicates that increase in phytic acid by

Table 5. Pearson correlation coefficient matrix for all biochemical traits

	Moisture	Ash	Protein	CF	DF	CHOAVL	Sugar	Starch	TPhos	Phytate	Anthocyanin	Total Phenol	Phy+ Anth+ Phe	CUPRAC	Cu	Zn	Fe
Ash	-0.25	1.00															
Protein	0.46*	0.21	1.00														
CF	-0.50*	0.42	-0.07	1.00													
DF	-0.06	-0.02	0.05	-0.08	1.00												
CHOAVL	-0.27	-0.48*	-0.77**	-0.29	-0.47*	1.00											
Sugar	0.00	0.02	0.18	-0.08	-0.09	-0.03	1.00										
Starch	-0.27	-0.37	-0.75**	-0.20	-0.45*	0.92**	-0.26	1.00									
TPhos	0.69**	0.07	0.80**	-0.22	-0.06	-0.57**	0.30	-0.56**	1.00								
Phytate	0.70**	0.06	0.79**	-0.25	-0.05	-0.56**	0.28	-0.54**	1.00**	1.00							
Anthocyanin	0.44*	0.03	0.55**	0.02	0.08	-0.50*	0.46*	-0.50*	0.72**	0.70**	1.00						
Total Phenol	0.43*	-0.34	0.14	-0.35	-0.10	0.07	0.49*	-0.09	0.44*	0.44*	0.57**	1.00					
Phy+ Anth+ Phe	.0.65**	0.01	0.72**	-0.18	-0.01	-0.54**	0.41	-0.55**	0.55**	0.94**	0.89**	0.60**	1.00				
CUPRAC	0.40	0.12	0.59**	0.00	0.01	-0.50*	0.43*	-0.47*	0.81**	0.81**	0.89**	0.62**	0.92**	1.00			
Cu	0.63**	0.02	0.81**	-0.20	-0.01	-0.58**	0.06	-0.57**	0.73**	0.73**	0.37	0.16	0.6**	0.46*	1.00		
Zn	0.11	0.42*	0.59**	0.36	-0.04	-0.61**	-0.25	-0.40	0.33	0.31	0.22	-0.26	0.25	0.28	0.56**	1.00	
Fe	-0.47*	0.54**	0.15	0.22	0.08	-0.21	-0.25	-0.04	-0.19	-0.20	-0.29	-0.50**	-0.30	-0.17	0.09	0.53*	1.00
Mg	0.00	-0.11	-0.16	-0.99*	-0.25	0.45*	0.11	0.29	-0.30	-0.29	-0.27	-0.02	-0.29	-0.42	-0.29	-0.25	-0.03

* Correlation is significant at the 0.05 level, ** Correlation is significant at the 0.01 level.

Table 6. Rice landraces superior for one or multiple nutritional traits studied

Landrace	Nutritional Trait
Chuhartu	High crude fat (7.29%)
Mirzag	Ash content (4.10%)
Mushk budji	High sugar (6.56%)
Begumi	High phenols (0.33 g/100g)
Lalbasmati	Low sugar (3.59%)
Bohana dhan	Dietary fibre (9.79%)
Rangdi	Low phytate (0.11 g/100g), high Fe (6.78 mg/100g)
Chittimuthyalu	Low phytate (0.11 g/100g), high Mg (145 mg/100g)
Kalajoha	High Cu (2.1 mg/100 g) and Zn content (8.26 mg/100 g)
Chakhao amubi	High protein (14.3%), high anthocyanin (15.89 mg/g), high total phenol (0.31 g/100g), high CUPRAC (8.1 mg GAE/g)
Chakhao poireiton	High protein (14.1%), total phosphorus (0.74 g/100g); high phytate (2.56 g/ 100g), high CUPRAC (10 mg GAE/g) and high anthocyanin (13.32 mg/g)

Table S1. Total variance explained (Eigen values) and its principal component traits

	PC1	PC2	PC3	PC4	PC5
Total	7.591	3.593	1.742	1.403	1.180
% of Variance	42.170	19.962	9.680	7.794	6.558
Cumulative %	42.170	62.132	71.811	79.605	86.163
Moisture	.642	.403	.471	-.141	-.152
Ash	.131	-.683	-.188	.327	.289
Protein	.853	-.261	.259	.069	.177
CF	-.094	-.625	-.559	.185	-.353
DF	.093	-.251	-.157	-.882	.230
CHOAVL	-.730	.588	.063	.277	-.100
Sugar	.337	.353	-.502	.240	.515
Starch	-.723	.402	.134	.310	-.295
TPhos	.948	.119	.127	.096	-.051
Phytate	.939	.135	.142	.085	-.052
Anthocyanin	.819	.177	-.358	.060	-.003
Total Phenol	.466	.703	-.267	.041	-.006
Phy+Anth+Phe	.954	.223	-.083	.080	-.034
CUPRAC	.865	.123	-.323	.154	-.089
Cu	.763	-.152	.462	-.003	-.079
Zn	.428	-.685	.262	.255	-.159
Fe	-.157	-.761	.203	.241	.217
Mg	-.361	.341	.370	.233	.641

2% can reduce starch digestibility up to 50% (Yoon *et al.*, 1983). It also increases intestinal immunity, and acts as natural anti-oxidant and anti-neoplastic (anti-cancer) agent (Fox and Eberl, 2002; Pacheco *et al.*, 2012; Goufo and Trindade, 2014). Therefore, genotypes containing higher or lower phytic acid can be appropriately used as per need.

The rice landraces with red, black or purple pericarp colors have comparatively higher amount of phenolic compounds, antioxidant activity and anthocyanin content than the light brown or white pericarp colored ones. Various previous studies also have shown that the concentration of bran colour is positively correlated with the antioxidant activity, total phenol and total flavonoids content (Shen *et al.*, 2009; Min *et al.*, 2011; Sutharut and Sudarat, 2012). In a similar study over sevenfold higher total antioxidant capacity and phenolic content was observed in Sri Lankan traditional red rice varieties than improved light brown-grained new-improved varieties (Gunaratne *et al.*, 2013). Several studies indicate that the antioxidant activity is cumulatively effect governed by anthocyanins, members of flavonoids family and other phenolic compounds (Iqbal *et al.*, 2005; Yawadio *et al.*, 2007; Tabart *et al.*, 2009). Anthocyanin also helps in slow release of glucose from rice starch during cooking of parboiled rice (Perera and Jansz, 2000).

Diversity analysis based on Duncan's Multiple Range Test (DMRT) and least significant differences (LSD) indicate that the rice landraces' nutritional diversity within and among the groups did not differ significantly for crude fat, dietary fibre, sugar and Fe content (Table 4 and Supplementary Table 2). Total available carbohydrate was observed as low as 61.2% in Chakhao poireiton which is significantly lower than the reported range of 75-85 % available carbohydrates in brown rice while white milled rice composed of as much as 90% carbohydrates. Higher starch content in the rice is considered as the poor quality food for diabetic patients, because rice starch is usually digested quite rapidly compared to other starch and this leads to a prompt and pronounced increase of the blood glucose level after the ingestion of rice, similar to that of white bread or pure glucose and can induce type II diabetes (i.e. non-insulin dependent diabetes) in adults. Frei and Becker (2003) also reported that rice landraces having slow digestion give a relatively long feeling of satiation.

Clustering analysis (Supplementary Figure 1) of landraces based on their biochemical composition reflects that nutritional parameters are randomly distributed with respect to their quantity among landraces irrespective of their geographical origin or their specialties like aleurone colour, aroma, etc. However, as discussed above each nutritional parameter might have influence of local food preferences or local rituals, etc.

Table S2.Group wise multiple comparisons for least significant differences (LSD) using mean values of biochemical traits.

Dependent Variable			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Moisture	Red rice	Black rice	-2.08*	.79	.01	-3.66	-.51
		White rice	-0.34	0.47	0.47	-1.27	0.60
		Aromatic rice	-0.66	0.57	0.25	-1.81	0.49
		Non-aromatic rice	-0.31	0.44	0.49	-1.20	0.59
		NEH rice	-1.87*	0.57	0.00	-3.03	-0.73
		NWH rice	0	0.49	0.99	-1.00	0.99
	Black rice	Red rice	2.08*	0.79	0.01	0.51	3.66
		White rice	1.74*	0.75	0.02	0.24	3.25
		Aromatic rice	1.42	0.82	0.09	-0.23	3.07
		Non-aromatic rice	1.77*	0.73	0.02	0.30	3.25
		NEH rice	0.2	0.82	0.81	-1.44	1.85
		NWH rice	2.07*	0.77	0.01	0.54	3.62
	White rice	Red rice	0.34	0.47	0.47	-0.60	1.27
		Black rice	-1.74*	0.75	0.02	-3.25	-0.24
		Aromatic rice	-0.33	0.52	0.53	-1.37	0.72
		Non-aromatic rice	0.03	0.37	0.93	-0.72	0.78
		NEH rice	-1.54*	0.52	0.00	-2.59	-0.50
		NWH rice	0.33	0.43	0.45	-0.54	1.20
	Aromatic rice	Red rice	0.66	0.57	0.25	-0.49	1.81
		Black rice	-1.42	0.82	0.09	-3.07	0.23
		White rice	0.33	0.52	0.53	-0.72	1.37
		Non-aromatic rice	0.36	0.50	0.48	-0.65	1.37
		NEH rice	-1.22	0.62	0.06	-2.46	0.03
		NWH rice	0.66	0.55	0.23	-0.44	1.76
	Non-aromatic rice	Red rice	0.31	0.44	0.49	-0.59	1.20
		Black rice	-1.77*	0.73	0.02	-3.25	-0.30
		White rice	-0.03	0.37	0.93	-0.78	0.72
		Aromatic rice	-0.36	0.50	0.48	-1.37	0.65
		NEH rice	-1.57*	0.50	0.00	-2.58	-0.57
		NWH rice	0.3	0.41	0.46	-0.52	1.12
	NEH rice	Red rice	1.87*	0.57	0.00	0.73	3.03
		Black rice	-0.2	0.82	0.81	-1.85	1.44
		White rice	1.54*	0.52	0.00	0.50	2.59
		Aromatic rice	1.22	0.62	0.06	-0.03	2.46
		Non-aromatic rice	1.57*	0.50	0.00	0.57	2.58
		NWH rice	1.87*	0.55	0.00	0.78	2.97
	NWH rice	Red rice	0	0.49	0.99	-0.99	1.00
		Black rice	-2.07*	0.77	0.01	-3.62	-0.54
		White rice	-0.33	0.43	0.45	-1.20	0.54
		Aromatic rice	-0.66	0.55	0.23	-1.76	0.44
		Non-aromatic rice	-0.3	0.41	0.46	-1.12	0.52
		NEH rice	-1.87*	0.55	0.00	-2.97	-0.78
Ash	Red rice	Black rice	0.51	0.68	0.46	-0.86	1.89
		White rice	0.36	0.41	0.38	-0.46	1.17
		Aromatic rice	0.29	0.50	0.57	-0.72	1.29
		Non-aromatic rice	0.24	0.39	0.53	-0.54	1.02
		NEH rice	0.4	0.50	0.42	-0.60	1.41
		NWH rice	-0.08	0.43	0.86	-0.94	0.79
	Black rice	Red rice	-0.51	0.68	0.46	-1.89	0.86
		White rice	-0.15	0.65	0.81	-1.46	1.15
		Aromatic rice	-0.22	0.71	0.76	-1.66	1.21
		Non-aromatic rice	-0.27	0.64	0.67	-1.56	1.02
		NEH rice	-0.11	0.71	0.88	-1.54	1.33
		NWH rice	-0.59	0.67	0.38	-1.93	0.75

Contd.

Dependent Variable			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
	White rice	Red rice	-0.36	0.41	0.38	-1.17	0.46
		Black rice	0.15	0.65	0.81	-1.15	1.46
		Aromatic rice	-0.07	0.45	0.88	-0.98	0.84
		Non-aromatic rice	-0.12	0.33	0.72	-0.77	0.54
		NEH rice	0.05	0.45	0.92	-0.86	0.96
		NWH rice	-0.43	0.38	0.25	-1.19	0.32
	Aromatic rice	Red rice	-0.29	0.50	0.57	-1.29	0.72
		Black rice	0.22	0.71	0.76	-1.21	1.66
		White rice	0.07	0.45	0.88	-0.84	0.98
		Non-aromatic rice	-0.05	0.44	0.92	-0.92	0.83
		NEH rice	0.12	0.54	0.83	-0.97	1.20
		NWH rice	-0.37	0.48	0.45	-1.32	0.59
	Non-aromatic rice	Red rice	-0.24	0.39	0.53	-1.02	0.54
		Black rice	0.27	0.64	0.67	-1.02	1.56
		White rice	0.12	0.33	0.72	-0.54	0.77
		Aromatic rice	0.05	0.44	0.92	-0.83	0.92
		NEH rice	0.16	0.44	0.71	-0.72	1.04
		NWH rice	-0.32	0.36	0.37	-1.03	0.39
	NEH rice	Red rice	-0.4	0.50	0.42	-1.41	0.60
		Black rice	0.11	0.71	0.88	-1.33	1.54
		White rice	-0.05	0.45	0.92	-0.96	0.86
		Aromatic rice	-0.12	0.54	0.83	-1.20	0.97
		Non-aromatic rice	-0.16	0.44	0.71	-1.04	0.72
		NWH rice	-0.48	0.48	0.32	-1.44	0.47
	NWH rice	Red rice	0.08	0.43	0.86	-0.79	0.94
		Black rice	0.59	0.67	0.38	-0.75	1.93
		White rice	0.43	0.38	0.25	-0.32	1.19
		Aromatic rice	0.37	0.48	0.45	-0.59	1.32
		Non-aromatic rice	0.32	0.36	0.37	-0.39	1.03
		NEH rice	0.48	0.48	0.32	-0.47	1.44
Protein	Red rice	Black rice	-4.28*	1.27	0.00	-6.83	-1.73
		White rice	-0.51	0.75	0.50	-2.03	1.00
		Aromatic rice	-1.67	0.93	0.08	-3.53	0.19
		Non-aromatic rice	-0.4	0.72	0.58	-1.84	1.04
		NEH rice	-3.54*	0.93	0.00	-5.40	-1.68
		NWH rice	0.07	0.80	0.93	-1.54	1.67
	Black rice	Red rice	4.2*	1.27	0.00	1.73	6.83
		White rice	3.76*	1.21	0.00	1.34	6.20
		Aromatic rice	2.61	1.32	0.05	-0.05	5.27
		Non-aromatic rice	3.88*	1.19	0.00	1.50	6.27
		NEH rice	0.74	1.32	0.58	-1.92	3.40
		NWH rice	4.34*	1.24	0.00	1.86	6.83
	White rice	Red rice	0.51	0.75	0.50	-1.00	2.03
		Black rice	-3.76*	1.21	0.00	-6.20	-1.34
		Aromatic rice	-1.16	0.84	0.18	-2.85	0.54
		Non-aromatic rice	0.12	0.60	0.85	-1.10	1.33
		NEH rice	-3.02*	0.84	0.00	-4.72	-1.33
		NWH rice	0.58	0.70	0.41	-0.82	1.98
	Aromatic rice	Red rice	1.67	0.93	0.08	-0.19	3.53
		Black rice	-2.61	1.32	0.05	-5.27	0.05
		White rice	1.16	0.84	0.18	-0.54	2.85
		Non-aromatic rice	1.27	0.81	0.12	-0.36	2.90
		NEH rice	-1.87	1.00	0.07	-3.88	0.14
		NWH rice	1.74	0.88	0.05	-0.04	3.51

Contd.

Dependent Variable			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
	Non-aromatic rice	Red rice	0.4	0.72	0.58	-1.04	1.84
		Black rice	-3.88*	1.19	0.00	-6.27	-1.50
		White rice	-0.12	0.60	0.85	-1.33	1.10
		Aromatic rice	-1.27	0.81	0.12	-2.90	0.36
		NEH rice	-3.14*	0.81	0.00	-4.77	-1.51
		NWH rice	0.47	0.66	0.48	-0.86	1.79
	NEH rice	Red rice	3.54*	0.93	0.00	1.68	5.40
		Black rice	-0.74	1.32	0.58	-3.40	1.92
		White rice	3.02*	0.84	0.00	1.33	4.72
		Aromatic rice	1.87	1.00	0.07	-0.14	3.88
		Non-aromatic rice	3.14*	0.81	0.00	1.51	4.77
		NWH rice	3.60*	0.88	0.00	1.83	5.38
	NWH rice	Red rice	-0.07	0.80	0.93	-1.67	1.54
		Black rice	-4.34*	1.24	0.00	-6.83	-1.86
		White rice	-0.58	0.70	0.41	-1.98	0.82
		Aromatic rice	-1.74	0.88	0.05	-3.51	0.04
		Non-aromatic rice	-0.47	0.66	0.48	-1.79	0.86
		NEH rice	-3.60*	0.88	0.00	-5.38	-1.83
CF	Red rice	Black rice	1.31	1.14	0.26	-0.99	3.61
		White rice	0.94	0.68	0.17	-0.42	2.30
		Aromatic rice	0.46	0.84	0.58	-1.22	2.14
		Non-aromatic rice	0.73	0.65	0.27	-0.57	2.02
		NEH rice	1.47	0.84	0.08	-0.21	3.15
		NWH rice	-0.32	0.72	0.66	-1.76	1.13
	Black rice	Red rice	-1.31	1.14	0.26	-3.61	0.99
		White rice	-0.37	1.09	0.74	-2.56	1.82
		Aromatic rice	-0.85	1.19	0.48	-3.25	1.55
		Non-aromatic rice	-0.59	1.07	0.59	-2.74	1.56
		NEH rice	0.16	1.19	0.89	-2.24	2.56
		NWH rice	-1.63	1.12	0.15	-3.87	0.61
	White rice	Red rice	-0.94	0.68	0.17	-2.30	0.42
		Black rice	0.37	1.09	0.74	-1.82	2.56
		Aromatic rice	-0.48	0.76	0.53	-2.01	1.04
		Non-aromatic rice	-0.21	0.54	0.69	-1.31	0.88
		NEH rice	0.53	0.76	0.49	-1.00	2.06
		NWH rice	-1.26	0.63	0.05	-2.52	0.01
	Aromatic rice	Red rice	-0.46	0.84	0.58	-2.14	1.22
		Black rice	0.85	1.19	0.48	-1.55	3.25
		White rice	0.48	0.76	0.53	-1.04	2.01
		Non-aromatic rice	0.27	0.73	0.72	-1.20	1.73
		NEH rice	1.01	0.90	0.27	-0.80	2.82
		NWH rice	-0.78	0.80	0.33	-2.37	0.82
	Non-aromatic rice	Red rice	-0.73	0.65	0.27	-2.02	0.57
		Black rice	0.59	1.07	0.59	-1.56	2.74
		White rice	0.21	0.54	0.69	-0.88	1.31
		Aromatic rice	-0.27	0.73	0.72	-1.73	1.20
		NEH rice	0.74	0.73	0.31	-0.72	2.21
		NWH rice	-1.04	0.59	0.09	-2.24	0.15
	NEH rice	Red rice	-1.47	0.84	0.08	-3.15	0.21
		Black rice	-0.16	1.19	0.89	-2.56	2.24
		White rice	-0.53	0.76	0.49	-2.06	1.00
		Aromatic rice	-1.01	0.90	0.27	-2.82	0.80
		Non-aromatic rice	-0.74	0.73	0.31	-2.21	0.72
		NWH rice	-1.78*	0.80	0.03	-3.39	-0.19

Contd.

Dependent Variable			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
	NWH rice	Red rice	0.32	0.72	0.66	-1.13	1.76
		Black rice	1.63	1.12	0.15	-0.61	3.87
		White rice	1.26	0.63	0.05	-0.01	2.52
		Aromatic rice	0.78	0.80	0.33	-0.82	2.37
		Non-aromatic rice	1.04	0.59	0.09	-0.15	2.24
		NEH rice	1.78*	0.80	0.03	0.19	3.39
DF	Red rice	Black rice	-0.46	1.35	0.74	-3.17	2.25
		White rice	0.37	0.80	0.65	-1.24	1.97
		Aromatic rice	0.88	0.98	0.37	-1.09	2.86
		Non-aromatic rice	-0.06	0.76	0.94	-1.59	1.47
		NEH rice	0.1	0.98	0.92	-1.87	2.08
		NWH rice	0.33	0.85	0.70	-1.37	2.04
	Black rice	Red rice	0.46	1.35	0.74	-2.25	3.17
		White rice	0.82	1.28	0.52	-1.76	3.40
		Aromatic rice	1.34	1.41	0.34	-1.49	4.17
		Non-aromatic rice	0.4	1.26	0.75	-2.13	2.93
		NEH rice	0.56	1.41	0.69	-2.26	3.39
		NWH rice	0.79	1.31	0.55	-1.85	3.43
	White rice	Red rice	-0.37	0.80	0.65	-1.97	1.24
		Black rice	-0.82	1.28	0.52	-3.40	1.76
		Aromatic rice	0.52	0.89	0.57	-1.28	2.32
		Non-aromatic rice	-0.43	0.64	0.51	-1.72	0.86
		NEH rice	-0.26	0.89	0.77	-2.06	1.54
		NWH rice	-0.03	0.74	0.96	-1.52	1.46
	Aromatic rice	Red rice	-0.88	0.98	0.37	-2.86	1.09
		Black rice	-1.34	1.41	0.34	-4.17	1.49
		White rice	-0.52	0.89	0.57	-2.32	1.28
		Non-aromatic rice	-0.94	0.86	0.28	-2.67	0.79
		NEH rice	-0.78	1.06	0.47	-2.92	1.36
		NWH rice	-0.55	0.94	0.56	-2.44	1.33
	Non-aromatic rice	Red rice	0.06	0.76	0.94	-1.47	1.59
		Black rice	-0.4	1.26	0.75	-2.93	2.13
		White rice	0.43	0.64	0.51	-0.86	1.72
		Aromatic rice	0.94	0.86	0.28	-0.79	2.67
		NEH rice	0.16	0.86	0.85	-1.57	1.89
		NWH rice	0.39	0.70	0.58	-1.02	1.80
	NEH rice	Red rice	-0.1	0.98	0.92	-2.08	1.87
		Black rice	-0.56	1.41	0.69	-3.39	2.26
		White rice	0.26	0.89	0.77	-1.54	2.06
		Aromatic rice	0.78	1.06	0.47	-1.36	2.92
		Non-aromatic rice	-0.16	0.86	0.85	-1.89	1.57
		NWH rice	0.23	0.94	0.81	-1.66	2.11
	NWH rice	Red rice	-0.33	0.85	0.70	-2.04	1.37
		Black rice	-0.79	1.31	0.55	-3.43	1.85
		White rice	0.03	0.74	0.96	-1.46	1.52
		Aromatic rice	0.55	0.94	0.56	-1.33	2.44
		Non-aromatic rice	-0.39	0.70	0.58	-1.80	1.02
		NEH rice	-0.23	0.94	0.81	-2.11	1.66
CHOAVL	Red rice	Black rice	5	2.56	0.06	-0.15	10.15
		White rice	-0.81	1.52	0.59	-3.87	2.24
		Aromatic rice	0.7	1.87	0.71	-3.06	4.46
		Non-aromatic rice	-0.21	1.45	0.89	-3.12	2.71
		NEH rice	3.44	1.87	0.07	-0.32	7.20
		NWH rice	0	1.61	1.00	-3.24	3.24

Contd.

Dependent Variable			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
	Black rice	Red rice	-5	2.56	0.06	-10.15	0.15
		White rice	-5.81*	2.44	0.02	-10.72	-0.91
		Aromatic rice	-4.3	2.67	0.11	-9.67	1.08
		Non-aromatic rice	-5.20*	2.40	0.03	-10.02	-0.39
		NEH rice	-1.56	2.67	0.56	-6.93	3.82
		NWH rice	-5	2.50	0.05	-10.02	0.02
	White rice	Red rice	0.81	1.52	0.59	-2.24	3.87
		Black rice	5.81*	2.44	0.02	0.91	10.72
		Aromatic rice	1.52	1.70	0.38	-1.90	4.93
		Non-aromatic rice	0.61	1.22	0.62	-1.84	3.06
		NEH rice	4.25*	1.70	0.02	0.84	7.67
		NWH rice	0.81	1.41	0.57	-2.02	3.65
	Aromatic rice	Red rice	-0.7	1.87	0.71	-4.46	3.06
		Black rice	4.3	2.67	0.11	-1.08	9.67
		White rice	-1.52	1.70	0.38	-4.93	1.90
		Non-aromatic rice	-0.91	1.64	0.58	-4.20	2.38
		NEH rice	2.74	2.02	0.18	-1.32	6.80
		NWH rice	-0.7	1.78	0.70	-4.28	2.88
	Non-aromatic rice	Red rice	0.21	1.45	0.89	-2.71	3.12
		Black rice	5.20*	2.40	0.03	0.39	10.02
		White rice	-0.61	1.22	0.62	-3.06	1.84
		Aromatic rice	0.91	1.64	0.58	-2.38	4.20
		NEH rice	3.64*	1.64	0.03	0.36	6.94
		NWH rice	0.2	1.33	0.88	-2.47	2.88
	NEH rice	Red rice	-3.44	1.87	0.07	-7.20	0.32
		Black rice	1.56	2.67	0.56	-3.82	6.93
		White rice	-4.25*	1.70	0.02	-7.67	-0.84
		Aromatic rice	-2.74	2.02	0.18	-6.80	1.32
		Non-aromatic rice	-3.64*	1.64	0.03	-6.94	-0.36
		NWH rice	-3.44	1.78	0.06	-7.02	0.14
	NWH rice	Red rice	0	1.61	1.00	-3.24	3.24
		Black rice	5	2.50	0.05	-0.02	10.02
		White rice	-0.81	1.41	0.57	-3.65	2.02
		Aromatic rice	0.7	1.78	0.70	-2.88	4.28
		Non-aromatic rice	-0.2	1.33	0.88	-2.88	2.47
		NEH rice	3.44	1.78	0.06	-0.14	7.02
Sugar	Red rice	Black rice	-0.29	0.70	0.68	-1.69	1.12
		White rice	1.12*	0.41	0.01	0.29	1.95
		Aromatic rice	0.72	0.51	0.16	-0.30	1.75
		Non-aromatic rice	0.58	0.39	0.15	-0.21	1.37
		NEH rice	0.55	0.51	0.28	-0.47	1.58
		NWH rice	0.4	0.44	0.36	-0.48	1.28
	Black rice	Red rice	0.29	0.70	0.68	-1.12	1.69
		White rice	1.41*	0.67	0.04	0.07	2.75
		Aromatic rice	1.01	0.73	0.17	-0.45	2.48
		Non-aromatic rice	0.87	0.65	0.19	-0.44	2.18
		NEH rice	0.84	0.73	0.25	-0.62	2.31
		NWH rice	0.69	0.68	0.32	-0.68	2.06
	White rice	Red rice	-1.12*	0.41	0.01	-1.95	-0.29
		Black rice	-1.41*	0.67	0.04	-2.75	-0.07
		Aromatic rice	-0.4	0.46	0.39	-1.33	0.53
		Non-aromatic rice	-0.54	0.33	0.11	-1.21	0.13
		NEH rice	-0.57	0.46	0.23	-1.50	0.36
		NWH rice	-0.72	0.38	0.07	-1.49	0.05

Contd.

Dependent Variable			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
	Aromatic rice	Red rice	-0.72	0.51	0.16	-1.75	0.30
		Black rice	-1.01	0.73	0.17	-2.48	0.45
		White rice	0.4	0.46	0.39	-0.53	1.33
		Non-aromatic rice	-0.14	0.45	0.75	-1.04	0.75
		NEH rice	-0.17	0.55	0.76	-1.27	0.94
		NWH rice	-0.32	0.49	0.51	-1.30	0.66
	Non-aromatic rice	Red rice	-0.58	0.39	0.15	-1.37	0.21
		Black rice	-0.87	0.65	0.19	-2.18	0.44
		White rice	0.54	0.33	0.11	-0.13	1.21
		Aromatic rice	0.14	0.45	0.75	-0.75	1.04
		NEH rice	-0.03	0.45	0.96	-0.92	0.87
		NWH rice	-0.18	0.36	0.63	-0.91	0.55
	NEH rice	Red rice	-0.55	0.51	0.28	-1.58	0.47
		Black rice	-0.84	0.73	0.25	-2.31	0.62
		White rice	0.57	0.46	0.23	-0.36	1.50
		Aromatic rice	0.17	0.55	0.76	-0.94	1.27
		Non-aromatic rice	0.03	0.45	0.96	-0.87	0.92
		NWH rice	-0.15	0.49	0.76	-1.13	0.82
	NWH rice	Red rice	-0.4	0.44	0.36	-1.28	0.48
		Black rice	-0.69	0.68	0.32	-2.06	0.68
		White rice	0.72	0.38	0.07	-0.05	1.49
		Aromatic rice	0.32	0.49	0.51	-0.66	1.30
		Non-aromatic rice	0.18	0.36	0.63	-0.55	0.91
		NEH rice	0.15	0.49	0.76	-0.82	1.13
Starch	Red rice	Black rice	4.51	2.37	0.06	-0.25	9.26
		White rice	-0.94	1.40	0.51	-3.76	1.88
		Aromatic rice	0.99	1.73	0.57	-2.48	4.47
		Non-aromatic rice	-0.45	1.34	0.74	-3.14	2.24
		NEH rice	2.92	1.73	0.10	-0.55	6.40
		NWH rice	-0.2	1.49	0.89	-3.19	2.79
	Black rice	Red rice	-4.51	2.37	0.06	-9.26	0.25
		White rice	-5.44*	2.25	0.02	-9.98	-0.92
		Aromatic rice	-3.51	2.47	0.16	-8.48	1.45
		Non-aromatic rice	-4.95*	2.21	0.03	-9.41	-0.51
		NEH rice	-1.58	2.47	0.52	-6.55	3.38
		NWH rice	-4.70*	2.31	0.05	-9.34	-0.07
	White rice	Red rice	0.94	1.40	0.51	-1.88	3.76
		Black rice	5.44*	2.25	0.02	0.92	9.98
		Aromatic rice	1.93	1.57	0.22	-1.23	5.09
		Non-aromatic rice	0.49	1.13	0.67	-1.78	2.75
		NEH rice	3.86*	1.57	0.02	0.71	7.02
		NWH rice	0.74	1.30	0.57	-1.88	3.35
	Aromatic rice	Red rice	-0.99	1.73	0.57	-4.47	2.48
		Black rice	3.51	2.47	0.16	-1.45	8.48
		White rice	-1.93	1.57	0.22	-5.09	1.23
		Non-aromatic rice	-1.44	1.51	0.34	-4.48	1.59
		NEH rice	1.93	1.87	0.31	-1.82	5.68
		NWH rice	-1.19	1.65	0.47	-4.50	2.12
	Non-aromatic rice	Red rice	0.45	1.34	0.74	-2.24	3.14
		Black rice	4.95*	2.21	0.03	0.51	9.41
		White rice	-0.49	1.13	0.67	-2.75	1.78
		Aromatic rice	1.44	1.51	0.34	-1.59	4.48
		NEH rice	3.37*	1.51	0.03	0.34	6.41
		NWH rice	0.25	1.23	0.84	-2.22	2.72

Contd.

Dependent Variable			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
	NEH rice	Red rice	-2.92	1.73	0.10	-6.40	0.55
		Black rice	1.58	2.47	0.52	-3.38	6.55
		White rice	-3.86*	1.57	0.02	-7.02	-0.71
		Aromatic rice	-1.93	1.87	0.31	-5.68	1.82
		Non-aromatic rice	-3.37*	1.51	0.03	-6.41	-0.34
		NWH rice	-3.12	1.65	0.06	-6.43	0.19
	NWH rice	Red rice	0.2	1.49	0.89	-2.79	3.19
		Black rice	4.70*	2.31	0.05	0.07	9.34
		White rice	-0.74	1.30	0.57	-3.35	1.88
		Aromatic rice	1.19	1.65	0.47	-2.12	4.50
		Non-aromatic rice	-0.25	1.23	0.84	-2.72	2.22
		NEH rice	3.12	1.65	0.06	-0.19	6.43
TPhosg100g	Red rice	Black rice	-.43*	0.12	0.00	-0.67	-0.20
		White rice	0.02	0.07	0.81	-0.12	0.15
		Aromatic rice	-0.03	0.08	0.74	-0.20	0.14
		Non-aromatic rice	-0.03	0.07	0.61	-0.16	0.10
		NEH rice	-.29*	0.08	0.00	-0.47	-0.13
		NWH rice	0.02	0.07	0.77	-0.12	0.17
	Black rice	Red rice	.43*	0.12	0.00	0.20	0.67
		White rice	.45*	0.11	0.00	0.23	0.67
		Aromatic rice	.40*	0.12	0.00	0.17	0.65
		Non-aromatic rice	.40*	0.11	0.00	0.19	0.62
		NEH rice	0.14	0.12	0.25	-0.10	0.38
		NWH rice	.45*	0.11	0.00	0.23	0.68
	White rice	Red rice	-0.02	0.07	0.81	-0.15	0.12
		Black rice	-.45*	0.11	0.00	-0.67	-0.23
		Aromatic rice	-0.04	0.08	0.57	-0.20	0.11
		Non-aromatic rice	-0.05	0.05	0.37	-0.16	0.06
		NEH rice	-.31*	0.08	0.00	-0.47	-0.16
		NWH rice	0	0.06	0.94	-0.12	0.13
	Aromatic rice	Red rice	0.03	0.08	0.74	-0.14	0.20
		Black rice	-.40*	0.12	0.00	-0.65	-0.17
		White rice	0.04	0.08	0.57	-0.11	0.20
		Non-aromatic rice	-0.01	0.07	0.93	-0.15	0.14
		NEH rice	-.26*	0.09	0.00	-0.45	-0.09
		NWH rice	0.05	0.08	0.55	-0.11	0.21
	Non-aromatic rice	Red rice	0.03	0.07	0.61	-0.10	0.16
		Black rice	-.40*	0.11	0.00	-0.62	-0.19
		White rice	0.05	0.05	0.37	-0.06	0.16
		Aromatic rice	0.01	0.07	0.93	-0.14	0.15
		NEH rice	-.26*	0.07	0.00	-0.41	-0.12
		NWH rice	0.05	0.06	0.37	-0.07	0.17
	NEH rice	Red rice	.29*	0.08	0.00	0.13	0.47
		Black rice	-0.14	0.12	0.25	-0.38	0.10
		White rice	.31*	0.08	0.00	0.16	0.47
		Aromatic rice	.26*	0.09	0.00	0.09	0.45
		Non-aromatic rice	.26*	0.07	0.00	0.12	0.41
		NWH rice	.31*	0.08	0.00	0.16	0.48
	NWH rice	Red rice	-0.02	0.07	0.77	-0.17	0.12
		Black rice	-.45*	0.11	0.00	-0.68	-0.23
		White rice	0	0.06	0.94	-0.13	0.12
		Aromatic rice	-0.05	0.08	0.55	-0.21	0.11
		Non-aromatic rice	-0.05	0.06	0.37	-0.17	0.07
		NEH rice	-.31*	0.08	0.00	-0.48	-0.16

Contd.

Dependent Variable			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Phytateg100g	Red rice	Black rice	-1.52*	0.40	0.00	-2.34	-0.71
		White rice	0.03	0.24	0.89	-0.45	0.52
		Aromatic rice	-0.1	0.30	0.75	-0.69	0.50
		Non-aromatic rice	-0.14	0.23	0.56	-0.60	0.32
		NEH rice	-1.05*	0.30	0.00	-1.65	-0.46
		NWH rice	0.07	0.25	0.77	-0.44	0.58
	Black rice	Red rice	1.52*	0.40	0.00	0.71	2.34
		White rice	1.55*	0.39	0.00	0.78	2.34
		Aromatic rice	1.42*	0.42	0.00	0.58	2.28
		Non-aromatic rice	1.39*	0.38	0.00	0.63	2.15
		NEH rice	0.47	0.42	0.27	-0.37	1.32
		NWH rice	1.59*	0.39	0.00	0.81	2.39
	White rice	Red rice	-0.03	0.24	0.89	-0.52	0.45
		Black rice	-1.55*	0.39	0.00	-2.34	-0.78
		Aromatic rice	-0.13	0.27	0.63	-0.67	0.41
		Non-aromatic rice	-0.17	0.19	0.38	-0.56	0.22
		NEH rice	-1.08*	0.27	0.00	-1.63	-0.54
		NWH rice	0.04	0.22	0.86	-0.41	0.49
	Aromatic rice	Red rice	0.1	0.30	0.75	-0.50	0.69
		Black rice	-1.42*	0.42	0.00	-2.28	-0.58
		White rice	0.13	0.27	0.63	-0.41	0.67
		Non-aromatic rice	-0.04	0.26	0.88	-0.56	0.48
		NEH rice	-.95*	0.32	0.00	-1.60	-0.31
		NWH rice	0.17	0.28	0.55	-0.40	0.74
	Non-aromatic rice	Red rice	0.14	0.23	0.56	-0.32	0.60
		Black rice	-1.39*	0.38	0.00	-2.15	-0.63
		White rice	0.17	0.19	0.38	-0.22	0.56
		Aromatic rice	0.04	0.26	0.88	-0.48	0.56
		NEH rice	-.91*	0.26	0.00	-1.44	-0.40
		NWH rice	0.21	0.21	0.33	-0.21	0.63
	NEH rice	Red rice	1.05*	0.30	0.00	0.46	1.65
		Black rice	-0.47	0.42	0.27	-1.32	0.37
		White rice	1.08*	0.27	0.00	0.54	1.63
		Aromatic rice	.95*	0.32	0.00	0.31	1.60
		Non-aromatic rice	.91*	0.26	0.00	0.40	1.44
		NWH rice	1.12*	0.28	0.00	0.56	1.69
	NWH rice	Red rice	-0.07	0.25	0.77	-0.58	0.44
		Black rice	-1.59*	0.39	0.00	-2.39	-0.81
		White rice	-0.04	0.22	0.86	-0.49	0.41
		Aromatic rice	-0.17	0.28	0.55	-0.74	0.40
		Non-aromatic rice	-0.21	0.21	0.33	-0.63	0.21
		NEH rice	-1.12*	0.28	0.00	-1.69	-0.56
Anthocyanin	Red rice	Black rice	-1.06*	0.28	0.00	-1.62	-0.51
		White rice	0.31	0.16	0.07	-0.02	0.64
		Aromatic rice	0	0.20	0.98	-0.40	0.41
		Non-aromatic rice	0.1	0.16	0.54	-0.22	0.41
		NEH rice	-0.27	0.20	0.19	-0.67	0.14
		NWH rice	0.06	0.17	0.73	-0.29	0.41
	Black rice	Red rice	1.06*	0.28	0.00	0.51	1.62
		White rice	1.37*	0.26	0.00	0.85	1.90
		Aromatic rice	1.07*	0.29	0.00	0.49	1.65
		Non-aromatic rice	1.16*	0.26	0.00	0.65	1.68
		NEH rice	.80*	0.29	0.01	0.22	1.38
		NWH rice	1.12*	0.27	0.00	0.59	1.67

Contd.

Dependent Variable			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
	White rice	Red rice	-0.31	0.16	0.07	-0.64	0.02
		Black rice	-1.37*	0.26	0.00	-1.90	-0.85
		Aromatic rice	-0.3	0.18	0.10	-0.67	0.06
		Non-aromatic rice	-0.21	0.13	0.11	-0.48	0.05
		NEH rice	-.57*	0.18	0.00	-0.94	-0.21
		NWH rice	-0.25	0.15	0.11	-0.55	0.06
	Aromatic rice	Red rice	0	0.20	0.98	-0.41	0.40
		Black rice	-1.07*	0.29	0.00	-1.65	-0.49
		White rice	0.3	0.18	0.10	-0.06	0.67
		Non-aromatic rice	0.09	0.18	0.60	-0.26	0.45
		NEH rice	-0.27	0.22	0.22	-0.71	0.17
		NWH rice	0.06	0.19	0.77	-0.33	0.44
	Non-aromatic rice	Red rice	-0.1	0.16	0.54	-0.41	0.22
		Black rice	-1.16*	0.26	0.00	-1.68	-0.65
		White rice	0.21	0.13	0.11	-0.05	0.48
		Aromatic rice	-0.09	0.18	0.60	-0.45	0.26
		NEH rice	-.36*	0.18	0.04	-0.72	-0.01
		NWH rice	-0.04	0.14	0.80	-0.33	0.25
	NEH rice	Red rice	0.27	0.20	0.19	-0.14	0.67
		Black rice	-.80*	0.29	0.01	-1.38	-0.22
		White rice	.57*	0.18	0.00	0.21	0.94
		Aromatic rice	0.27	0.22	0.22	-0.17	0.71
		Non-aromatic rice	.36*	0.18	0.04	0.01	0.72
		NWH rice	0.33	0.19	0.09	-0.06	0.71
	NWH rice	Red rice	-0.06	0.17	0.73	-0.41	0.29
		Black rice	-1.12*	0.27	0.00	-1.67	-0.59
		White rice	0.25	0.15	0.11	-0.06	0.55
		Aromatic rice	-0.06	0.19	0.77	-0.44	0.33
		Non-aromatic rice	0.04	0.14	0.80	-0.25	0.33
		NEH rice	-0.33	0.19	0.09	-0.71	0.06
Total Phenol	Red rice	Black rice	-0.1	0.08	0.21	-0.25	0.06
		White rice	0.09	0.05	0.06	0.00	0.18
		Aromatic rice	0.06	0.06	0.31	-0.06	0.17
		Non-aromatic rice	0.04	0.04	0.40	-0.05	0.12
		NEH rice	0	0.06	0.94	-0.11	0.12
		NWH rice	0.03	0.05	0.52	-0.07	0.13
	Black rice	Red rice	0.1	0.08	0.21	-0.06	0.25
		White rice	.18*	0.07	0.01	0.04	0.34
		Aromatic rice	0.16	0.08	0.06	-0.01	0.32
		Non-aromatic rice	0.14	0.07	0.07	-0.01	0.28
		NEH rice	0.1	0.08	0.21	-0.06	0.26
		NWH rice	0.13	0.08	0.09	-0.02	0.28
	White rice	Red rice	-0.09	0.05	0.06	-0.18	0.00
		Black rice	-.18*	0.07	0.01	-0.34	-0.04
		Aromatic rice	-0.03	0.05	0.54	-0.13	0.07
		Non-aromatic rice	-0.05	0.04	0.16	-0.13	0.02
		NEH rice	-0.09	0.05	0.10	-0.19	0.02
		NWH rice	-0.06	0.04	0.17	-0.14	0.03
	Aromatic rice	Red rice	-0.06	0.06	0.31	-0.17	0.06
		Black rice	-0.16	0.08	0.06	-0.32	0.01
		White rice	0.03	0.05	0.54	-0.07	0.13
		Non-aromatic rice	-0.02	0.05	0.67	-0.12	0.08
		NEH rice	-0.05	0.06	0.38	-0.18	0.07
		NWH rice	-0.03	0.05	0.62	-0.13	0.08

Contd.

Dependent Variable			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
	Non-aromatic rice	Red rice	-0.04	0.04	0.40	-0.12	0.05
		Black rice	-0.14	0.07	0.07	-0.28	0.01
		White rice	0.05	0.04	0.16	-0.02	0.13
		Aromatic rice	0.02	0.05	0.67	-0.08	0.12
		NEH rice	-0.03	0.05	0.51	-0.13	0.07
		NWH rice	-0.01	0.04	0.89	-0.09	0.07
	NEH rice	Red rice	0	0.06	0.94	-0.12	0.11
		Black rice	-0.1	0.08	0.21	-0.26	0.06
		White rice	0.09	0.05	0.10	-0.02	0.19
		Aromatic rice	0.05	0.06	0.38	-0.07	0.18
		Non-aromatic rice	0.03	0.05	0.51	-0.07	0.13
		NWH rice	0.03	0.05	0.62	-0.08	0.13
	NWH rice	Red rice	-0.03	0.05	0.52	-0.13	0.07
		Black rice	-0.13	0.08	0.09	-0.28	0.02
		White rice	0.06	0.04	0.17	-0.03	0.14
		Aromatic rice	0.03	0.05	0.62	-0.08	0.13
		Non-aromatic rice	0.01	0.04	0.89	-0.07	0.09
		NEH rice	-0.03	0.05	0.62	-0.13	0.08
PhyAnthPhe	Red rice	Black rice	-2.69*	0.65	0.00	-4.00	-1.39
		White rice	0.43	0.38	0.27	-0.34	1.20
		Aromatic rice	-0.03	0.47	0.94	-0.99	0.92
		Non-aromatic rice	0	0.37	1.00	-0.74	0.73
		NEH rice	-1.31*	0.47	0.01	-2.27	-0.36
		NWH rice	0.16	0.41	0.69	-0.66	0.98
	Black rice	Red rice	2.69*	0.65	0.00	1.39	4.00
		White rice	3.12*	0.62	0.00	1.88	4.37
		Aromatic rice	2.65*	0.68	0.00	1.30	4.02
		Non-aromatic rice	2.69*	0.61	0.00	1.47	3.91
		NEH rice	1.37*	0.68	0.05	0.02	2.74
		NWH rice	2.85*	0.63	0.00	1.59	4.13
	White rice	Red rice	-0.43	0.38	0.27	-1.20	0.34
		Black rice	-3.12*	0.62	0.00	-4.37	-1.88
		Aromatic rice	-0.47	0.43	0.28	-1.33	0.40
		Non-aromatic rice	-0.43	0.31	0.17	-1.05	0.19
		NEH rice	-1.74*	0.43	0.00	-2.61	-0.88
		NWH rice	-0.27	0.36	0.46	-0.98	0.45
	Aromatic rice	Red rice	0.03	0.47	0.94	-0.92	0.99
		Black rice	-2.65*	0.68	0.00	-4.02	-1.30
		White rice	0.47	0.43	0.28	-0.40	1.33
		Non-aromatic rice	0.03	0.41	0.94	-0.80	0.86
		NEH rice	-1.28*	0.51	0.02	-2.31	-0.25
		NWH rice	0.2	0.45	0.66	-0.71	1.10
	Non-aromatic rice	Red rice	0	0.37	1.00	-0.73	0.74
		Black rice	-2.69*	0.61	0.00	-3.91	-1.47
		White rice	0.43	0.31	0.17	-0.19	1.05
		Aromatic rice	-0.03	0.41	0.94	-0.86	0.80
		NEH rice	-1.31*	0.41	0.00	-2.15	-0.48
		NWH rice	0.17	0.34	0.63	-0.51	0.84
	NEH rice	Red rice	1.31*	0.47	0.01	0.36	2.27
		Black rice	-1.37*	0.68	0.05	-2.74	-0.02
		White rice	1.74*	0.43	0.00	0.88	2.61
		Aromatic rice	1.28*	0.51	0.02	0.25	2.31
		Non-aromatic rice	1.31*	0.41	0.00	0.48	2.15
		NWH rice	1.47*	0.45	0.00	0.57	2.38

Contd.

Dependent Variable			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
	NWH rice	Red rice	-0.16	0.41	0.69	-0.98	0.66
		Black rice	-2.85*	0.63	0.00	-4.13	-1.59
		White rice	0.27	0.36	0.46	-0.45	0.98
		Aromatic rice	-0.2	0.45	0.66	-1.10	0.71
		Non-aromatic rice	-0.17	0.34	0.63	-0.84	0.51
		NEH rice	-1.47*	0.45	0.00	-2.38	-0.57
CUPRAC	Red rice	Black rice	-4.81*	1.54	0.00	-7.91	-1.72
		White rice	2.05*	0.91	0.03	0.22	3.89
		Aromatic rice	0.83	1.12	0.46	-1.43	3.09
		Non-aromatic rice	0.68	0.87	0.44	-1.07	2.43
		NEH rice	-1.44	1.12	0.21	-3.70	0.82
		NWH rice	0.45	0.97	0.64	-1.49	2.40
	Black rice	Red rice	4.81*	1.54	0.00	1.72	7.91
		White rice	6.86*	1.47	0.00	3.92	9.82
		Aromatic rice	5.64*	1.61	0.00	2.42	8.88
		Non-aromatic rice	5.49*	1.44	0.00	2.60	8.39
		NEH rice	3.38*	1.61	0.04	0.15	6.61
		NWH rice	5.26*	1.50	0.00	2.25	8.29
	White rice	Red rice	-2.05*	0.91	0.03	-3.89	-0.22
		Black rice	-6.86*	1.47	0.00	-9.82	-3.92
		Aromatic rice	-1.22	1.02	0.24	-3.28	0.83
		Non-aromatic rice	-1.37	0.73	0.07	-2.85	0.10
		NEH rice	-3.48*	1.02	0.00	-5.54	-1.43
		NWH rice	-1.6	0.85	0.06	-3.30	0.10
	Aromatic rice	Red rice	-0.83	1.12	0.46	-3.09	1.43
		Black rice	-5.64*	1.61	0.00	-8.88	-2.42
		White rice	1.22	1.02	0.24	-0.83	3.28
		Non-aromatic rice	-0.15	0.98	0.88	-2.13	1.83
		NEH rice	-2.27	1.21	0.07	-4.71	0.18
		NWH rice	-0.38	1.07	0.73	-2.53	1.78
	Non-aromatic rice	Red rice	-0.68	0.87	0.44	-2.43	1.07
		Black rice	-5.49*	1.44	0.00	-8.39	-2.60
		White rice	1.37	0.73	0.07	-0.10	2.85
		Aromatic rice	0.15	0.98	0.88	-1.83	2.13
		NEH rice	-2.11*	0.98	0.04	-4.09	-0.14
		NWH rice	-0.22	0.80	0.78	-1.83	1.38
	NEH rice	Red rice	1.44	1.12	0.21	-0.82	3.70
		Black rice	-3.38*	1.61	0.04	-6.61	-0.15
		White rice	3.48*	1.02	0.00	1.43	5.54
		Aromatic rice	2.27	1.21	0.07	-0.18	4.71
		Non-aromatic rice	2.11*	0.98	0.04	0.14	4.09
		NWH rice	1.89	1.07	0.08	-0.27	4.04
	NWH rice	Red rice	-0.45	0.97	0.64	-2.40	1.49
		Black rice	-5.26*	1.50	0.00	-8.29	-2.25
		White rice	1.6	0.85	0.06	-0.10	3.30
		Aromatic rice	0.38	1.07	0.73	-1.78	2.53
		Non-aromatic rice	0.22	0.80	0.78	-1.38	1.83
		NEH rice	-1.89	1.07	0.08	-4.04	0.27
Cumg100g	Red rice	Black rice	-.88*	0.35	0.02	-1.59	-0.18
		White rice	-0.23	0.21	0.27	-0.65	0.18
		Aromatic rice	-0.51	0.26	0.05	-1.03	0.00
		Non-aromatic rice	-0.13	0.20	0.53	-0.52	0.27
		NEH rice	-.99*	0.26	0.00	-1.51	-0.48
		NWH rice	-0.02	0.22	0.92	-0.47	0.42

Contd.

Dependent Variable			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
	Black rice	Red rice	.88*	0.35	0.02	0.18	1.59
		White rice	0.65	0.33	0.06	-0.02	1.32
		Aromatic rice	0.37	0.37	0.32	-0.37	1.10
		Non-aromatic rice	.75*	0.33	0.03	0.10	1.42
		NEH rice	-0.11	0.37	0.76	-0.85	0.62
		NWH rice	.86*	0.34	0.02	0.17	1.55
	White rice	Red rice	0.23	0.21	0.27	-0.18	0.65
		Black rice	-0.65	0.33	0.06	-1.32	0.02
		Aromatic rice	-0.28	0.23	0.23	-0.75	0.19
		Non-aromatic rice	0.11	0.17	0.52	-0.23	0.44
		NEH rice	-.76*	0.23	0.00	-1.23	-0.29
		NWH rice	0.21	0.19	0.28	-0.18	0.60
	Aromatic rice	Red rice	0.51	0.26	0.05	0.00	1.03
		Black rice	-0.37	0.37	0.32	-1.10	0.37
		White rice	0.28	0.23	0.23	-0.19	0.75
		Non-aromatic rice	0.39	0.22	0.09	-0.06	0.84
		NEH rice	-0.48	0.28	0.09	-1.04	0.07
		NWH rice	.49*	0.24	0.05	0.00	0.98
	Non-aromatic rice	Red rice	0.13	0.20	0.53	-0.27	0.52
		Black rice	-.75*	0.33	0.03	-1.42	-0.10
		White rice	-0.11	0.17	0.52	-0.44	0.23
		Aromatic rice	-0.39	0.22	0.09	-0.84	0.06
		NEH rice	-.87*	0.22	0.00	-1.32	-0.42
		NWH rice	0.1	0.18	0.58	-0.26	0.47
	NEH rice	Red rice	.99*	0.26	0.00	0.48	1.51
		Black rice	0.11	0.37	0.76	-0.62	0.85
		White rice	.76*	0.23	0.00	0.29	1.23
		Aromatic rice	0.48	0.28	0.09	-0.07	1.04
		Non-aromatic rice	.87*	0.22	0.00	0.42	1.32
		NWH rice	.97*	0.24	0.00	0.48	1.46
	NWH rice	Red rice	0.02	0.22	0.92	-0.42	0.47
		Black rice	-.86*	0.34	0.02	-1.55	-0.17
		White rice	-0.21	0.19	0.28	-0.60	0.18
		Aromatic rice	-.49*	0.24	0.05	-0.98	0.00
		Non-aromatic rice	-0.1	0.18	0.58	-0.47	0.26
		NEH rice	-.97*	0.24	0.00	-1.46	-0.48
Znmg100g	Red rice	Black rice	-0.65	1.29	0.62	-3.24	1.95
		White rice	-0.07	0.77	0.93	-1.61	1.47
		Aromatic rice	-0.88	0.94	0.35	-2.78	1.01
		Non-aromatic rice	0.14	0.73	0.84	-1.32	1.61
		NEH rice	-1.33	0.94	0.16	-3.23	0.56
		NWH rice	-0.24	0.81	0.77	-1.87	1.39
	Black rice	Red rice	0.65	1.29	0.62	-1.95	3.24
		White rice	0.58	1.23	0.64	-1.89	3.05
		Aromatic rice	-0.23	1.35	0.86	-2.94	2.47
		Non-aromatic rice	0.79	1.21	0.51	-1.63	3.22
		NEH rice	-0.68	1.35	0.61	-3.39	2.02
		NWH rice	0.41	1.26	0.74	-2.12	2.94
	White rice	Red rice	0.07	0.77	0.93	-1.47	1.61
		Black rice	-0.58	1.23	0.64	-3.05	1.89
		Aromatic rice	-0.81	0.86	0.35	-2.54	0.91
		Non-aromatic rice	0.21	0.61	0.73	-1.02	1.45
		NEH rice	-1.26	0.86	0.15	-2.99	0.46
		NWH rice	-0.17	0.71	0.81	-1.60	1.26

Contd.

Dependent Variable			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
	Aromatic rice	Red rice	0.88	0.94	0.35	-1.01	2.78
		Black rice	0.23	1.35	0.86	-2.47	2.94
		White rice	0.81	0.86	0.35	-0.91	2.54
		Non-aromatic rice	1.03	0.82	0.22	-0.63	2.68
		NEH rice	-0.45	1.02	0.66	-2.50	1.60
		NWH rice	0.65	0.90	0.48	-1.16	2.45
	Non-aromatic rice	Red rice	-0.14	0.73	0.84	-1.61	1.32
		Black rice	-0.79	1.21	0.51	-3.22	1.63
		White rice	-0.21	0.61	0.73	-1.45	1.02
		Aromatic rice	-1.03	0.82	0.22	-2.68	0.63
		NEH rice	-1.48	0.82	0.08	-3.13	0.18
		NWH rice	-0.38	0.67	0.57	-1.73	0.97
	NEH rice	Red rice	1.33	0.94	0.16	-0.56	3.23
		Black rice	0.68	1.35	0.61	-2.02	3.39
		White rice	1.26	0.86	0.15	-0.46	2.99
		Aromatic rice	0.45	1.02	0.66	-1.60	2.50
		Non-aromatic rice	1.48	0.82	0.08	-0.18	3.13
		NWH rice	1.1	0.90	0.23	-0.71	2.90
	NWH rice	Red rice	0.24	0.81	0.77	-1.39	1.87
		Black rice	-0.41	1.26	0.74	-2.94	2.12
		White rice	0.17	0.71	0.81	-1.26	1.60
		Aromatic rice	-0.65	0.90	0.48	-2.45	1.16
		Non-aromatic rice	0.38	0.67	0.57	-0.97	1.73
		NEH rice	-1.1	0.90	0.23	-2.90	0.71
Femg100g	Red rice	Black rice	1.16	1.08	0.29	-1.00	3.33
		White rice	-0.46	0.64	0.47	-1.75	0.82
		Aromatic rice	-0.12	0.79	0.88	-1.70	1.46
		Non-aromatic rice	-0.16	0.61	0.79	-1.39	1.06
		NEH rice	0.1	0.79	0.90	-1.48	1.68
		NWH rice	0.02	0.68	0.97	-1.34	1.39
	Black rice	Red rice	-1.16	1.08	0.29	-3.33	1.00
		White rice	-1.62	1.03	0.12	-3.69	0.44
		Aromatic rice	-1.28	1.12	0.26	-3.54	0.98
		Non-aromatic rice	-1.33	1.01	0.19	-3.35	0.70
		NEH rice	-1.06	1.12	0.35	-3.32	1.20
		NWH rice	-1.14	1.05	0.28	-3.25	0.98
	White rice	Red rice	0.46	0.64	0.47	-0.82	1.75
		Black rice	1.62	1.03	0.12	-0.44	3.69
		Aromatic rice	0.34	0.72	0.63	-1.09	1.78
		Non-aromatic rice	0.3	0.51	0.56	-0.73	1.33
		NEH rice	0.56	0.72	0.44	-0.88	2.00
		NWH rice	0.49	0.59	0.42	-0.70	1.68
	Aromatic rice	Red rice	0.12	0.79	0.88	-1.46	1.70
		Black rice	1.28	1.12	0.26	-0.98	3.54
		White rice	-0.34	0.72	0.63	-1.78	1.09
		Non-aromatic rice	-0.05	0.69	0.95	-1.43	1.34
		NEH rice	0.22	0.85	0.80	-1.49	1.92
		NWH rice	0.14	0.75	0.85	-1.36	1.65
	Non-aromatic rice	Red rice	0.16	0.61	0.79	-1.06	1.39
		Black rice	1.33	1.01	0.19	-0.70	3.35
		White rice	-0.3	0.51	0.56	-1.33	0.73
		Aromatic rice	0.05	0.69	0.95	-1.34	1.43
		NEH rice	0.26	0.69	0.71	-1.12	1.65
		NWH rice	0.19	0.56	0.74	-0.94	1.31

Contd.

Dependent Variable			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
	NEH rice	Red rice	-0.1	0.79	0.90	-1.68	1.48
		Black rice	1.06	1.12	0.35	-1.20	3.32
		White rice	-0.56	0.72	0.44	-2.00	0.88
		Aromatic rice	-0.22	0.85	0.80	-1.92	1.49
		Non-aromatic rice	-0.26	0.69	0.71	-1.65	1.12
		NWH rice	-0.07	0.75	0.92	-1.58	1.43
	NWH rice	Red rice	-0.02	0.68	0.97	-1.39	1.34
		Black rice	1.14	1.05	0.28	-0.98	3.25
		White rice	-0.49	0.59	0.42	-1.68	0.70
		Aromatic rice	-0.14	0.75	0.85	-1.65	1.36
		Non-aromatic rice	-0.19	0.56	0.74	-1.31	0.94
		NEH rice	0.07	0.75	0.92	-1.43	1.58
Mgmg100g	Red rice	Black rice	9.25	13.64	0.50	-18.16	36.66
		White rice	-7.12	8.09	0.38	-23.38	9.14
		Aromatic rice	-7.44	9.96	0.46	-27.45	12.58
		Non-aromatic rice	-1.86	7.71	0.81	-17.36	13.63
		NEH rice	1.59	9.96	0.87	-18.43	21.61
		NWH rice	1.85	8.57	0.83	-15.38	19.08
	Black rice	Red rice	-9.25	13.64	0.50	-36.66	18.16
		White rice	-16.37	12.99	0.21	-42.48	9.74
		Aromatic rice	-16.68	14.23	0.25	-45.29	11.92
		Non-aromatic rice	-11.11	12.76	0.39	-36.75	14.53
		NEH rice	-7.66	14.23	0.59	-36.26	20.94
		NWH rice	-7.4	13.30	0.58	-34.12	19.33
	White rice	Red rice	7.12	8.09	0.38	-9.14	23.38
		Black rice	16.37	12.99	0.21	-9.74	42.48
		Aromatic rice	-0.31	9.06	0.97	-18.51	17.88
		Non-aromatic rice	5.26	6.50	0.42	-7.79	18.32
		NEH rice	8.71	9.06	0.34	-9.48	26.91
		NWH rice	8.98	7.50	0.24	-6.10	24.05
	Aromatic rice	Red rice	7.44	9.96	0.46	-12.58	27.45
		Black rice	16.68	14.23	0.25	-11.92	45.29
		White rice	0.31	9.06	0.97	-17.88	18.51
		Non-aromatic rice	5.57	8.72	0.53	-11.94	23.09
		NEH rice	9.03	10.76	0.41	-12.60	30.65
		NWH rice	9.29	9.49	0.33	-9.78	28.36
	Non-aromatic rice	Red rice	1.86	7.71	0.81	-13.63	17.36
		Black rice	11.11	12.76	0.39	-14.53	36.75
		White rice	-5.26	6.50	0.42	-18.32	7.79
		Aromatic rice	-5.57	8.72	0.53	-23.09	11.94
		NEH rice	3.45	8.72	0.69	-14.06	20.97
		NWH rice	3.71	7.09	0.60	-10.53	17.96
	NEH rice	Red rice	-1.59	9.96	0.87	-21.61	18.43
		Black rice	7.66	14.23	0.59	-20.94	36.26
		White rice	-8.71	9.06	0.34	-26.91	9.48
		Aromatic rice	-9.03	10.76	0.41	-30.65	12.60
		Non-aromatic rice	-3.45	8.72	0.69	-20.97	14.06
		NWH rice	0.26	9.49	0.98	-18.81	19.33
	NWH rice	Red rice	-1.85	8.57	0.83	-19.08	15.38
		Black rice	7.4	13.30	0.58	-19.33	34.12
		White rice	-8.98	7.50	0.24	-24.05	6.10
		Aromatic rice	-9.29	9.49	0.33	-28.36	9.78
		Non-aromatic rice	-3.71	7.09	0.60	-17.96	10.53
		NEH rice	-0.26	9.49	0.98	-19.33	18.81

Contd.

This study suggest that the high nutritional quality of rice landraces can form a solid basis for changing priorities in rice breeding, putting more emphasis on the grain nutritional value. Combining high yields and high grain nutritional values with the aid of modern conventional breeding techniques, including molecular marker-assisted selection, may be very useful in accelerating the development of more nutritious rice varieties. However, the current prevalence of consuming milled rice may also nullify the efforts, as nutrient rich bran layer is removed in the milling process. Therefore, in addition to developing more nutritious varieties, awareness of the benefits of eating brown rice should be raised among rice consumers. Such a combined approach would ultimately result in a sustainable enhancement of the essential nutrient supply in rice-based diets and also will encourage the traditional farmers to continue and maintain local rice landrace diversity. The nutritional diversity in diverse rice landraces of same crop can be utilized for particular nutrient supply or in favourable combination thereof to improve nutritional status of human society. This will have much impact on the diets of the most economically disadvantaged people which are mainly dependent on rice as the main food crop. These rice landraces which are specialized for at least one or a few particular nutrient components can be utilized for supplying various nutrients as a single source or in combination of more than one specialized landraces to enhance final bioavailability of nutrients to human body. Thus rice bio-fortification appears to be an easy way by combining genetic background of high yielding varieties and nutrient rich rice landraces.

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RESEARCH ARTICLE

Selection Strategy and Estimation of Interrelationships for Improvement of Seed Yield and Other Related Traits in Linseed (*Linum usitatissimum* L.)

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Thirty five genotypes of linseed (*Linum usitatissimum* L.) were evaluated to determine the selection strategy and interrelationships of seed yield and yield related traits, during *rabi* 2013-14. Analysis of variance revealed significant differences among all the genotypes for all the traits under study. Sufficient genetic variability was observed in the present material selected for the study, through various genetic variability parameters, indicating the scope for selection of suitable initial breeding material for crop improvement. The genotypic and phenotypic correlation coefficient between different traits was similar in direction, whereas, in magnitude, genotypic correlation was higher than the corresponding phenotypic correlations. Seed yield per plant had significant and positive association with primary and secondary branches per plant. Secondary branches per plant exhibited the higher and positive direct effect on seed yield per plant indicating that selection for this character would lead to increase in the yield. Primary branches per plant contributed indirectly through biological yield per plant. The principal component analysis showed that primary branches per plant, secondary branches per plant and seed yield per plant had the maximum contributions, interpreting almost all the variation of traits. For the development of high yielding linseed varieties, breeding strategies should focus on secondary branches per plant prior to the primary branches per plant.

Key Words: Biplot, Correlation, Interrelationship, Linseed, Principal component analysis

Introduction

Linseed (*Linum usitatissimum* L.; n = 15), belongs to genus *Linum*, the largest genus of family Linaceae with about 200 species displaying great diversity in Karyotype. Linnaeus in 1857 was first to give a botanical name *L. usitatissimum* to the cultivated species. Linseed is an annual, self pollinating and non-edible oil crop. It is the sole species of agricultural importance within the family Linaceae and belongs to group of founder crops that initiated agriculture in "old world" (Zohary, 1999). The somatic chromosome number in other species of the genus *Linum* is reported to vary from 16 to 80 (Darlington and Wylie, 1956; Gill, 1966; Harris, 1968). Almost every part of the linseed plant is utilized commercially either directly or after processing. Linseed oil and meal are the two products provided by the seeds on account of its quick drying properties. Linseed oil is extensively used in industry for the manufacturing of high quality paints and varnishes. The oil content of the seed generally varies from 33 to 45%. About 20% of the total linseed oil is used for edible and domestic purposes and 80% goes for industrial utilization. The oil is also utilized for manufacturing paints, varnishes, oilcloth, linoleum, pad-ink, printers ink, soap etc. Across the globe it covers 2270.35 thousand hectare area with production of 2238.94 thousand tons having productivity of 986.16 kg/ha, where as in India its area is limited to 338 thousand hectares and production 147 thousand tons with the productivity of 434.91 kg/ha, (Anonymous, 2013). In spite of being an important oilseed crop, its average productivity in India is very low, because of various factors *viz*., narrow genetic base, raising of crop by the resource poor farmers in marginal and sub-marginal areas, non-availability of high yielding varieties and susceptibility to biotic and abiotic stresses, etc.

Selection is an integral part of a breeding programme by which genotypes with high productivity in a given environment can be developed. Yield per unit area is the end product of components of several characters, which are polygenic in inheritance and are thus highly influenced by environment. Genetic variability is a measure of the tendency of individual genotypes in a population to vary from one another. The measurement of genetic variation and mode of inheritance of quantitative and qualitative

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traits are of prime importance in planning the breeding programme efficiently and effectively (Shah *et al.*, 2015). Selection for yield has many complexities because it is end product of various yield components, which are naturally polygenic inherited and maximally influenced by environment. Therefore, only little progress could be made over a long span of time through direct selection for yield. Indirect selection through yield components has been proved more effective (Ford, 1964). Proper understanding of association of different traits, provide more reliable selection criteria to achieve high seed yield (Akbar *et al.*, 2001). This selection criteria takes into account the information on interrelationships among agronomic characters, their relationship with seed yield as well as direct influence on seed yield. However, selection for yield via highly correlated characters becomes easy if the contribution of different characters to yield is quantified using path coefficient analysis (Dewey and Lu, 1959). So path coefficient analysis technique is a statistical approach which is based on multiple regression and is useful for revealing the direct and indirect effects of characters in a network of factors like agro/morpho/physio/biochemical traits which is able to separate correlation coefficients into their components of direct and indirect effects (Dewey and Lu, 1959; Wright, 1960). However, information on the extent and nature of interrelationship among characters helps in formulating efficient scheme of multiple traits selection. The present study was conducted to study the selection strategy and interrelationships for the improvement of seed yield and other related traits in linseed.

Materials and Methods

Experimental material consisted of 35 linseed genotypes (Table 1), evaluated at the Experimental Farm of the Department of Crop Improvement, CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur, Himachal Pradesh, India, situated at an elevation of 1300 m. above m.s.l with 3606'N latitude and 7603'E longitude, representing mid-hill zone of Himachal Pradesh, during *rabi* 2013-14. The experiment was laid out in a Randomized Block Design (RBD) with two replications. The plot size of each genotype consisted of three rows of 3 m with row to row and plant to plant spacing of 25 cm and 5 cm respectively. The experimental field was well prepared and recommended doses of fertilizers were applied @ 50 kg N, 40 kg, P_2O_5 and 20 kg K_2O per hectare. Half dose of N and full dose of P_2O_5 and K_2O were applied as basal and the remaining half nitrogen was top dressed after 45 days of sowing and regular weeding was done to keep the experimental field weed free. Data were recorded for plant height, primary branches per plant, secondary branches per plant, capsules per plant, seeds per capsule, 1000-seed weight and seed yield per plant for five randomly selected plants in each replication, whereas days to 50% flowering and days to 75% maturity were recorded on plot basis.

Table 1. List of linseed genotypes and their parentage/source used in the study

S. No.	Genotype	Source/pedigree
1	KL-241	Giza-7 × KLS-1
2	KL-242	Gaurav × KLS-1
3	KL-243	RL-904 × Exotic-2
4	KL-244	(RLC-29 × Jeevan) X RLC-29
5	KL-245	Jeevan × KLS-1
6	KL-246	Him Alsi-2 × RLC-29
7	KL-247	Neelam × Nagarkot
8	KL-248	Selection from KL-178
9	KL-249	–
10	KL-250	–
11	KL-251	–
12	KL-252	–
13	KL-253	Chambal × 89D-2B/5
14	KL-254	Him Alsi-2 × 8 9D-2B/5
15	KL-255	Binwa × Baner
16	KL-256	Binwa × Flak-1
17	KL-257	LC-2323 × KLS-1
18	KL-258	Janki × Surbhi
19	KL-259	KL-187 × Flak-1
20	KL-260	Binwa × KLS-1
21	KL-261	KL-233 × KLS-1
22	KL-262	Himalini × Flak-1
23	KL-263	Him Alsi-2 × Baner
24	KL-264	LC-2232 × KLS-1
25	KL-265	KL-168 × Baner
26	KL-266	LCK-9826 × 89D-2B/5
27	KL-267	Him Alsi-2 × Flak-1
28	KL-268	Early selection of Him Alsi-2
29	KL-269	Medium selection of Him Alsi-2
30	Bhagsu	RL-50-3 × Surbhi
31	Binwa	Flak-1 × SPS 47/ 7-10-3
32	Baner	EC 21741 × LC 216
33	Surbhi	LC-216 × LC-185
34	Nagarkot	New River × LC-216
35	American Flax	–

Statistical Analysis

The recorded data was subjected to analysis of variance (Panse and Sukhatme, 1985).

The genotypic coefficient variation (GCV), phenotypic coefficient of variation (PCV) and heritability

(H2) in broad sense were computed according to Burton and Devane (1953). Genetic advance (GA) was estimated following Johnson *et al.* (1955). The genotypic and phenotypic correlation coefficients were calculated as suggested by Al- Jibouri *et al.* (1958) and the path coefficient analysis was conducted as suggested by Dewey and Lu (1959).

Principal component analysis (PCA) analysis was performed using XLSTAT software to determine the best relationships among characters.

Results and Discussion

The analysis of variance revealed significant differences among the genotypes for all the traits (Table 2) indicating existence of sufficient variability in the material selected for the study and scope for selection of suitable breeding material for crop improvement.

The general mean and range of characters revealed a wide variability for seed yield per plant, 1000 seed weight, primary branches, secondary branches, plant height and capsules per plant indicating greater scope for selection. The PCV values were greater than the GCV values for all the traits studied. Therefore, caution has to be exercised in making selection for these characters on the basis of phenotype alone as environmental variation is unpredictable in nature. Higher estimates of PCV and GCV (>25%) were obtained for seed yield per plant Similar findings were also reported by Chandrashekhar *et al.* (1998); Pradhan *et al.* (1999); Rafiq *et al.* (2014). Moderate PCV and GCV (15-25%) was recorded for primary branches per plant, and 1000 seed weight. Whereas, moderate PCV and low GCV was recorded for secondary branches per plant. Low PCV and GCV (<15) were recorded for days to 50% flowering, days to 75% maturity, plant height, capsules per plant and seeds per suggested that the environment had little role and predominance of additive gene effects in the expression of these traits. All the characters studied in the present investigation expressed high heritability except seeds per capsule. High heritability indicates the scope of genetic improvement of these characters through selection. Similar results have been reported by Tewari (1999); Rai *et al.* (2000); Rama Kant *et al.* (2005); Pali and Mehta (2013); Rafiq *et al.* (2014).

Heritability estimates along with genetic advance are normally more helpful in predicting gain under selection than heritability estimates alone. The variability parameter for different characters are given in Table 3.

Table 2. Analysis of variance for various traits in linseed

S. No.	Traits	Replication df = 1	Genotypes df = 34	Error df = 34	CV (%)
1	Days to 50% flowering	25.14	11.53**	1.85	1.08
2	Days to 75% maturity	4.48	13.60**	2.46	0.86
3	Plant height (cm)	37.05	115.47**	5.22	3.83
4	Primary branches per plant	0.34	1.34**	0.22	10.05
5	Secondary branches per plant	0.06	0.66**	0.06	6.12
6	Capsules per plant	2.53	19.79**	4.95	7.13
7	Seeds per capsule	1.03	0.69**	0.20	5.73
8	1000 Seed weight (g)	0.99	3.59**	0.13	4.81
9	Seed yield per plant (g)	0.01	0.05**	0.01	19.57

* P≤0.005and ** P≤0.001

Table 3. Genetic parameters of variability for different agro-morphological traits of linseed

S. No.	Traits	Mean ± S.E.(m)	Range	PCV (%)	GCV (%)	h²bs (%)	GA (%)	GAM
1	Days to 50% flowering	126.00±0.96	121-130	2.05	1.75	72.36	3.86	3.06
2	Days to 75% maturity	183.29±1.11	179-189	1.55	1.29	69.39	4.05	2.21
3	Plant Height (cm)	59.69±1.62	47.70-75.20	13.01	12.44	91.35	14.62	24.49
4	Primary branches per plant	4.62±0.33	2.80-7.00	19.11	16.25	72.35	1.32	28.48
5	Secondary branches per plant	3.83±0.17	2.50-5.50	15.63	14.38	84.66	1.04	27.26
6	Capsules per plant	31.22±1.57	25.75-44.90	11.26	8.72	59.99	4.35	13.92
7	Seeds per capsule	7.81±0.32	6.50-9.30	8.54	6.33	54.96	0.76	9.67
8	1000 Seed weight (g)	7.42±0.25	4.78-9.99	18.37	17.73	93.16	2.61	35.26
9	Seed yield per plant (g)	0.41±0.07	0.15-0.79	42.52	36.64	74.27	.27	65.05

The present study revealed that all the traits under study showed low genetic advance (<15), coupled with high heritability except seeds per capsule. High heritability coupled with low genetic advance indicates non-additive gene action. The heritability exhibited due to favourable influence of environment rather than genotypes and selection for such traits may not be rewarding. High heritability coupled with high genetic advance as percentage of mean (GAM) were also more useful than heritability alone in predicting the resultant effect during selection of best individual genotype. Genetic advance is the measure of genetic gain under selection and expression in percentage of mean (Johnson *et al.*, 1955). In the present experiment high heritability and GAM (>30) was recorded for 1000 seed weight and seed yield per plant. Simple selection based on phenotypic performance of these traits would be more effective. High heritability and moderate GAM (15-30), were observed for the traits, plant height, primary branches per plant and secondary branches per plant. These traits could be exploited through manifestation of dominance and epistatic components through heterosis. Other traits except seeds per capsule showed high heritability along with low GAM (<15) indicating the presence of non-additive gene action. Hence selection can be postponed for these characters or these characters can be improved by intermating of superior genotypes of segregating population from recombination breeding.

Correlation Coefficient Analysis

The possible enhancements of high yield through yield attributes, as primary target of crop improvement, requires understanding the amount of correlations among various yield contributing characters or in fact, yield components. The correlation coefficients between different characters are given in Table 4. The primary and secondary branches per plant had positive correlation with seed yield per plant at both genotypic and phenotypic levels, whereas, days to 50% flowering showed negative significant correlation with seed yield per plant. Seed yield per plant had negative and non-significant correlation with days to 75% maturity but a positive and non-significant correlation with plant height, capsules per plant, seeds per capsule and 1000 seed. The genotypic and phenotypic correlation coefficients were similar in directions, in magnitude, genotypic correlations were mostly higher for most of the traits than corresponding phenotypic correlations. Similar findings were reported by Sohan *et al.* (2004); Joshi (2004). Thus the low phenotypic correlation could results due to the masking and modifying effects of environment on the association of characters at genotypic level. On the basis of correlation analysis studies, it can be concluded that the selection based on traits *viz*., primary and secondary branches per plant can provide better results for improvement of seed yield per plant in linseed, as reported by most of the workers in linseed (Mishra and Yadav, 1999; Savita *et al.,* 2011).

Table 4. Estimates of correlation coefficients at phenotypic (P) and genotypic (G) levels among different traits of linseed

		DTM	PH	PB	SB	CPP	SPC	SW	SYP
DTF	P	0.2466*	0.0276	0.0473**	-0.1600	0.2699*	-0.0333	0.0134	-0.2532*
	G	0.3233**	-0.0062	0.1200	-0.1655	0.3922**	-0.0241	0.0202	-0.3377**
DTM	P		0.3253**	0.2215	0.0855	0.1023	-0.1347	0.2285	-0.1287
	G		0.3928**	0.3944**	0.1514	0.4850**	-0.1786	0.3310**	-0.0939
PH	P			0.3722**	0.0661	0.3032*	-0.3144**	-0.0111	0.2132
	G			0.4769**	0.0748	0.4021**	-0.4482**	-0.0067	0.2164
PB	P				0.6481**	0.3554 **	0.0638	0.2873*	0.4839**
	G				0.7686**	0.4521 **	0.0449	0.3426**	0.5664**
SB	P					0.3829**	0.3625**	0.2591*	0.6873**
	G					0.4631**	0.3804**	0.2495*	0.6994**
CPP	P						0.0249	-0.0508	0.2165
	G						0.0572	-0.1064	0.0679
SPC	P							0.0906	0.0553
	G							0.0511	0.0422
SW	P								0.0608
	G								0.0189

*Significant at 5% level; **Significant at 1% level

DTF-Days to 50% flowering; DTM-Days to 75% maturity; PH-Plant height; PB-Primary branches per plant ; SB-Secondary branches per plant; CPP-Capsules per plant; SPC-Seeds per capsule; SW-1000 Seed weight; SY-Seed yield per plant

Path Coefficient Analysis

Correlation coefficient measures the mutual association between two variables but does not permit the cause and effect relationship of traits contributing directly or indirectly towards economic yield. Whereas, the path coefficient analysis specifies the causes and measures their relative importance (Shivanna *et al.*, 2007). The economic characters like seed yield is dependent on several component characters which are mutually related. Slight changes in any one component will ultimately disturb the complex. Hence character hence to be analysed for its action namely direct effect of component character on seed yield and the indirect effect through other component characters on seed yield. Therefore, correlations were partitioned into direct and indirect effects (Table 5). Such an analysis helps the breeders to identify the characters that could be used as selection criteria in linseed breeding programmes. The direct and indirect effects of genotypic path coefficients were mostly higher in magnitude than the corresponding phenotypic path coefficients. Similar finding with respect to path coefficients have been reported (Gauraha and Rao, 2011; Reddy *et al.,* 2013). Secondary branches per plant had higher and positive direct effect on seed yield per plant. This traits also had significant positive correlation with seed yield per plant indicating that selection for secondary branches per plant would indirectly select for higher seed yield. On partitioning the components for correlation of seed yield per plant with characters showing positive correlation, direct effects were found to be low indicating that while selecting these characters seed yield per plant cannot be improved through these characters. Their indirect effects through secondary branches per plant were high, therefore primary branches per plant contributed indirectly through secondary branches per plant. Path coefficient analysis revealed the importance of secondary branches per plant as major yield contributing component in linseed.

Principal Component Analysis of Seed Yield and other Traits

The principal component analysis (PCA) was performed for traits (Table 6 and Fig. 2.) which revealed five most informative principal components with eigenvalues 2.796, 1.868, 1.326, 1.144 and 0.614 respectively, which together accounted 86.089% of the total variance (Fig. 1), for all the characters. Thus, according to principal component 1, characters such as primary branches per plant, secondary branches per plant and seed yield per plant had relatively higher contributions to the total morphological variability, a truth which proved by other statistical approaches similar to path coefficients analysis which discussed at earlier parts of this paper. The first two principal components biplot including loadings of the various characters along with the genotypes spread over is given in Fig. 2. This Figure indicates that the

Table 5. Estimates of direct and indirect phenotypic (P) and genotypic (G) effects of different traits on seed yield in linseed

		DTF	DTM	PH	PB	SB	CPP	SPC	SW	Correlation with SYP
DTF	P	-0.0321	-0.0638	0.0060	0.0004	-0.1331	-0.0363	0.0068	-0.0011	-0.2532*
	G	0.4448	-0.0018	-0.0039	-0.0899	-0.3240	-0.3664	0.0095	-0.0060	-0.3377**
DTM	P	-0.0079	-0.2586	0.0703	0.0017	0.0712	-0.0137	0.0275	-0.0191	-0.1287
	G	0.1438	-0.0054	0.2473	-0.2954	0.2964	-0.4531	0.0702	-0.0978	-0.0939
PH	P	-0.0009	-0.0841	0.2160	0.0029	0.0550	-0.0407	0.0641	0.0009	0.2132
	G	-0.0027	-0.0021	0.6295	-0.3572	0.1465	-0.3757	0.1762	0.0020	0.2164
PB	P	-0.0015	-0.0573	0.0804	0.0078	0.5392	-0.0477	-0.0130	-0.0240	0.4839**
	G	0.0534	-0.0021	0.3002	-0.7491	1.001	-0.4223	-0.0176	-0.1012	0.5664**
SB	P	0.0051	-0.0221	0.0143	0.0050	0.8319	-0.0514	-0.0739	-0.0216	0.6873**
	G	-0.0736	-0.0008	0.0471	-0.5757	1.000	-0.4326	-0.1496	-0.0737	0.6994**
CPP	P	-0.0087	-0.0265	0.0655	0.0028	0.3185	-0.1343	-0.0051	0.0042	0.2165
	G	0.1745	-0.0026	0.2531	-0.3386	0.9068	-0.9342	-0.0225	0.0314	0.0679
SPC	P	0.0011	0.0348	-0.0679	0.0005	0.3016	-0.0034	-0.2039	-0.0076	0.0553
	G	-0.0107	0.0010	-0.2821	-0.0336	0.7450	-0.0534	-0.3932	-0.0151	0.0422
SW	P	-0.0004	-0.0591	-0.0024	0.0022	0.2156	0.0068	-0.0185	-0.0834	0.0608
	G	0.0090	-0.0018	-0.0042	-0.2566	0.4886	0.0994	-0.0201	-0.2954	0.0189

*Significant at 5% level; **Significant at 1% level

DTF-Days to 50% flowering; DTM-Days to 75% maturity; PH-Plant height; PB-Primary branches per plant ; SB-Secondary branches per plant; CPP-Capsules per plant; SPC-Seeds per capsule; SW-1000 Seed weight; SY-Seed yield per plant

Table 6. Eigenvectors and eigenvalues of 5 principal components for nine characters of 35 linseed genotypes

	DTF	DTM	PH	PB	SB	CPP	SPC	SW	SYP	Eigen value	Proportion	Cumulative %
PC1	0.003	0.215	0.280	0.528	0.516	0.348	0.074	0.197	0.406	2.796	31.065	31.065
PC2	0.448	0.476	0.383	0.039	-0.291	0.253	-0.390	0.006	-0.350	1.868	20.759	51.824
PC3	0.450	0.186	-0.456	0.012	0.117	0.103	0.561	0.345	-0.311	1.326	14.735	66.559
PC4	-0.287	0.285	0.020	0.074	-0.060	-0.525	-0.201	0.713	-0.042	1.144	12.707	79.267
PC5	0.552	-0.498	-0.251	0.162	-0.024	-0.065	-0.468	0.246	0.273	0.614	6.822	86.089

DTF-Days to 50% flowering; DTM-Days to 75% maturity; PH-Plant height; PB-Primary branches per plant ; SB-Secondary branches per plant; CPP-Capsules per plant; SPC-Seeds per capsule; SW-1000 Seed weight; SY-Seed yield per plant

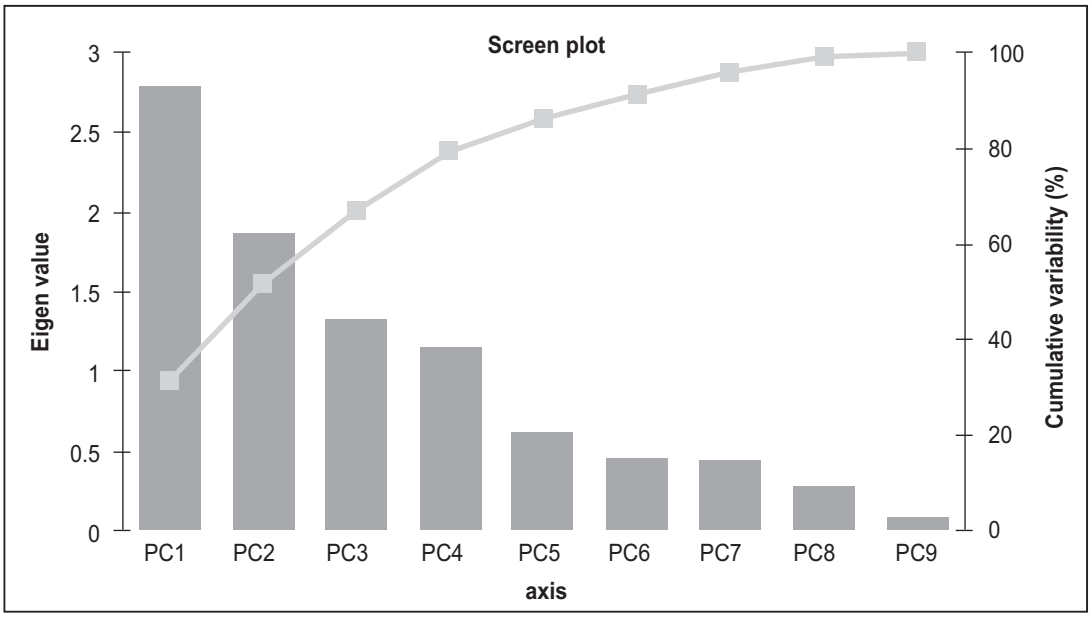

Fig. 1. Eigenvalues of principal components

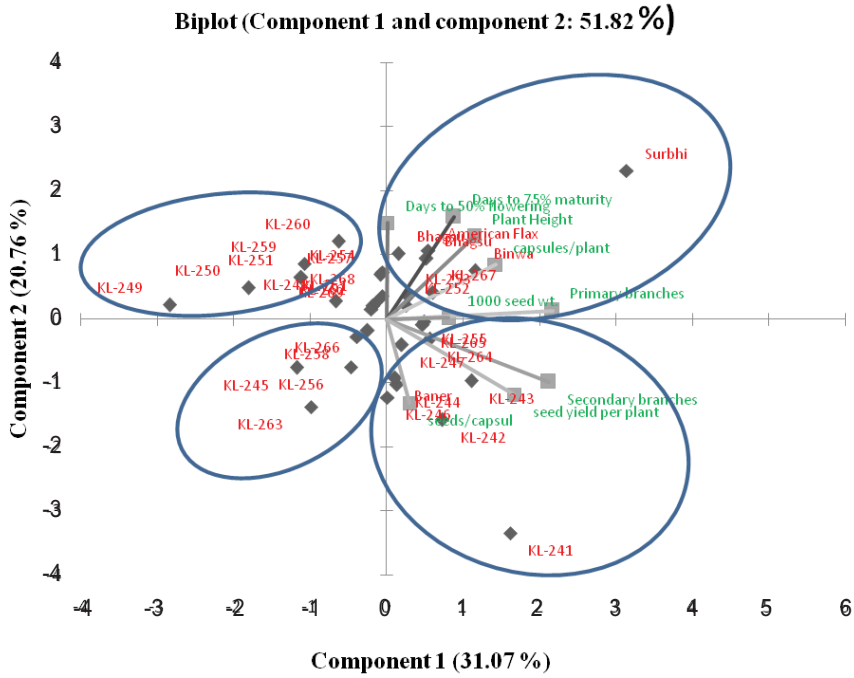

Fig. 2. Biplot of principal component

35 genotypes could be categorized at four groups. The group comprising of Surbhi, Nagarkot, Bhagsu, Binwa, American Flax, KL-252, KL-253 and KL-267 had higher days to 50% flowering, days to 75% maturity, plant height, primary branches per plant, capsules per plant and 1000 seed weight; the second group including varieties Baner, KL-241, KL-242, KL-243, KL-244, KL-241, KL-246, KL-247, KL-255, KL-264 and KL-265 contained more secondary branches per plant, seeds/capsules and seed yield per plant and rest of the genotypes were found in third and fourth group. PCA showed a clear differentiation between flax cultivars from each other. The variation (31.065%) is explained by first principal component and in total this biplot explained 51.82% of the variation. Thus, this biplot can interpret the near-real differentiation of the linseed cultivars and morphological characters studied in this experiment. Vromans (2006) also reported that the biplot can be used as a vital instrument to categorize, differentiate and address the genetic entities in breeding decisions.

From the present investigation it is observed that analysis of variance showed significant differences between genotypes for all the characters, indicating the scope for selection from diverse genotypes for various important traits. There were positive significant correlation between seed yield per plant with primary branches per plant and secondary branches per plant. The PCA showed that the first two components explained 51.82 percent of the existing variation in genotypes as depicted by biplot. The present studies and its results can play an important role in effective selection and breeding strategies for linseed improvement in future endeavours.

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RESEARCH ARTICLE

Field Book – Mobile App for Plant Genetic Resources Management

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Digitalization is the process of converting information into a digital format. Digital India, launched in 2015, aims towards the promotion of digital literacy and creation of digital infrastructure for empowering rural communities. Considering the need of digitalization and handling of huge data, a mobile based application technology for data collection named Field book has been developed by Kansas State University (KSU), United States of America and International Maize and Wheat Improvement Centre (CIMMYT), Mexico. Such app could be very useful to collect data related to plant genetic resources (PGR). Efforts have been made by ICAR-Indian Institute of Millets Research (IIMR), Hyderabad to customize the field book to use as one stop solution for plant genetic resource management. We have customized the app for collection of characterization data of eight crops namely sorghum, pearl millet, finger millet, foxtail millet, barnyard millet, proso millet, kodo millet and little millet. The app is customized for DUS characterization of sorghum and collecting passport data for PGR exploration. Twenty-seven agro-morphological traits are created as SOR GERM file for sorghum characterization. The trait screen has several advantages including saving of note book data collection. The off-line data collection reduces threat of sharing the data without owner knowledge. ICAR-IIMR-Hyderabad has initiated the training on field book to the researchers in the country with a target of 5000 researchers by 2020. This mobile app has potential to reduce paper usage, cutting of large number of trees and thus saving carbon foot prints and also facilitating more efficient data collection, analysis and storage.

Key Words: Characterization, Digitalization, Exploration, Field book, PGR

Introduction

Digitalization is the integration of digital technologies into everyday life (Ochs and Riemann, 2018). Digital Information and Media are excellent tools to upsurge knowledge which is an important production factor in agriculture (Afroz *et al.*, 2015). In 2017, the number of mobile phone users in India is expected to rise to 730.7 million. In this same year the number of smartphone users in India is predicted to reach 340 million and could reach almost 468 million by 2021 (Tableau, Statistica, 2019). Digital India, launched in 2015 aims towards the promotion of digital literacy and creation of digital infrastructure for empowering rural communities. Considering that 58% of rural households depend on agriculture as a major source of livelihood, the role of Digital Agriculture needs to be considered within Digital India.

Advances in digital technologies offer the potential for transformational change in agriculture such as being observed in providing new ways to improve productivity and profitability in Australia. Rapid developments in computing power, robotics, big data, and cloud computing are creating opportunities for more data-driven approaches into farm management, referred to as 'smart farming' (Zhang *et al.*, 2017).

Conventional approach of data collection on paper has several disadvantages including the delay in data availability for analysis, errors during the transcription, vulnerability to loss or damage (Johnston, 2017). Collecting data using mobile technology, especially when the data is uploaded to a properly administered campus server, makes the data much more secure and is immediately available for review and analysis. The use of mobile technology also introduces additional functionality. This includes more reliable data through the use of authority controls and validity checks. It also enables use of GPS coordinates, photographs, audio, and video in addition to textual and numeric data. An increased use of digitalization, remote sensing, and ICT would improve efficiency, quality, and timeliness of controls and audits (Karner, 2017).

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The use of mobile application for collecting the field or lab data is very limited in research institutes in India and other developing countries. Recently, a mobile based application technology for data collection named Field book was developed by KSU and CIMMYT. This mobile app can be used to collect any kind of field or lab data and reduces manpower and papers; increases accuracy and authenticity. Field book is an open-source Android app that is used to collect data on field which is very useful for the field functionaries for collection of geographical information, information with geo-tagged photos and audio records. This app is developed as an open source tool that can be used to collect data on all kinds of experiments. Hence has potential to replace paper field books and is projected to increase the speed of collection and analysis that will enable the rate of genetic gain. The ability to keep data organized in digital form allows technicians and breeders to focus on other tasks, leading to further innovation and growth of plant breeding programs (Rife and Poland, 2014).

The improvising of the field book applications has added many new data collection formats, quality and requirements on the increasing and improving demand in the digital technology arena. The three new data formats such as barcode scanning, field mapping and survey developed and upgraded by Courtney (2017) opens a new open-source domain for acquisition of data on farmlands to accompany the research of plant breeders.

Phenotypical data acquisition has been completed by hand or accompanied by expensive software, which may be unavailable to most developing countries (Rife and Poland, 2014). Nielsen and Gangadhara (2016) opined that field book is useful with the explosion of genomic data and need for increased speed of data collection. Karner (2017) stated that digital society is not just a technology, but a comprehensive social organization, where the information and its exchange play a major role. Digitalization of farming sector would contribute to its competitiveness, help to raise farmers' income, and attract young people to join the traditional activity, which is vital for the whole society.

Customization of App for PGR Management

Though the field book was initially used for collecting the field related experimental data, an effort has been made by ICAR-Indian Institute of Millets Research (IIMR), Hyderabad to customize the field book to use as one stop solution for PGR management.

Downloading and Installing

Field book can be downloaded from Google Play Store on your mobile phones and tablets. Upon installation, the app will ask the user if they wish to load sample data and turn on the tutorial. The source code for Field book is available on GitHub.

Data Storage and Folders

Once field book has been installed and opened. It will create nine different folders in the devices memory with specific purpose to handle and store the data. The folders are as below:

1. **Field export:** This folder stores exported data files after collecting the data
2. **Field import:** The field book file are created in MS-Excel with information about the field or lab experiments and imported in .csv format (comma delimited)
3. **Plot data:** The data associated with plots (audio and photos) are organized into this folder based on the name of the imported field or lab experimental file individually
4. **Resources:** This folder can hold pictures and files that can be helpful when out in the field and are accessible from the main data collection screen
5. **Database:** This folder contains files that are exported when backing up the database
6. **Trait:** This folder contains backed up trait files which you can create by add, delete, and edit traits from the sample file available. For example, each crop germplasm will have separate descriptor trait file for characterization
7. **Archives:** This folder contains archive files
8. **Error:** This folder contains error files
9. **Updated:** This folder contains update files

Settings Menu

This is the first menu to be seen while installing the Field book. A user cannot collect the data until two steps are taken – importing the field file and loading/create the trait file. The settings menu is the main location for configuring field book. The options in this menu are used to import files, load traits, change profile information, export data, change the language, and toggle different advanced settings.

Creating Field Files for PGR Characterization

Field files can be created in MS-Excel and can load both CSV and XLS files. File names and column headers should exclude the following characters: / ? < > \ * | ” . Field book import files should, at the least, include three columns: a unique ID, a primary order, and a secondary order. To create field file for PGR characterization, each accession number should be assigned a unique identifier. This unique ID is used internally to associate data with a specific accession and should be globally unique. The unique ID for the experiment on characterization of sorghum germplasm can be created viz., crop code (three letters), season (K for Kharif, LK for Late Kharif, R for Rabi and S for Summer) and year of characterization (two digit number). For example, the Sorghum-Kharif-2018 and the first genotype can be read as SORK18001. When importing files, field book will check to see if the unique ID is unique within the file being imported. In addition, two columns must be included as a basic navigation ordering. These are referred to as the Primary and Secondary Order. The user can select these columns based on how they traditionally walk through the field. The related information on crop name, accession number, IC/EC number, type of material, received from, category and bed number can be used as primary or secondary order. Similar way field file for different crop germplasm characterization can be created. Once a field file has been designed, it can be transferred to the Android device via apps like shareit, drop box or manually with a USB cable.

Creation of Trait Files for PGR Characterization

There are 12 different data formats available to create the trait file. They are numeric, categorical, date, percent, rust rating, text, boolean, multi-category, location, counter, photo and audio. Each trait formats utilize different layouts and features to streamline how the data is collected. Data is collected in field book by creating traits. The different data formats and its input style is presented in Fig. 1.

For example, 27 agro-morphological traits are created as SOR GERM file for sorghum characterization. The trait screen allows the user to create new traits or modify existing traits. The new traits can be created by pressing the add trait button (+) at the bottom of the screen. Each trait has a custom creation menu where the user can mention the name of the trait and other related states of

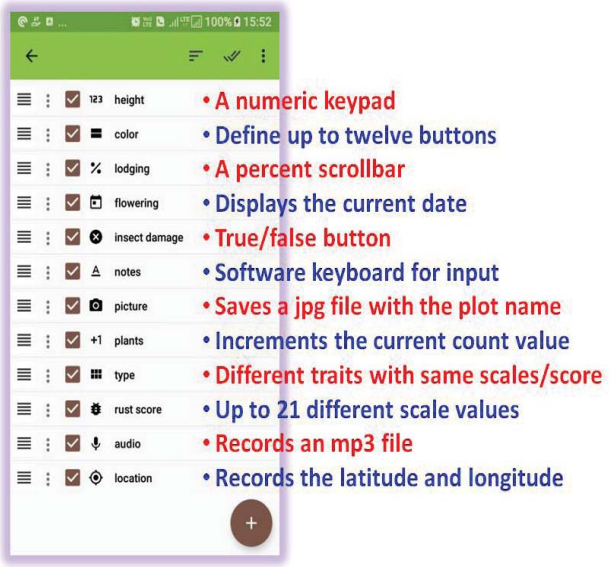


Fig. 1. Twelve different data formats and its input style

the trait. Traits name are unique and cannot share the same name. Each trait has options to make data collection easier. The menu icon on each line allows each trait to be delete, copy or edit. The checkbox allows traits to be active or hidden on the main layout screen. Traits can be reordered by dragging and dropping the icon on the right of each trait line. Lists of traits can be created and transferred between different devices using the import/ export option on the toolbar. The newly created trait files are stored in the trait folder. The example traits and its format to be selected while creating trait file for plant genetic resources characterization and plant genetic resources exploration in general are presented in Table 1 and Table 2 respectively.

The different trait files customized for different crop germplasm descriptors for characterization is presented in Fig 2. a. Sorghum, b. Finger millet, c. Pearl millet, d. Lentil, e. Wheat and f. Barley. The trait files customized for collecting exploration data / passport data is presented in Fig. 3.

Exporting Data

Collected data can be exported to as CSV files. The export dialog allows the user to customize how collected data is exported. Two different formats can be selected viz., database or table. The export file can include only the unique identifier or all field columns that were imported. The export file can contain all traits currently active or all traits for which data has been collected. The filename is automatically generated by the app

Table 1. Common traits used for PGR characterization and its data type for creation of trait file

S.No.	Type of data	Traits
1	Numeric	Number of tillers, Flag leaf blade length (cm), Flag leaf blade width (cm), Peduncle length (cm), Lower raceme length (cm), Culm branching, Panicle length (cm), Panicle width (cm), Plant height (cm), Grain yield per plant (g), 1000- seed weight (g), Pod length (cm), fruit length (cm) etc.
2	Categorical	Growth habit, Plant pigmentation, Inflorescence shape, Inflorescence colour, Panicle compactness, Spikelet arrangement on the rachis, Lowest raceme shape, Lowest raceme thickness, Lower raceme branching, Lodging, Grain colour, Grain shape
3	Percent	Staygreen, Lodging, resistance to pest / disease
4	Date	Days to flowering, Harvesting date
5	Boolean	Insect / Pest damage
6	Text	Remarks
7	Photo	Panicle, seed, other variable traits
8	Counter	No. of plants in a row/plot
9	Multicat	Multiple trait selection
10	Rust rating	Screening for leafy diseases, pest damage
11	Audio	Describe the accession other than the available traits
12	Position	Displays latitude and longitude of the data location

Table 2. Common traits used for PGR exploration and its data type for creation of trait file

S.No.	Type of data	Traits
1	Numeric	Latitude, Longitude, Altitude
2	Categorical	Status, Frequency, Material, Sample type, Sample method, Habitat, Disease symptoms, IPN infection, Cultural practices, Season, Associated crop, Soil colour, Soil texture, Stoniness, Land aspect, Slope, Topography, Agronomic score, Ethno useful parts, Kind, Informants
3	Percent	Drought/Flood/Natural calamity
4	Date	Date of collection
5	Boolean	Logical scores on availability of traits
6	Text	Common name, Botanical name, Vernacular name, Village, Taluk , District, State
7	Photo	Field view, Panicle, Seed, Other variable traits
8	Counter	Minimum available trees
9	Multicat	Multi-traits with same scale/score
10	Rust rating	Pest/Disease rating
11	Audio	Farmers interview , additional description of germplasm
12	Position	GPS data

based on the current date (YYYY-MM-DD), current time (HH-MM-SS) and the name of the field file that was imported into the app.

Database

The database can be backed up and transferred between tablets. When exporting the database, two different files are exported: one containing the database, and one containing the user settings when the database was exported. To re-import the database, both files need to be present in the database folder.

Need for Digitalization of Data Collection

Many electronic versions of information dissemination has happened in the near past such as e-book, e-journal, e-thesis, e-mail etc. The modern day electronic information like lengthy e-mails has become short message SMS, whatsapp, twitter etc. The mobiles have taken over the cameras for photo capturing. The atlas book has become google maps. The cassettes/CDs have become mp3 audio files. The video coverage has become video clips. The hard discs have become SD cards. There is need to change our paper note books to smart phone for collecting data. The digitalization of data can be done using the field book mobile app as ICT (Information and Communication Technology) tool for any form of data collection.

Challenges in Implementing Digitalization of PGR Management

The main challenges for implementation of digitalization are shifting from field note book to mobile field book due to several reasons including faith in the older technology. A study by Hirose *et al.* (2017) revealed that the mobile users i) perceived ease of use positively influences perceived usefulness ii) perceived ease of use

a) Sorghum b) Finger millet c) Pearl millet

d) Lentil e) Wheat f) Barley

Fig. 2. Crop descriptors customized using the field book mobile app

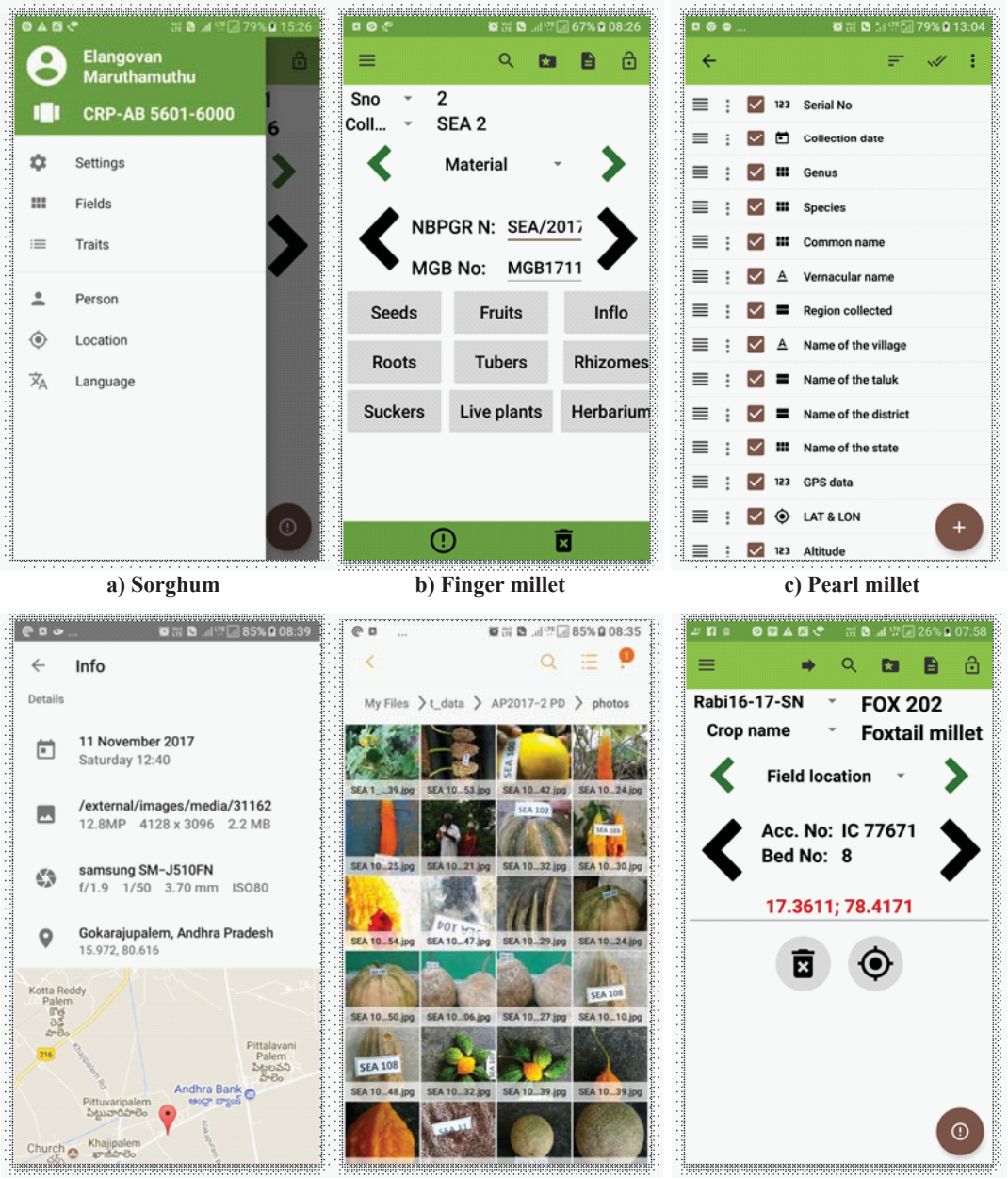
positively influences attitudes toward mobile apps usage, iii) perceived usefulness positively influences attitudes toward mobile apps usage iv) attitudes toward apps positively influence intentions to use mobile apps. It's really high time to for the user researchers to change their attitude towards the field book mobile app in place of the paper field book.

Mobile App for Seed Genebank Management

Angala and Diaego (2015) reported that the adoption and implementation of mobile computing as a tool to improve efficiency in data collection and information management for the genebank operations. On the other hand Hernández and Gonzalez (2015) suggested following challenges for collecting data by mobile i) No full coverage of wireless points in the work areas of Genetic Resources Program ii) No connectivity with experimental stations (Popayan, Santander de Quilichao, and Tenerife).

Action Plan to Implement Mobilization in PGR Management

The field book mobile app can also be used as a platform to manage the accession conserved in the seed genebank. A working model is being customized for this purpose at ICAR-IIMR Hyderabad.



a) Sorghum **b) Finger millet** **c) Pearl millet**

d) Lentil **e) Wheat** **f) Barley**

Fig. 3. Trait files customized for plant genetic resources exploration

The action plans includes, organizing learning workshop for the PGR workers especially young researchers, identifying their ongoing experimental problems and converting them to mobile field book, build a confidence on data security related doubts, monitoring the progress on implementation and make them the trainer.

More Apps Available for Researchers for Characterization of Plant Genotypes

There are more and more mobile apps being developed for strengthening the data collection in field related experiments – NDVI images to judge the vigour rating, leaf area index (LAI) and leaf biomass around flowering and to very late senescence rating (Liebisch *et al.*, 2017).

Cresente *et al.* (2017) developed novel open source software called Phenobook, which consists of web-based software for experiment design, data input and visualization, and exportation, combined with a mobile application for remote data collecting. Phenobook

is a software tool that can be easily implemented in collaborative research and development projects involving data collecting and forward analyses.

Roxanne *et al.* (2017) developed a smart phone application to integrate a low-cost Bluetooth colour sensor with a GPS-enabled smart phone.

Pengcheng *et al.* (2017) reported that the colours of soil and proofread cards can be acquired by the smartphone, converted into RGB signals and then after simple processing can help in-rapid soil classification.

Conclusion

The hard copy recording of data in the field consumes lot of paper. Replacing the paper field book can only be possible by increase use of field book mobile app. ICAR-IIMR Hyderabad has initiated the training on field book to the researchers in the country. We have trained 2,632 researchers so far from 5 State Agricultural Universities (SAUs), 6 ICAR institutes, 3 AICRP's on Sorghum, Small millets and Pearl millet, 4 Agricultural Colleges, 2 Private Seed Industries and 30 lectures/practical's on Digital Field Book in various training programmes. A target of 5000 trained researchers by 2020 has been aimed. In continuance of efforts many scientists at ICAR institutes (IIMR-Hyderabad, IISR-Indore, NBPGR-New Delhi, IIHR-Baengaluru, NRCP-Solapur, DOGR-Pune) and SAUs (PJTSAU-Hyderabad, Dr PDKV-Akola, PAU-Ludhiana, CCSHAU-Hisar, TNAU-Coimbatore and NAU-Navsari) have started using the field book. With the average use of 10,000 paper sheets per year by a scientist, all the 5000 researchers' uses field book mobile app, it has potential to save 5.0 crore paper sheets per year which is equal to saving of 6,000 trees annually. All institutions should take advantage of the latest technologies like tablets, computers and smart phones to record and maintain digital files and notes.

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RESEARCH ARTICLE

Note on Diversity in Legumes and Oilseeds and their Wild Relatives in Eastern Ghats of India: Utilization and Conservation Concerns

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Investigation was carried out during 2013-2016 for collection, conservation and updating the present status of legumes, oilseeds and their wild relatives in Eastern Ghats of India. Frequent field surveys were conducted and the plant specimens at flowering and fruiting stages were collected. A total of 66 wild species of legume and oilseeds were collected. These belong to 11 genera and two families. The genus *Rhynchosia* represented maximum number of species with 16 followed by *Vigna* (13), *Flemingia* (10), *Cajanus Mucuna* (6), *Sesamum* (5), *Canavalia* (4), *Macroptelium* (3) and *Dunbaria*, *Dolichos*, *Paracalyx* represented one species each. These wild relatives possess important traits such as resistance to abiotic and biotic stresses and high nutritional value can be utilized for trait specific crop improvement.

Key Words: Conservation, Eastern Ghats, Legumes, Oilseeds, Wild relatives

Introduction

Grain legumes are an important source of nutrients and renowned as poor man's meat especially in developing countries (Hayat *et al.*, 2014). Legumes provide an exceptionally varied nutrient profile, including proteins, fibers, vitamins and minerals (Wang *et al.*, 2003; Mitchell *et al.*, 2009). Climate change is another converging force, which potentially decrease crop productivity (Varshney *et al.*, 2010; McClean *et al.*, 2011). Wild relatives of domesticated crops possess genetic diversity useful for developing more productive, nutritious and resilient crop varieties (Nora *et al.*, 2016). Crop wild relatives are species with a close genetic similarity to crops and many of them have the potential or actual ability to contribute beneficial traits to these crops such as resistance to biotic and abiotic stresses, higher and stable yields (Meilleur and Hodgkin, 2004; Hajjar and Hodgking, 2007; Maxted *et al.*, 2010; Elangovan *et al.*, 2012). Crop wild relatives share a relatively recent common ancestry with domesticated species and due to that close relationship are reservoirs of genetic traits that can be useful in crop improvement (Guarino and Lobell, 2011; Dempewolf *et al.*, 2014). The efficient utilization of wide diversity of plant genetic resources improves the collection and conservation of more distant relatives of the crop plants (Khoury *et al.*, 2015).

Grain legumes provide an unparalleled solution to food and nutritional security problem because of their inherent capacity for symbiotic atmospheric nitrogen fixation, which provides economically sustainable advantages for farming (Foyer *et al.*, 2016). Food legumes are second most important group of crops after cereals which have been a vital ingredient of balanced human diet since millennia and second most valuable plant source for human and animal nutrition (Bhatt and Karim, 2009). Underutilized legumes make a significant contribution to the diet of the rural households particularly, during drought, famine and dry season. These are the life-savers for millions of resource poor people in the regions where ensuring food and nutritional security is one of the significant problems, particularly in traditional subsistence farming systems. Legumes have the ability to quench the demands for human consumption as well as supply of quality supplementary livestock feed and also exhibit high potential for soil conservation strategies (Pengelly and Maass, 2001). Legumes encompass the ability to fix atmospheric nitrogen (N) through their symbiosis with rhizobia and hence supply N to

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Fig. A. **1. *Flemingia stricta*** **2. *Cajanus scaraboides*** **3. *Canavalia gladiata***

subsequent crops (Wortmann *et al.*, 2000). Due to its high nutritive value and agronomic traits, legumes can play an increasing role in low input production systems in India, East Africa and elsewhere in the dry sub-tropics and tropics. Oilseeds are next to food grains in terms of area of cultivation and production value in India. The genetic base of the currently cultivated oilseed varieties is narrow which can be further broadened by utilization of diverse germplasm/wild relatives.

Eastern Ghats is one of the major hill ranges of India form an assembly of discontinuous ranges of hills, plateaus and narrow basin which are and spread over to an area of 7500km^2. The Eastern Ghats cover parts of Odisha, Andhra Pradesh, Telangana, Tamil Nadu and smaller area of Chhattisgarh, Maharashtra and Karnataka states with rich infloristic diversity. More than 2500 species of Angiosperms represented from this area which constitute about 13 percent of the flowering plants of India. Identifying both the cultivated and their wild relatives are a subject of contemporary investigations. Based on the experience of these initiatives and to meet the chanllaenges of Indian agriculture this paper aims to outline coherent information on legumes, oil seeds and their wild relatives with respect to conservation and its utilization in future.

Materials and Methods

The present study was carried out during 2013-2016. Wild species of legumes and oilseeds were collected from different phyto-geographical regions of the Eastern Ghats of India. Information on species distribution was were prepared based on published literature, local, regional, and state floras and monographs. Frequent field surveys were conducted and plant specimens were collected at flowering and fruiting stages.Collected fresh plant materials were dried for herbarium specimen's preparation and specimens collected in Formalin Acetic Acid (FAA) (Alcohol 70% + Acetic acid 5% + Formaldehyde 5% + Distilled water 20%) solutions were used for morphological charactersiations for species identifications. All the collected plants were identified up to species level using regional and local floras (Pullaiah and Ramamurthy, 2000; Pradheep *et al.*, 2014). The wild relatives of crop plants were recorded and herbarium specimens were prepared following standard methodology and finally validated through reference material at the herbarium of National Herbarium on Cultivated Plants (NHCP), ICAR-National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India.

Results

A total of 66 wild species of legumes and oilseeds were identified. These comprised of 11 genera belonging to two families in Eastern Ghats. The genus Rhynchosia were represented by maximum number of species (16), followed by Vigna (13), Flemingia (10), Cajanus and *Mucuna* (6), *Sesamum* (5), *Canavalia* (4), *Macroptelium* (3) and *Dunbaria*, *Dolichos* and *Paracalyx* are represented by single species, respectively. The wild relatives with their available location are listed in Table 1 along with their photographs (Fig. A&B). The details of crop wise wild relatives of legumes and oilseeds present in Eastern Ghats are given below:

Legumes

Cultivated grain legumes or pulses belong to the family Fabaceae (Leguminosae), which comprises 800 genera and 20,000 species, is the third largest family of flowering plants, after the Orchidaceae and Asteraceae. The family Papilionoideae is divided into four clades: (1) Phaseoloids (*Glycine* Willd., *Phaseolus* L., *Cajanus* L. and *Vigna savi*), (2) *Galegoids* (*Pisum* L., Lens Mill., *Lathyrus* L., *Vicia* L., *Medicago* L. and *Cicer* L.), (3) Genistoids (*Lupinus* L.) and (4) Dalbergoids (*Arachis* L. and Stylosanthes Sw.) (Lewis *et al.*, 2005; Petr *et al.*, 2015).

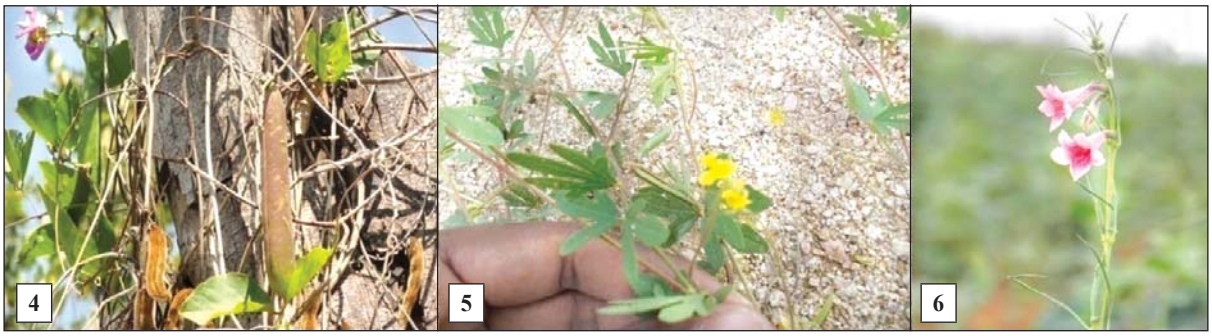

Fig. B. **4. *Mucuna* and *Canavalia*** **5. *Vigna aconitifolia*** **6. *Sesamum alatum***

Table 1. List of wild species of legumes and oil seeds from Eastern Ghats of India

S.No.	Scientific Name	Chromosome No.	Location
1	*Cajanus albicans* (Wight & Arn). Van der maesen	2n=22	KNL, PKSM
2	*C. cajanifolius* (Haines) van der maesan	2n=22	CTR, KDP, KNL, PKSM
3	*C. rugosus* (Wight & Arn) van der maesen		CTR, KD, KNL, MBNR
4	*C. scarabaeoides* (L.) du Petit	2n=22	Throughout EG
5	*C. sericeus* (Benth ex Baker) van der maesen	2n=22	Throughout EG
6	*C. volubilis* (Blanco) Blanco (Syn. C. crassus)	2n=22	CTR, KNL, PKSM
7	*Canavalia cathartic* Thouars (Syn. C. africana)	2n=22	CTR, KNL, PKSM
8	*C. ensiformis* (L.)Dc	2n=22	CTR, KNL, PKSM
9	*C. lineata* DC	2n=22	CTR, VSKP
10	*C. mollis* Wall ex Wight & Arn	2n=22	CTR, KNL, MBNR
11	*Dunbaria ferruginea* Wight & Arn		Throughout EG
12	*Dolichos trilobatus* L.	2n=20	Throughout EG
13	*Flemingia bracteata* (Roxb)Wight		KNL, PKSM
14	*F. chappar* Bush. Ham		CTR, KDP, VSKP
15	*F. lineata* (L.) Roxb		KNL, PKSM, MBNR
16	*F. macrophylla* (Willd.) Prain ex Merr.	2n=22	KNL, PKSM
17	*F. nana* Roxb		CTR, KDP, KNL, MBNR
18	*F. praecox* C.B. Clarke ex prain var. *robusta* (Mukerjee) Benet		KNL, PKSM, MBNR
19	*F. semialata* Roxb		KNL, PKSM, VSKP
20	*F. stricta* Roxb		Throughout EG
21	*F. strobilifera* (L.) R.Br		CTR, KNL, PKSM
22	*F. wallichii* Wight & Arn		CTR, KDP, KNL
23	*Macroptilium atropurpureum* (DC.) Urban		Throughout EG
24	*M. lathyroides* (L.) Urban var. *semierectum* (L.) Urban		VSKP
25	*M. uniflorum* (Lam.) verde		Throughout EG
26	*Mucuna atropurpurea* DC. Prodr		Throughout EG
27	*M. gigantea* D.C. Prodr.		CTR, KNL, VSKP
28	*M. monosperma* DC ex Wight		CTR, KNL, MBNR
29	*M. nigricans* (Lour.) Steud		KNL, PKSM, CTR
30	*M. pruriens* (L.) DC Prodr		Throughout EG
31	*M. pruriens* var. *Hirsuta* (Wight & Arn) Wilmot Dean		Throughout EG
32	*Paracalys scariosus*		KNL, PKSM, CTR
33	*Rhynchosia beddomei* Baker		Throughout EG
34	*R. bracteata* Benth		CTR, KDP, KNL, MBNR
35	*R. cana* (Willd.) DC. Prodr		CTR, KDP, KNL
36	*R. capitata* DC Prodr		Throughout EG
37	*R. courtallensis* van der Maesen		CTR, KNL, VSKP
38	*R. densiflora* (Roth) Roth. Prodr		CTR, KDP, KNL, MBNR
39	*R. filipes* Benth		CTR, KDP

Contd.

S.No.	Scientific Name	Chromosome No.	Location
40	*R. hainesiana* Satyan & Thoth		CTR, KDP
41	*R. heynei* Wight & Arn		CTR, KNL
42	*R. hirta* (Andr.) Meikle & Verdc.		Throughout EG
43	*R. minima* (L.) DC Prodr		Throughout EG
44	*R. ravii*		ATP
45	*R. rothii* Benth ex Aitch		CTR, KDP, KNL, MBNR
46	*R. rufescens* (Willd.) DC. Prodr		Throughout EG
47	*R. suaveolens* (L.f.) DC. Prodr		CTR, KDP, KNL
48	*R. viscosa* (Roth) DC. Prodr		CTR, KNL
49	*Vigna aconitifolia* (Jacq) Marechal	2n=22, 44	Throughout EG
50	*V. dalzelliana* (O. Kuntze) Verdc	2n=22	CTR, VSKP
51	*V. grahamiana* (Wight & Arn) Verdc	2n=22	VSKP
52	*V. hainiana* Babu *et al.*		Throughout EG
53	*V. mungo* (L.) Hepper	2n=22	Throughout EG
54	*V. pilosa* (Willd) Baker	2n=22	KNL, PKSM, VSKP
55	*V. radiata* Wilczek	2n=22	Throughout EG
56	*V. sublobata* (Roxb) Babu *et al.*	2n=22	Throughout EG
57	*V. trilobata* (Thunb) Ohwi & Ohashi	2n=22, 44	Throughout EG
58	*V. umbellata* (Thumb.) Ohwi & Ohashi	2n=22, 44	Throughout EG
59	*V. unguiculata* (L.) walp subsp. *cylindrica* (L.) ESH	2n=22, 44	VSKP
60	*V. unguiculata* subsp. *sesquipedalis*		Throughout EG
61	*V. vexillata* (L.) A. Rch var. Stocksii Benth ex Baker	2n=22	VSKP
62	*Sesamum alatum* Thonn	2n=26	KNL, PKSM
63	*S. laciniatum* Klein ex willd	2n=32	CTR, KNL
64	*S. indicum* L. var. *indicumorientale* L.	2n=26	CTR, KDP, KNL
65	*S. prostratum* Retz.	2n=32	KNL, MBNR
66	*S. radiatum* Schumach &Tginn	2n=64	KNL

CTR: Chittor; KDP: Kadapa; KNL: Kurnool; MBNR: Mahabubnagar; PKSM: Prakasam; VSKP: Visakhapatnam

In Eastern Ghats regions, several legume crops such as Pigeon pea (*Cajanus cajan*), chickpea (*Cicer aritinum*), Soybean (*Glycine max*), horse gram (*Macrotyloma uniflorum*), groundnut (*Arachis hypogea*), green gram (*Vigna radiata),* black gram (*Vigna mungo*), cowpea (*Vigna unguiculata*) and Indian bean (*Lablab purpureus*), cluster bean (*Cymopsis tetragonoloba*), sword bean (*Canavalia gladiata*) are cultivated for livelihood and commercial purposes. Although Chickpea and groundnut are extensively cultivated in Eastern Ghats but we did not find any wild relatives of these crops in the surveyed region area. Similarly, soybean and and cluster bean are cultivated in small pockets but their wild relatives are not reported so far.

Cajanus: Pigeonpea (*Cajanus cajan* (L.) Millsp.), a sub-tropical and tropical grain legume that originated in the northern region of the Indian sub-continent, spreading to East Africa at least 4000 years BC and then to Southeast Asia, West Africa, Latin America, and the Caribbean (Khoury *et al.*, 2015). Smartt (1990) classified the wild relatives of *Cajanus* into primary, secondary and tertiary gene pools based on their crossability. Upadhyaya *et al.* (2013) reported seven genera closely related to pigeonpea. These included *Cajanus, Dunbaria, Flemengia, Paracalyx* and *Rhynchosia* which occur in Eastern Ghats. Bio-systematic studies encompassing morpho-cytological and electrophoretic analysis of *Cajanus cajan* (7), one of *Rhynchosia* revealed that *C. cajanifolius* is closest to *C. cajan* followed by *C. lineatus*, *C. scarabaeoides*, *C. sericeus*, *C. albicans*, *C. volubilis*, *C. platycarpus* and *R. rothii* (Pundir and Singh, 1985a). Except *C. lineatus* and *C. platycarpus*, all other species are present in Eastern Ghats.

Cajanus cajanifolius is the progenitor of the present day cultivated pigeon pea (Kassa *et al.*, 2012) and it is the donor for dwarf genes and cytoplasmic male sterility and having high protein content (Jha and Ohri, 1996), salinity tolerant, tolerant to pod borer, pod fly and pod wasp and a source for A5 cytoplasm, crossable with pigeon pea (Singh *et al.*, 1990; Sharma *et al.*, 2006).

C. scarabaoides is resistant to pod-borer, bruchids and drought and water logging and it exhibit early flowering, resistance to wilt, sterility mosaic disease, phytophthora blight, alternaria blight and also source for A2 cytoplasm and crossable with pigeon pea. While, *C. sericeus*, *C. albicans* and *C. volubilis* is resistant to pigeon pea sterility mosaic virus, Phytophthora blight, pod fly and pod borer (Pundir and Singh, 1985b; Sharma, 2006). *C. crassus* is a Source for A3 Cytoplasm (Upadhyaya *et al.*, 2013). All these wild relatives are highly tolerant to drought, salinity and high protein content. Many wild relatives including those of pigeon pea have survived drought, floods, extreme heat and cold and have developed resistance to various biotic and abiotic stresses.

Jack bean: The Jack bean (*Canavalia ensiformis*) is very closely related to the sword bean but the seeds can be distinguished by the length of the hilum which is nearly as long as the seed in the sword bean and less than half its length in the jack bean (Smartt, 1976). According to Herklots (1972), the plant *C. gladiate* is considered as an Old World Tropic, whereas *C. ensiformis* is a New World Tropic. The mature seeds of *Canavalia gladiata* have been originally consumed by people of ancient India and are now consumed even by urbanized population (Vishnu Mittra, 1981). Jack bean and sword bean are advocated to be good sources for protein since the protein quality is similar to most edible food legumes both as a food and a feed (Bressani *et al.*, 1987). Tribal people of ancient India consume Canavalia seeds traditionally (Rajaram and Janardhanan, 1987).

Lablab bean: Lablab is an ancient domesticated crop, widely distributed in Africa, the Indian sub-continent and Southeast Asia . This is a drought tolerant plant. It is used as a grain legume and vegetable for more than 3500 years (Maass *et al.*, 2006).

Mucuna: Underutilized legumes are in demand as alternate protein sources to meet the ever increasing demand of vegetable protein (Pugalenthi *et al.*, 2005).

Rhynchosia: The genus *Rhynchosia* comprises about 250 species and closely related to the genus *Cajanus* and distributed throughout the tropics (Ramcharan *et al.*, 1973). In India the genus is represented by 25 species, as well as one variety and one subspecies of which 7 species are endemic to India.

In India, a great diversity of the *Rhynchosia* species (~60 found in the Eastern Ghats) have been reported (Pullaiah and Sriramamurthy, 2000). *Rhynchosia* species are used as anti-nociceptive and anti-inflammatory agents and some species also have anti-fertility and antipyretic effects (Vimala *et al.*, 1997).

Vigna: Vigna is a pan-tropical genus comprising 104 species distributed widely in tropical and subtropical regions (Lewis *et al.*, 2005; Schrire, 2005). Maréchal *et al.* (1978) recognized seven subgenera in the genus *Vigna* sp. l.: *Ceratotropis, Haydonia, Lasiospron, Macrorhynchus, Plectotropis, Sigmoidotropis* and *Vigna*. *Vigna* subg. *Macrorhynchus* is now included in *Wajira* (Thulin *et al.*, 2004). Among the listed accessions species, five subgenera presently recognized (*Ceratotropis, Haydonia, Lasiospron, Plectrotropis* and *Vigna*), and cultivated species have been developed only from three subgenera (*Ceratotropis, Plectrotropis* and *Vigna*) (Takahasi *et al.*, 2016). The sub-genus *Ceratotropis* has its centre of diversity in Asia with 21 species (Tomooka *et al.*, 2011). The genus *Vigna* has a complex taxonomy because of its relationship with *Phaseolus* (Maréchal *et al.*, 1978). About ten *Vigna* species are domesticated or cultivated as food, feed and for ground cover. Mungbean (*V. radiata* (L.) Wilczek), blackgram (*V. mungo* (L.) Hepper), cowpea (*V. unguicul*ata (L.) Walps) and Bambara groundnut (*V. subterranea* (L.) Verdc.) are the most important crops of the genus *Vigna* and grown as a main component in cropping systems (Tomooka *et al.*, 2002). Babu *et al.*, (1987) enumerated 23 species of *Vigna* including naturalized and cultivated ones from India and a total of 13 *Vigna* species (56%) present in Eastern Ghats.

Moth bean: It is reported as a crop most tolerant to drought and heat in the subgenus *Ceratotropis* (Jain and Mehra, 1980). The wild form of moth bean was documented to be distributed in India (Arora and Nayer, 1984). Takahashi *et al.* (2016) collected three *V. aconitifolia* accessions as wild forms from Tamil Nadu and Andhra Pradesh. The collection sites of these three accessions suggest that the primary habitat of the wild form of moth bean is south-eastern India, Andhra Pradesh, some parts of Tamil Nadu under Eastern Ghats region. *Vigna aconitifolia* is resistant to Mung bean Yellow Mosaic Virus (MYMV) (Bhaskar *et al.*, 1990, Yaqoob, 2007; Meghwal *et al.*, 2015).

Vigna unguiculata subsp. *sesquipedalis* are believed to have been selected and developed for their long tender pods in South-East Asia. This is from vegetable types of *Vigna unguiculata* introduced from India (Steele and Mehra, 1980). It is recently identified and collected from coastal regions of Andhra Pradesh and Odisha of Eastern

Ghats (Kamala *et al.*, 2014). This crop has potential uses in the cosmetic, food, textile and pharmaceutical sectors because of their therapeutic properties (Singh and Basu, 2012). It is a source of several vitamins, minerals and other trace elements (Singh *et al.*, 2003).

Vigna vexillata (L.) A. Rich. belongs to sub genus *Plectotropis* of *Vigna* within the subtribe Phaseolinae (Smartt 1990; Karuniawan *et al.*, 2006). It is considered to be closely related to the cowpea (*V. unguiculata*) (Sonnante *et al.*, 1996; Garba and Pasquet, 1998). The cultivation of *V. vexillata* as a root crop has been reported in East and North East India, where it is locally known as 'halunda' (Bhattacharyya *et al.*, 1984) and in the foothills of the Himalaya region (Sasikumar and Sardana, 1988). Seeds of *Vigna vexillata* are boiled and consumed by the tribal people living in the hilly region of Pune district, India (Siddhuraju *et al.*, 1994).

Oilseeds

In Eastern Ghats several oil yielding crops are cultivated like groundnut (*Arachis hypogea*), oil palm (*Elaeis guineensis*), safflower (*Carthamus tinctorius*), mustard (*Brassica* sp.), soybean (*Glycine max*), castor (*Ricinus communis*), sunflower (*Helianthus annuus*) and coconut (*Cocos nucifera*). Wild relatives of only sesamum crop are reported and found in this region.

Sesamum: Sesamum orientale and *Sesamum indicum* are the alternatively used scientific names of sesame (Bedigian 2003). Nicolson and Wieserma (2004) proposed *S. indicum* name against *S. orientale*, which was conserved against *S. orientale* and is in use since 2005. More than 38 species have been described in this genus, which are classified into different groups on the basis of their geographic distribution, morphologic and cytogenetic information (Kobayashi 1991). Nayer *et al.*, (2006) categorized reported six species of *Sesamum* into in India of which 5 of them are reported from Eastern Ghats. The genus *Sesamum* L. contains about 19 species and is widely distributed in Old world tropics and South Africa. Earlier research and reports on sesame have mainly focused on quantitative genetics (Wei *et al.*, 2009), traditional genetic breeding (Sarwar and Hussain, 2010), mutation breeding (Sengupta and Datta, 2005) and genetic relationships and diversity among sesame germplasm collections (Laurentin and Karlovsky, 2006; Abdellatef *et al.*, 2008; Uzun and Çağırgan, 2009) and screening for biotic stresses (Gupta 2004; Kumar *et al.*, 2010; Mishra *et al.*, 2016). Wild species are reservoir of potential traits which can help in the breeding activities to meet the oil demand. *S. radiatum* having large number of capsules and wide adaptation for diverse climatic conditions, is resistance to powdery mildew, leaf-webber, phyllody and drought tolerance (Thangavelu, 1994). *S. alatum* is resistant to phyllody and antigastra, *S. malabaricum* is resistant to water logging, donor for cytoplasmic male sterility, *S. mulayanum* is resistant to *fusarium* wilt and gall fly, *S. prostratum* is resistant to antigastra, salinity and non-shattering capsules whereas *S. laciniatum* is resistant to antigastra, drought and non-shattering habit Sesame is susceptible to phyllody disease caused by phytoplasma, resulting stunted plant growth and yield losses (Singh *et al.*, 2007), whereas *S. prostratum* is resistance to powdery mildew, phyllody and highly tolerant to drought and salinity and less seed shattering in nature (Pandey *et al.*, 2008).

Discussion

Grain legumes have a narrow genetic base since they are self-pollinated (though cross-pollination does take place, it is at very low frequency). Thus, there is a need to study legume genetics and genomics in depth to understand the biology of legume crops to widen the genetic base, support legume breeding programs and introgression of traits of interest (Varshney and Kudapa, 2013).Bean cultivars with tolerance to low temperatures and salinity derived from wild *Vigna* are in the pipeline (Bayuelo-Jimenez *et al.*, 2002). *Vigna* wild relatives are currently being screened for resistances to web blight, rust, white mold, bean golden yellow mosaic, bruchids, and seed storage insects (Hajjar and Hodgkin, 2007). Pest and disease resistance in wild species are still the foremost criteria for utilization. Elangovan and Kiran Babu (2015) reported 111 cultivated crops belonging to 120 genera with 139 species of 74 families from Andhra Pradesh and Telangana states, of which nine belonged to legume crops and eight belonged to oilseed crops. Khoury *et al.* (2015) proposed, priority species such as *C. crassus* and *C. scarabaeoides* occur outside these regions, thustargeted collecting throughout the geographic distributions of the species is necessary in order to form germplasm collections that are comprehensive at the population level. Non-native distributions of widespread species, particularly *C. scarabaeoides*, may also be considered for further collection for useful traits for crop improvement.

Further collecting for *ex situ* conservation of this diversity, securing long-term funding for this

conservation and associated research, ensuring safety duplication of unique germplasm and sharing of this diversity with the global research community are critical to this process (Esquinas-Alcázar, 2005). Greater investment in genotypic and phenotypic characterization and evaluation for traits of interest (Mallikarjuna *et al.*, 2011; Varshney *et al.*, 2011) and in breeding programs using crop wild relatives (Tester and Langridge, 2010; Khoury *et al.*, 2015). The wild relatives have not received due attention from germplasm collectors and plant breeders, and remain under-represented, accounting for lessthan two percent of the global germplasm collections of major food crops (Kameshwara Rao, 2003). Neglected and under-valorised crop has great untapped potential to support smallholder rural farming communities by providing income, food and nutritional security as well as sustaining the genetic resources needed to address present and future environmental challenges (Kahane *et al.*, 2013). The cultivation of grain legumes over cereals is a promising way for resource-poor farmers to increase income as they can be grown well with low inputs (Siddique *et al.*, 2012). Wild relatives of crop plants potentially rich with various sources of resistance (biotic and abiotic stresses) can be improved suitably for adoptability to changing climate and meet the increasing food demand.

Conservation

Increasing awareness of the extent of habitat destruction, invasive species and other threats to the habitats of the CWR of major crops has given urgency to efforts to identify important species, determine their distributions, and to ensure their conservation for the long-term and thus their availability to plant breeders (Jarvis *et al.*, 2008; FAO, 2010). Indian National Genebank holds ~0.44 million accessions of germplasm, of which, legumes represent 11,185 accessions and sesame clasps 9,954 accessions respectively. The species wise accessions conserved in Indian National Genebank given in Table 2. A gap analysis for collected and conserved crop wild relatives in Indian National Genebank revealed that out of a total of ~2000 taxa (Arora and Nayar, 1984; Pradheep *et al.*, 2014) crop wild relatives reported from India only 94 species are conserved in Indian National Genebank (Gupta *et al.*, 2016).There is an urgency to ensure that the diversity in landraces is sampled and conserved in *ex situ* genebanks, especially as farming evolves from subsistence to a market orientated endeavor dominated by modern cultivars and resulting in the erosion of crop genetic diversity (Petr *et al.*, 2015).

Table 2. List of wild germplasm accessions of legumes and sesame conserved at Indian National Genebank

S.No.	Name of the species	No. of Acc.
1	*Cajanus albicans*	3
2	*C. cajanifolius*	3
3	*C. platycarpa*	4
4	*C. scaraboides*	15
5	*Canavalia africana*	3
6	*C. cathartica*	50
7	*C. ensiformis*	62
8	*C. gladiata*	34
9	*C. obtusifolia*	2
10	*C. virosa*	7
11	*Flemingia sp.*	1
12	*Mucuna capirata*	1
13	*Mucuna gigantea*	2
14	*Mucuna monosperma*	5
15	*Sesamum laciniatum*	3
16	*S. prostratum*	6
17	*S. radiatum*	31
18	*Vigna angularis*	171
19	*V. heyniana*	2
20	*V. mungo var. sylvestris*	13
21	*V. pilosa*	3
22	*V. vexillata*	115
23	Yardlong bean	13
	Total	549

Further development in targeting germplasm for key traits is to use a GPS map of landrace collection sites, which can be overlaid with climate data corresponding to vegetative and reproductive growth stages and to identify landraces corresponding to sites with severe abiotic stresses during the previous 25 years (Petr *et al.*, 2015). Priorities and strategies for collection followed by their conservation will be defined based on the economic value of cultivated species, distribution of wild species and its potential use in crop improvement program for enhancing the food and nutritional security (Tyagi, 2016). The genus *Cajanus* holds 25 wild accessions, *Canavalia* (158), *Flemingia* (1), *Mucuna* (8), *Vigna* (304) and *Sesame* (40) and yardlong bean (13), respectively. This figure indicates that there is an urgent need for collection and conservation of trait specific wild legumes and oil seeds for future needs. At the same time, identification of trait specific germplasm for biotic, abiotic stresses needs to be identifiedexplored by characterization and evaluation for its best utilization and value added products.

Conclusion

Eastern Ghats are rich in grain legumes and oilseed

plant resources and indigenous livelihood traditions, therefore under the framework of proper policy and guidelines, these resources can be more effectively used to benefit for crop improvement programs, sustainable utilization and conservation strategies. Conservation and development in this region need to be specifically suited to the environmental fragility and unique cultural aspects. Thrust area need to be for plant genetic resource based conservation, sustainable use and management of species and ecosystems that are unique to the region. The increased number of species at risk as a result of the changing climatic conditions will force the National Active Germplasm Sites (NAGS) which is responsible for crop specific to refocus, to strengthen their conservation policies and to increase their participation in recovery programs for trait specific germplasm, landraces, primitive cultivars and threatened species, including crop wild relatives. It is necessary to network and link institutions involved in agro-biodiversity related research, management and training in this region.

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RESEARCH ARTICLE

Assessment of Character Contribution to the Divergence in Rice (*Oryza sativa* L.) Germplasm

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Sixty-five rice accessions were evaluated for eleven characters to study genetic diversity. Result indicated presence of considerable genetic divergence among the accessions and their pattern of distribution into various clusters was random and independent of geographical origin. Cluster I, III and II contain 28, 19 and 12 accessions, respectively, while clusters IV, V, VI, VII, VIII and IX were monogenotypic. The highest intra cluster distance was observed for cluster III (7.72) followed by cluster II (4.85). Cluster VII had highest mean value for filled grains per panicle (210.06), total grains per panicle (251.33), spikelet fertility per cent (89.19) and grain yield per plant (30.81 g) and cluster VIII for days to 50 per cent flowering (155.33), days to maturity (180.33) and plant height (177.21 cm). Days to flowering had maximum contribution (47.74 %) to the total divergence followed by days to maturity (13.7 %) and plant height (13.65 %). The accessions falling in different clusters with high mean grain yield and high mean for component characters can be utilized for hybridization programme to obtain elite segregants.

Key Words: Character contribution, Clustering, D^2 analysis, Genetic divergence, *Oryza sativa*

Introduction

Genetic diversity is a pre-requisite for any crop improvement breeding programme as it helps in estimating and establishing the genetic relationship in the collection of accessions, identifying diverse parental combinations to create segregating progenies with maximum genetic variability and superior recombinations (Thompson *et al.*, 1998; Islam *et al.*, 2012; Ramadan *et al.*, 2015). Parents identified on the basis of divergence would be more promising for breeding program (Kwon *et al.*, 2002). Thus, accurate assessment of the levels and patterns of genetic diversity can be invaluable in crop breeding for diverse applications including introgression of desirable genes from diverse accessions into the available genetic base and for widening the narrow genetic base of the developed varieties (Roy, 2013). The D^2 technique based on multivariate analysis developed by Mahalanobis (1936) had been found to be a potent tool in quantifying the degree of divergence in accessions and provides a measurement of relative contribution of different components on diversity both in inter and intra-cluster level and genotypes drawn from widely divergent clusters are likely to produce heterotic combinations and wide variability in segregating generation. Thus, the present investigation was intended to characterize the rice accessions on the basis of D^2 analysis (Mahalanobis, 1936).

Materials and Methods

Experimental Material and Site

The present investigation analysed 65 rice genotypes listed in Table 1, received from DBT Networking Project, Institute of Agricultural Sciences, Banaras Hindu University. The plants were raised in Randomized Block Design with three replications during the kharif season 2015 at Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University. The 21 days old seedlings were used for transplanting at row to row 20 cm spacing and plant to plant was 15 cm, respectively.

Traits Observed

Observations were recorded on randomly selected plants from each entry for eleven quantitative traits viz., days to 50 per cent flowering (DF), days to maturity (DM), number of effective tillers per plant (ET), plant height

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Table 1. Genotypes used under study

S. No.	Genotype	S. No.	Genotype	S. No.	Genotype
1	OR 1946-2-1	2	IR 83142-76	3	Vandana
4	IR 82635-B-B-47-1	5	IR 82589-B-B-7-2	6	IR 82635-B-B-23-1
7	CRR 660-2	8	CRR 428-237-1-3-1	9	Rewa 1208-15
10	IR 83399-B-B-52-1	11	PAU 3832-79-4-3-1	12	IR 83182-6-4
13	IR 78755-190-B-1-3	14	IR 82635-B-B-25-4	15	RP 5345-9-6-3
16	RRF-48	17	IR 55423-01	18	CB 10-504
19	GK 5022	20	Anjali	21	IR 10L-105
22	B 11576F-MR-18-2	23	CR 3631-1-3	24	BAU 411-05
25	IR 82921-B-B-1	26	BD 104	27	IR 77298-14-1-2-13
28	CR 422-63-51-B-2-1-1-1-B	29	IR 1718-59-1-2-3	30	IR 82635-B-B-145-1
31	NDR 1140	32	CR 3633-1-2	33	IR 368B-TB-25-MP-2
34	UPLRI – 7	35	IR 87694-28-7-2-1	36	RP 5330-63-5-2-1-B
37	MGD 1206	38	IR83867-B-B-250-CRA-1-1	39	IR 83926-B-B-71-4
40	IR 60080-46A	41	BD 108	42	BVS 1
43	BVD 111	44	BVD 203	45	BAU 389-02
46	BAU/IRRI 497	47	LC -1	48	LC -2
49	LC -3	50	LC -4	51	LC -5
52	LC – 6	53	LC – 7	54	LC – 8
55	LC – 9	56	LC – 10	57	LC – 11
58	LC – 12	59	LC – 13	60	LC – 14
61	LC – 15	62	LC – 16	63	LC – 17
64	LC – 18	65	LC – 19		

(PH), panicle length (PL), panicle weight (PW), filled grains per panicle (FG), total grains per panicle (TG), spikelet fertility percent (SFP), test weight (TW) and grain yield per plant (GY).

Statistical Analysis

Analysis of variance was carried out by the method of Panse and Sukhatme (1967) for each of the sixty-five genotypes. Genetic divergence was estimated by Mahalanobis' D^2 statistics (1936). After arranging the D^2 values of all combinations of one genotype with the others in ascending order of magnitudes, the genotypes were grouped into a number of clusters by Tocher's method described by Rao (1952). Each character was ranked on the basis of values in all the combination of genotype for estimation of contribution of individual characters towards divergence.

Results and Discussion

Analysis of variance revealed that the genotypes differed significantly for all the characters under study and might be of diverse origin (Table 2). These findings were in accordance with the findings of Paikhomba *et al.* (2014) and Islam *et al.* (2016).

Cluster Distances and Composition

D^2 analysis revealed the presence of considerable diversity in the set of sixty-five accessions under study. The accessions were observed to be distributed in nine different clusters. Clustering pattern indicated that twenty-eight out of 65 accessions belong to the same cluster i.e. cluster I (Table 3). On the other hand, 19 accessions belong to cluster III and 12 belong to cluster II. Rest other clusters i.e. cluster IV, V, VI, VII, VIII and IX consists of one accessions each for which the intra-cluster distance was observed to be zero. Intra-cluster and inter-cluster distances i.e. $\sqrt{D^2}$-values have been presented in Table 4. Cluster III showed the maximum intra-cluster distance i.e. 7.72 followed by cluster II (4.85) and I (4.48). Genotypes included in the same cluster indicated their close relationship among themselves as compared to genotypes in other clusters. The maximum inter-cluster distance was observed between cluster II and VIII (23.33) indicating that the hybridization between the most diverse genotypes would yield desirable segregants with the accumulation of favorable genes in the segregating generations. The next two diverse clusters were cluster VII and VIII (22.61) and cluster V and VIII (19.75). The clustering pattern indicated wide diversity between different groups of genotypes. The inter-cluster distances were higher than the intra-cluster distances which indicate the existence of substantial diversity among the parents (Kumar *et*

Table 2. Analysis of variance (ANOVA) for eleven quantitative traits in sixty-five rice genotypes

Source of variation	df	Mean Sum of Squares										
		DF	DM	ET	PH	PL	PW	FG	TG	SFP	TW	GY
Replication	2	1.00	10.23	7.45	114.74	0.51	0.07	5.17	15.32	25.18	7.34	1.65
Treatment	64	994.01**	960.59**	12.90**	2060.52**	23.77**	2.48	3692.41**	4949.83**	181.33**	44.57**	95.23**
Error	128	4.99	4.19	2.55	88.75	0.72	0.11	244.88	157.38	40.46	3.01	8.36

**Significant at p< 0.01.
(DF) days to 50 per cent flowering, (DM) days to maturity, (ET) number of effective tillers per plant, (PH) plant height, (PL) panicle length, (PW) panicle weight, (FG) filled grains per panicle, (TG) total grains per panicle, (SFP) spikelet fertility percent, (TW) test weight and (GY) grain yield per plant.

Table 3. Grouping of sixty-five rice genotypes into nine clusters (by Tocher method)

Clusters	Germplasm	Number
I	IR 55423-01, LC – 18, LC – 13, IR 1718-59-1-2-3, LC – 16, BVS 1, BAU 389-02, BVD 203, LC – 11, LC – 17, LC – 7, LC – 6, LC – 14, Vandana, OR 1946-2-1, LC – 19, LC – 3, LC – 4, IR 368B-TB-25-MP-2, LC – 2, CR 422-63-51-B-2-1-1-1-B, IR 10L-105, CR 3633-1-2, LC – 9, CB 10-504, R RF-48, IR 83867-B-B-250-CRA-1-1, LC – 5	28
II	BAU 411-05, LC – 12, IR 82635-B-B-25-4, IR 60080-46A, MGD 1206, CRR 660-2, IR 78755-190-B-1-3, CRR 428-237-1-3-1, RP 5345-9-6-3, RP 5330-63-5-2-1-B, BD 104, IR 83926-B-B-71-4	12
III	IR 82589-B-B-7-2, IR 87694-28-7-2-1, IR 77298-14-1-2-13, IR 83399-B-B-52-1, IR 83182-6-4, UPLRI – 7, BD 108, IR 82635-B-B-47-1, IR 83142-76, Rewa 1208-15, BVD 111, BAU/IRRI 497, LC – 1, CR 3631-1-3, LC – 15, B 11576F-MR-18-2, LC – 8, IR 82635-B-B-145-1, PAU 3832-79-4-3-1	19
IV	GK 5022	1
V	NDR 1140	1
VI	Anjali	1
VII	IR 82921-B-B-1	1
VIII	IR 82635-B-B-23-1	1
IX	LC – 10	1

Table 4. Average Intra- and Inter-cluster √D2 values among nine clusters (by Tocher's method)

Clusters	I	II	III	IV	V	VI	VII	VIII	IX
I	4.48	7.43	11.84	7.17	7.65	6.94	8.31	18.86	7.96
II		4.85	16.09	11.48	9.86	11.14	9.11	23.33	12.59
III			7.72	10.18	11.44	10.85	14.89	11.13	11.13
IV				0	8.56	9.63	8.12	16.26	8.25
V					0	9.32	6.95	19.75	12.64
VI						0	11.56	16.92	6.21
VII							0	22.61	12.97
VIII								0	15.23
IX									0

(Figure in diagonal indicate intra-cluster √D2 value)

al., 2014). Similar results of inter and intra cluster distances in rice were reported by Senapati and Sarkar (2005), Kuchanur *et al.* (2009), Roy (2013) and Singh *et al.* (2015).

Cluster Mean for Different Characters under Study

A perusal of cluster means indicated that there existed considerable differences in the mean values of different traits (Table 5). Cluster VII exhibited highest mean value for filled grains per panicle (210.06), grains per panicle (251.33), spikelet fertility per cent (89.19) and grain yield per plant (30.81), while cluster VIII had highest mean value for days to 50 per cent flowering (155.33), days to maturity (180.33) and plant height (177.21) likewise cluster IX had highest mean value for panicle length (33.38) and test weight (29.72). Cluster II and IV had highest mean value for number of tillers

Table 5. Cluster mean values for different eleven characters

Cluster	DF	DM	ET	PH	PL	PW	FG	TG	SFP	TW	GY
I	97.86	132.56	8.94	112.79	26.48	3.22	128.61	160.87	79.54	23.10	22.65
II	85.91	117.00	**11.57**	104.97	22.78	2.21	89.30	106.41	82.60	22.68	20.22
III	128.63	160.42	9.93	133.79	24.94	2.96	139.28	168.91	82.66	19.39	22.65
IV	111.66	140.66	7.46	125.45	27.25	**5.76**	198.00	220.73	82.74	28.67	32.36
V	103.33	137.00	9.80	98.11	23.41	3.00	199.86	226.13	85.73	14.88	22.25
VI	107.33	138.33	9.60	123.09	31.49	2.45	137.80	177.06	77.68	16.17	16.86
VII	95.00	122.33	8.20	142.71	24.94	5.41	**210.06**	**251.33**	**89.19**	22.13	**30.81**
VIII	**155.33**	**180.33**	10.33	**177.21**	25.47	2.78	80.73	80.73	72.00	24.89	16.23
IX	109.33	142.33	8.06	155.487	**33.38**	4.32	142.86	142.86	89.06	**29.72**	28.63

*Bold numbers are highest mean value

(DF) days to 50 per cent flowering, (DM) days to maturity, (ET) number of effective tillers per plant, (PH) plant height, (PL) panicle length, (PW) panicle weight, (FG) filled grains per panicle, (TG) total grains per panicle, (SFP) spikelet fertility percent, (TW) test weight and (GY) grain yield per plant.

(11.57) and panicle weight (5.76), respectively. Thus, the genotypes in cluster VII, VIII and IX seem to be quite promising for these mentioned traits. Similar findings were observed by Singh *et al.* (2011) and Chouhan *et al.* (2014) for days to maturity, plant height, total number of tiller per plant and test weight. It has been observed that distribution of highest and lowest mean values for different traits under study in distinct cluster suggested that the traits contributing to the total divergence.

Contribution of Different Traits towards Total Genetic Divergence

The perusal of the comparison of contribution of different characters towards genetic diversity was estimated based on ranking method (Fig. 1) and it was observed that days to 50 per cent flowering contributed maximum (47.74%). The second largest contributor was days to maturity (13.7%) followed by panicle length (13.65%) and panicle weight (6.59%). The character like test weight, spikelet fertility, total grain per panicle and filled grain per panicle was found to be least contributing traits. Similar results were reported by Khare *et al.* (2014).

Conclusion

A wide range of variation was evident among 65 rice germplasms evaluated. The sixty-five rice accessions were grouped into nine clusters which were in concordance with the clustering pattern obtained by Mahalanobis D^2 statistics. The parents for hybridization program should be selected based on the magnitude of genetic distance, contribution of different characters' towards the total divergence and magnitude of cluster

Fig. 1. Percentage contribution of each character towards total genetic divergence in 65 germplasm

means for different characters performance having maximum heterosis. A higher heterosis could be produced from the crosses between genetically distant parents. Therefore, crosses between the genotypes of clusters II and VIII, VII and VIII, V and VIII would give high manifestation of heterosis and is expected to be effective in accumulation of favorable genes in segregating generations.

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RESEARCH ARTICLE

Genetic Studies based on Selected Morpho–physiological Parameters in Garden Pea (*Pisum sativum* L.)

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The experiment was planned to study genetic variability and association among different morpho-physiological traits. Forty five genotypes derived from five intervarietal crosses with variable plant and pod characteristics were evaluated in Randomized Complete Block Design with three replications during winter 2014–15 and 2015–16 at Palampur. Significant differences were observed among all genotypes for all the characters over years. High GCV and PCV along with high heritability (>60%) and genetic advance (>30%) were observed for pods/plant and pod yield/plant during respective years, suggesting additive gene inheritance and selection in early generations would be effective. Significant positive correlation of pod yield/plant was recorded with internodal length (0.235), seeds/pod (0.441), shelling percentage (0.306), pods/plant (0.885) and average pod weight (0.349). The positive association was mainly due to direct effects of these traits with pod yield/plant (0.90 and 0.43, respectively) and also indirectly contributed for positive association with other traits. Therefore, these traits provide an important criterion of selection procedures for achieving enhanced performance of garden pea genotypes for higher pod yield.

Key Words: Garden pea, Genetic parameters, Pod yield, Variability

Introduction

Garden pea (*Pisum sativum* L.) is a leading vegetable crop in the north–western Himalayan region of India comprising the states of Himachal Pradesh, Jammu and Kashmir, and Uttarakhand (Sharma *et al.*, 2010). It is an important source of proteins, vitamins, minerals and also lysine, a limiting essential amino acid in cereals (Sharma and Sharma, 2016). It is an important off–season vegetable crop of Himachal Pradesh, grown over the year and green pods are available during the period when its supply from plain areas almost ceases, and hence, provides lucrative returns to the growers.

The prime objective of breeding work is to increase the yield (Tyagi and Srivastva, 2002) and to prepare the background for the future research. High yield, desirable pod characteristics and resistance to biotic stresses are the main criteria, taken into consideration by the breeders for its genetic improvement. Despite continuous breeding efforts, the average yield of garden pea is low due to its narrow genetic base and consumer preference for some specific traits such as lush green well-filled pods. Also, genetic drift in the extensively grown age old few cultivars and emergence of new pathogen races also lead to low/stagnant yield. The age old varieties like 'Azad P–1', 'Lincoln', 'Arkel' etc., are still preferred by the growers due to desirable horticultural traits though the varieties have become vulnerable to a plethora of biotic and abiotic stresses (Sharma *et al.*, 2013).

Crop improvement with heritable characters, estimation of genetic parameters and their association is of prime importance in breeding (Ajmal *et al.*, 2009; Bozokalfa *et al.*, 2010). The estimate of heritability acts as a predictive instrument in expressing the reliability of phenotypic values (Unche *et al.*, 2008). Yield is highly influenced by the environment, hence selection based on yield alone may limit the improvement. On the other hand, the yield component traits are comparatively less complex in inheritance and are influenced to a lesser extent by the environment (Alkuddsi *et al.*, 2013). Thus, effective improvement in yield may be brought about through selection for yield component characters. Favourable associations between desirable attributes will help improvement in a joint manner whereas, unfavourable associations between the desirable attributes under selection may limit them. Hence, knowledge of associations between the yield components and also

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Table 1. Genotypes and their sources

Code	Genotype	Source	Code	Genotype	Source	Code	Genotype	Source
1	Palam Priya	CSK HPKV, Palampur	18	DPP-2011-SP-22	CSK HPKV, Palampur	35	DPP-2011-SN-16	CSK HPKV, Palampur
2	Pb 89	PAU, Ludhiana	19	DPP-2011-SP-23	CSK HPKV, Palampur	36	DPP-2012-SN-1	CSK HPKV, Palampur
3	Azad P-1	CSAUA&T, Kanpur	20	DPP-2011-SP-24	CSK HPKV, Palampur	37	DPP-2012-SN-2	CSK HPKV, Palampur
4	Palam Sumool	CSK HPKV, Palampur	21	DPP-2011-SP-25	CSK HPKV, Palampur	38	DPP-2012-SN-4	CSK HPKV, Palampur
5	Palam Triloki	CSK HPKV, Palampur	22	DPP-2011-SP-28	CSK HPKV, Palampur	39	DPP-2012-SN-7	CSK HPKV, Palampur
6	DPP-2011-SP-3	CSK HPKV, Palampur	23	DPP-2011-SP-29	CSK HPKV, Palampur	40	DPP-2012-SN-8	CSK HPKV, Palampur
7	DPP-2011-SP-6	CSK HPKV, Palampur	24	DPP-2011-SP-32	CSK HPKV, Palampur	41	DPP-2012-SN-9	CSK HPKV, Palampur
8	DPP-2011-SP-7	CSK HPKV, Palampur	25	DPP-2011-SP-33	CSK HPKV, Palampur	42	DPP-2012-SN-10	CSK HPKV, Palampur
9	DPP-2011-SP-8	CSK HPKV, Palampur	26	DPP-2011-SP-38	CSK HPKV, Palampur	43	DPP-2012-SN-11	CSK HPKV, Palampur
10	DPP-2011-SP-10	CSK HPKV, Palampur	27	DPP-2011-SN-1	CSK HPKV, Palampur	44	DPP-2012-SN-12	CSK HPKV, Palampur
11	DPP-2011-SP-11	CSK HPKV, Palampur	28	DPP-2011-SN-4	CSK HPKV, Palampur	45	DPP-2012-SA-1	CSK HPKV, Palampur
12	DPP-2011-SP-14	CSK HPKV, Palampur	29	DPP-2011-SN-5	CSK HPKV, Palampur	46	DPP-2012-SA-3	CSK HPKV, Palampur
13	DPP-2011-SP-15	CSK HPKV, Palampur	30	DPP-2011-SN-6	CSK HPKV, Palampur	47	DPP-2012-SA-4	CSK HPKV, Palampur
14	DPP-2011-SP-16	CSK HPKV, Palampur	31	DPP-2011-SN-8	CSK HPKV, Palampur	48	DPP-2011-ST-1	CSK HPKV, Palampur
15	DPP-2011-SP-17	CSK HPKV, Palampur	32	DPP-2011-SN-10	CSK HPKV, Palampur	49	DPPMR-09-1	CSK HPKV, Palampur
16	DPP-2011-SP-20	CSK HPKV, Palampur	33	DPP-2011-SN-13	CSK HPKV, Palampur	50	DPPMR-09-2	CSK HPKV, Palampur
17	DPP-2011-SP-21	CSK HPKV, Palampur	34	DPP-2011-SN-15	CSK HPKV, Palampur			

among themselves is essential for planning a sound breeding programme.

Simple correlation analysis that relates yield to a single variable may not provide a complete understanding of the importance of each component in determining pod yield (Dewey and Lu, 1959; Okuyama *et al.*, 2004). In such cases, it becomes necessary to use a method which takes into account the causal relationship between the variables, in addition to their magnitude. Path coefficient analysis permits a critical examination of specific factors that produce a given correlation and can be successfully employed in formulating an effective selection strategy. It allows separating the direct effect and their indirect effects through other attributes by apportioning the correlations (Wright, 1921) for better interpretation of cause and effect relationship. Similar studies were conducted by many researchers in various crops by using different genetic materials (Sharma *et al.*, 2016; Ashok *et al.*, 2018). Selection based on the detailed knowledge of magnitude and direction of association between yield and its attributes is very important in identifying the key characters, which can be exploited for crop improvement through suitable breeding programme. Keeping this in view, 45 F_7 progenies with variable pod and plant characteristics derived from five intervarietal crosses following pedigree method of selection were used to study the genetic variability along with five varieties recommended for cultivation in Himachal Pradesh.

Material and Methods

The present investigation was undertaken at the Research Farm, Department of Vegetable Science and Floriculture, CSK HPKV, Palampur (1290.8 m above mean sea level, with latitude 32º6' N, longitude 76º3' E). The soil of experimental field was clay loam with pH 5.7. The experimental material comprised 50 genotypes (Table 1 and Table 2) which were evaluated in Randomized Complete Block Design with three replications for two consecutive years (November to April 2014–15 and 2015–16). Each genotype was raised in two rows spaced at 45 cm with intra-plant distance of 10 cm. The observations were recorded on randomly selected ten plants of each genotype over the replications for 15 traits, viz., first flower node, days to flowering, days to first picking, number of branches, internodal length (cm), nodes/plant, plant height (cm) at final harvest,

Table 2. Intervarietal crosses attempted during 2006-07 and genotypes selected for evaluation during 2014-15 and 2015-16

S. No.	Crosses attempted	Coding of Genotypes	No. of genotypes in F7 for evaluation
1	Palam Sumool × Palam Priya	SP series	21
2	Palam Sumool × Pb-89	SN series	9
3	Palam Sumool × Azad P-1	SA series	12
4	Palam Sumool × Palam Triloki	ST series	1
5	VRPMR10 × Sugar Giant	DPPMR series	1
6	Green Pearl × DPP-9411	DPPMR series	1

pod length (cm), seeds/pod, shelling (%), pods/plant, pod yield/plant (g) and average pod weight (g). Besides, quality parameters such as total soluble solids (obrix) using hand refractometer and ascorbic acid (mg/100g fresh weight basis) as described by Ranganna (1979) were also estimated.

Statistical Analysis

Analysis of variance was performed for individual season and error variance was tested for homogeneity (Gomez and Gomez, 1983). The combined analysis of variance of two season's data was performed for each trait. The genotypic and phenotypic variations and heritability were calculated as per Burton and DeVane (1953). Heritability and genetic advance (GA) was calculated using formulae of Burton and DeVane (1953) and Johanson *et al.* (1955). Coefficients of correlation were calculated as suggested by Al–Jibouri *et al.* (1958) while path coefficients of different traits with pod yield/plant were carried out as per methodology suggested by Dewey and Lu (1959).

Results and Discussion

Phenotypic Coefficient of Variation (PCV) and Genotypic Coefficient of Variation (GCV)

An effective breeding programme for developing varieties of improved quality requires preliminary information on the nature and magnitude of genetic variability, degree of transmission of traits and their inter–relationship (Selvi *et al.*, 2016). The knowledge of phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) is helpful in predicting the amount of variation present in the given genetic stock which in turn helps in formulating an efficient breeding programme. In this context, high values of PCV and GCV were recorded for pod yield/plant and pods/plant for both years and pooled years (Table 3). This indicates the existence of immense inherent variability that remains unaltered by environmental conditions among the genotypes, which in turn is more useful for exploitation in selection and hybridization programs (Jalal and Ahmed, 2012). Such high estimates of PCV and GCV for pods/plant and pod yield/plant were also reported in different studies using variable genetic material and environments (Sharma *et al.*, 2009). Moderate PCV and GCV estimates were observed for average pod weight during both years and pooled years while number of branches, nodes/plant, plant height, seeds/pod and ascorbic acid exhibited moderate PCV and GCV estimates during 2014–15 and 2015–16. Besides, moderate estimates of PCV were observed for internodal length and shelling (%) during 2014–15 and pooled years. Differences in the magnitude of PCV and GCV (high to moderate or moderate to low) for some of the traits in pool years indicated the role of environment in the manifestation of particular trait(s).

Heritability and Genetic Advance

Heritability of a trait is an index of transmission of

Table 3. Estimates of parameters of variability for different characters in garden pea during 2014-15, 2015-16 and pooled years

Traits	PCV (%)			GCV (%)			h2bs			GA (% of mean)		
	2014-15	2015-16	Pooled	2014-15	2015-16	Pooled	2014-15	2015-16	Pooled	2014-15	2015-16	Pooled
First flower node	9.17	7.68	8.63	7.88	6.72	5.90	73.80	76.63	46.60	13.94	12.12	8.29
Days to flowering	4.75	6.57	5.74	3.91	5.36	4.41	67.80	66.56	58.93	6.63	9.01	6.97
Days to first picking	4.51	2.98	3.86	3.89	2.51	2.81	74.21	70.74	53.11	6.90	4.35	4.23
Number of branches	12.81	14.57	13.71	10.00	11.33	6.84	59.70	60.46	24.86	15.76	18.14	7.02
Internodal length (cm)	12.56	9.95	11.47	12.42	9.37	8.76	97.78	88.54	58.35	25.29	18.16	13.78
Nodes/plant	14.81	13.65	14.33	14.04	10.67	8.43	89.88	61.07	34.68	27.41	17.17	10.24
Plant height (cm)	13.83	11.78	12.81	10.78	10.40	7.71	60.84	78.06	36.24	17.33	18.94	9.57
Pod length (cm)	9.70	8.80	9.27	8.75	7.60	7.77	81.50	74.59	70.19	16.28	13.52	13.42
Seeds/pod	13.36	11.36	12.28	12.03	10.26	9.00	81.07	81.67	53.78	22.32	19.09	13.60
Shelling (%)	12.79	9.72	11.12	10.01	7.79	7.75	61.20	64.33	48.52	16.13	12.88	11.12
Pods/plant	23.49	28.76	29.52	21.41	27.12	22.42	83.03	88.95	57.67	40.18	52.70	35.08
Pod yield/plant (g)	27.39	29.91	31.01	25.83	29.23	24.15	88.94	95.52	60.65	50.18	58.85	38.74
Average pod weight (g)	13.27	14.02	13.56	12.44	12.57	10.18	87.71	80.33	56.31	23.72	23.20	15.74
Total soluble solids (°brix)	8.45	5.01	7.03	6.84	4.73	4.19	65.64	88.95	35.59	11.41	9.19	5.16
Ascorbic acid (mg)	11.91	12.48	12.18	10.14	10.00	8.09	72.52	64.12	44.09	17.79	16.50	11.07

Abbreviations: GCV and PCV represent genotypic and phenotypic coefficients of variations, respectively,
h2bs: Heritability in broad sense,
GA (%): Genetic advance (%) of mean.

characters from parents to progeny (Bagati *et al.*, 2016). The estimates of heritability help the plant breeder in selection of elite genotypes from diverse genetic population, hence prior knowledge about the heritability of the traits is a prerequisite for the selection programme (Singh *et al.*, 2011; Bagati *et al.*, 2016). However, high heritability alone is not enough to make sufficient improvement through selection generally in advance generations unless accompanied by substantial amount of genetic advance (Bhargava *et al.*, 2003). Thus, genetic advance is yet another important selection parameter that aids breeder in a selection programme (Shukla *et al.*, 2004). On the same lines, high estimates of heritability (>60%) along with high genetic advance (>30%) were observed for pod yield/plant and pod/plant during 2014–15 and 2015–16 (Table 3) suggesting that these are simply inherited traits and the heritability is most likely due to additive gene effects and phenotypic selection may be effective in early generations for these traits (Katoch *et al.*, 2016; Shrimali *et al.*, 2017). High heritability along with moderate genetic advance was observed for pod length, first flower node, internodal length, nodes/plant, plant height, seeds/pod, shelling (%), average pod weight and ascorbic acid during 2014–15 and 2015–16 and for internodal length number of branches during 2015–16 which indicated the preponderance of additive and non–additive gene effects for their inheritance (Ranjan *et al.*, 2006; Saxesena *et al.*, 2016). High heritability and low genetic advance was observed for days to flowering and days to first picking during both the years and that of total soluble solids in 2015–16. Dar *et al.* (2013) and Habtamu and Million (2013) also reported such estimates for days to flowering indicating the role of non–additive gene action in their inheritance. As, in the present study expected genetic advance values were based on broad sense heritability, which incorporate both additive and non–additive components of gene action, much reliance cannot be placed on expected genetic advance. However, the traits, which had high heritability and also showed high expected genetic advance, could be substantially considered for making selections as these traits were mainly influenced by the major effects of additive gene action (Jalal and Ahmed, 2012).

Correlation and Path Analysis

For shifting the mean population under study in the desired direction, a thorough understanding of nature and extent of association between characters is essential. It would be very difficult to obtain the desired combinations, if negative association between characters is due to pleiotropic effects. However, if linkage is involved, special breeding programmes are needed to break these linkage blocks. Knowledge of the correlation that exists between important characteristics may facilitate the interpretation of results and provide the basis for planning more efficient breeding programs. It also helps to improve different characters simultaneously (Falconer, 1981). In this regard, pod yield/plant had shown a positive and significant correlation with pods/plant (0.885), seeds/pod (0.441), average pod weight (0.349), shelling percentage (0.306) and internodal length (0.235) during both the years, with pod length during 2014–15, and with number of branches, nodes/plant and plant height during 2015–16. Pooling of data over the years also revealed similar associations of these traits with pod yield suggesting that the selection on the basis of these traits might lead to higher yield. Earlier reports have also indicated significant and positive association for majority of these traits with variable magnitude in diverse breeding material (Kumar *et al.*, 2015; Katoch *et al.*, 2016) which emphasized special focus on these traits. Pod yield/plant had revealed negative association with first flower node (-0.194), days to flowering (-0.317) and days to first picking (-0.248) during both the years and pooled years suggesting to adopt cautious approach while selecting for early genotypes. Negative association of pod yield/plant with total soluble solids (-0.151) and ascorbic acid (-0.153) was also observed during 2015–16 and pooled years. Earlier reports of many research workers have also indicated negative correlation for pod yield/plant with days to flowering and days to first picking (Rathi and Dhaka, 2007).

Correlation analysis indicates the association pattern of component traits with yield and simply represents the overall influence of a particular trait on yield rather than providing cause and effect relationship. Path coefficient analysis measures the direct influence of one variable upon the other, and permits separation of correlation coefficients into components of direct and indirect effects. Therefore, for assessing the cause–effect relationship as well as effective selection, path coefficient analysis is used. Pods/plant and average pod weight had maximum positive direct effect on fresh pod yield/plant during both the years and pooled years suggesting the importance of these traits in selection programme for improving yield (Table 4). Selvi *et al.* (2016) have also reported

Table 4. Estimates of path analysis at phenotypic level (P) for different pairs of horticultural traits in garden pea in 2014-15 (I), 2015-16 (II) and pooled years (III).

Trait		First flower node	Days to flowering	Days to first picking	Number of branches	Internodal length (cm)	Nodes /plant	Plant height (cm)	Pod length (cm)	Seeds / pod	Shelling (%)	Pods / plant	Average pod weight (g)	Total soluble solids (° brix)	Ascorbic acid (mg)	Pod yield /plant (g)
First flower node	I	0.046	-0.018	-0.010	0.004	0.000	-0.001	-0.002	0.000	-0.004	-0.013	-0.197	-0.033	-0.001	0.001	-0.228**
	II	-0.010	-0.003	0.009	0.002	0.000	-0.005	0.000	-0.001	-0.010	-0.002	-0.206	0.003	0.000	-0.001	-0.224**
	III	0.016	-0.003	-0.005	0.000	0.002	0.000	-0.001	-0.001	-0.009	-0.005	-0.170	-0.017	-0.001	0.000	-0.194**
Days to flowering	I	0.033	-0.026	-0.012	0.003	0.000	-0.001	-0.002	0.001	-0.006	-0.015	-0.268	-0.027	0.000	0.002	-0.316**
	II	-0.004	-0.008	0.012	0.001	0.000	-0.002	0.001	-0.003	-0.004	-0.001	-0.322	0.003	0.000	0.000	-0.325**
	III	0.008	-0.006	-0.006	0.000	0.003	0.000	0.001	-0.003	-0.007	-0.005	-0.297	-0.009	0.001	0.001	-0.317**
Days to first picking	I	0.019	-0.012	-0.024	0.002	0.000	-0.001	-0.002	0.001	-0.006	-0.015	-0.292	0.030	0.001	0.002	-0.298**
	II	-0.005	-0.005	0.019	0.002	0.000	-0.002	0.001	-0.004	-0.001	-0.003	-0.311	0.012	0.000	0.001	-0.297**
	III	0.007	-0.003	-0.012	0.000	0.002	0.000	0.000	-0.004	-0.008	-0.007	-0.248	0.021	0.001	0.001	-0.248**
Number of branches	I	0.007	-0.003	-0.002	0.027	0.000	-0.006	0.001	0.001	0.001	-0.008	0.041	0.044	0.001	0.002	0.105
	II	0.002	0.001	-0.003	-0.010	0.000	0.010	-0.001	0.001	0.003	-0.001	0.231	-0.005	0.000	0.000	0.228**
	III	0.000	0.000	0.000	-0.004	-0.001	0.007	-0.001	0.000	0.002	-0.003	0.166	0.017	0.000	0.001	0.185**
Internodal length (cm)	I	-0.013	0.007	0.003	-0.001	0.001	0.000	-0.007	0.002	0.004	0.010	0.223	0.127	0.001	-0.003	0.351**
	II	0.000	0.002	-0.004	-0.003	0.000	0.000	-0.003	0.001	0.003	0.004	0.132	0.089	0.000	-0.001	0.219**
	III	-0.003	0.001	0.002	0.000	-0.013	0.000	-0.006	-0.002	0.006	0.006	0.142	0.104	0.001	-0.002	0.235**
Nodes/plant	I	0.005	-0.002	-0.001	0.013	0.000	-0.014	0.002	0.001	0.002	-0.003	0.160	-0.040	0.002	0.000	0.123
	II	0.002	0.001	-0.002	-0.004	0.000	0.023	0.000	0.001	0.007	-0.002	0.276	-0.054	0.000	0.000	0.248**
	III	0.000	0.000	0.000	-0.002	0.000	0.017	0.001	0.000	0.005	-0.002	0.206	-0.044	0.002	0.000	0.183**
Plant height (cm)	I	0.005	-0.003	-0.003	-0.001	0.000	0.002	-0.016	0.001	-0.001	0.000	-0.047	0.099	0.000	-0.002	0.037
	II	0.000	0.002	-0.003	-0.003	0.000	0.001	-0.005	0.001	0.000	0.001	0.168	0.034	0.000	-0.001	0.195*
	III	0.001	0.001	0.000	0.000	-0.006	-0.001	-0.012	-0.001	-0.001	0.001	0.085	0.065	0.000	-0.001	0.129*
Pod length (cm)	I	0.003	-0.004	-0.004	0.003	0.000	-0.001	-0.003	0.007	0.002	-0.003	0.021	0.237	0.002	0.005	0.264**
	II	-0.001	-0.002	0.009	0.001	0.000	-0.002	0.000	-0.009	0.007	-0.002	-0.092	0.210	0.000	0.001	0.119
	III	0.002	-0.001	-0.003	0.000	-0.002	0.000	-0.001	-0.014	0.005	-0.003	-0.048	0.213	0.003	0.003	0.154**
Seeds/pod	I	-0.012	0.010	0.009	0.002	0.000	-0.002	0.001	0.001	0.015	0.022	0.431	0.072	0.001	-0.003	0.546**
	II	0.003	0.001	0.000	-0.001	0.000	0.005	0.000	-0.002	0.034	0.003	0.364	0.031	0.000	-0.001	0.436**
	III	-0.004	0.001	0.003	0.000	-0.002	0.003	0.000	-0.002	0.033	0.009	0.352	0.049	0.002	-0.002	0.441**
Shelling (%)	I	-0.011	0.007	0.006	-0.004	0.000	0.001	0.000	0.000	0.006	0.056	0.268	0.056	0.000	-0.005	0.379**
	II	0.002	0.001	-0.005	0.001	0.000	-0.003	-0.001	0.001	0.009	0.013	0.215	0.076	0.000	0.000	0.308**
	III	-0.003	0.001	0.003	0.000	-0.003	-0.001	-0.001	0.001	0.011	0.026	0.209	0.063	0.001	-0.002	0.306**
Pods/plant	I	-0.011	0.008	0.009	0.001	0.000	-0.003	0.001	0.000	0.008	0.019	0.814	0.016	0.001	0.000	0.863**
	II	0.002	0.003	-0.006	-0.002	0.000	0.007	-0.001	0.001	0.013	0.003	0.936	-0.061	0.000	-0.004	0.890**
	III	-0.003	0.002	0.003	-0.001	-0.002	0.004	-0.001	0.001	0.013	0.006	0.900	-0.035	0.002	-0.004	0.885**
Average pod weight (g)	I	-0.003	0.002	-0.002	0.003	0.000	0.001	-0.003	0.004	0.002	0.007	0.030	0.452	0.000	0.000	0.492**
	II	0.000	0.000	0.001	0.000	0.000	-0.003	0.000	-0.004	0.002	0.002	-0.128	0.448	0.000	0.000	0.317**
	III	-0.001	0.000	-0.001	0.000	-0.003	-0.002	-0.002	-0.007	0.004	0.004	-0.073	0.430	0.001	0.000	0.349**
Total soluble solids (° brix)	I	0.006	-0.001	0.002	-0.002	0.000	0.003	0.000	-0.002	-0.001	0.001	-0.043	0.004	-0.009	0.000	-0.044
	II	0.002	0.003	-0.003	0.000	0.000	-0.001	0.000	0.002	-0.008	-0.003	-0.223	-0.073	0.001	0.003	-0.302**
	III	0.001	0.001	0.001	0.000	0.001	-0.003	0.000	0.003	-0.005	-0.002	-0.113	-0.022	-0.014	0.001	-0.151**
Ascorbic acid (mg)	I	0.002	-0.002	-0.002	0.002	0.000	0.000	0.001	0.001	-0.002	-0.011	-0.013	0.001	0.000	0.026	0.003
	II	0.001	0.000	0.002	0.000	0.000	0.000	0.000	-0.001	-0.003	0.000	-0.250	0.006	0.000	0.014	-0.232**
	III	0.000	0.000	-0.001	0.000	0.001	0.000	0.001	-0.002	-0.004	-0.003	-0.167	0.003	-0.001	0.019	-0.153**

*P < .05, **P < .01; Unexplained variation (P) I: 0.0374 (P) II: 0.0084 (P) III: 0.0349

direct effect of these traits in different studies in different environments. The positive and significant association of different traits with pod yield/plant was mainly due to indirect effects via pods/plant and average pod weight over the years. Therefore, these traits provide an important criterion of selection procedures for achieving enhanced performance of genotypes for higher pod yield. Patel *et al.* (2006) and Katoch *et al.* (2016) also suggested pods/plant as most reliable component in breeding programme in pea for increased yield potential. The low magnitude of residual effects indicated that the traits included in the present investigation accounted for most of the variation present in the dependent variable. A more detailed study of the relationships obtained by path analysis revealed that relationship between yield and component traits are somewhat different from that

of simple correlation. In correlation studies, internodal length was the main trait related to yield increase while its manifestation was secondary as depicted from path analysis. The apparent divergence is due to the analytical approach since correlation simply identifies the mutual associations among the parameters while path analysis allows determination of the relative magnitude of each effect (Okuyama *et al*., 2004). This indicates that path coefficient analysis is a more efficient method than the correlation analysis (Dewey and Lu, 1959) when the objective is to establish relationships among the variables that affect yield. Thus, it can be concluded that pods/plant and average pod weight should be considered as selection criteria for yield improvement of garden pea.

Conclusion

On the basis of parameters of variability, it can be concluded that for pods/plant and pod yield/plant selection in early generations would be effective. Significant correlation of pod yield/plant was recorded with internodal length, seeds/pod, shelling (%), pods/plant and average pod weight. The positive association of these traits with pod yield/plant was mainly due to direct and indirect effects via pods/plant and average pod weight. Therefore, these traits provide an important criterion of selection procedures for achieving enhanced performance of genotypes for higher pod yield.

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RESEARCH ARTICLE

Assessment of Distinctiveness, Uniformity and Stability of Zinc Fortified Rice (*Oryza sativa*) Genotypes based on Morphological Descriptors

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Twenty five rice genotypes including indigenous and improved zinc lines were characterized using morphological descriptors adopted from the DUS guidelines of PPV & FR Authority and subsequently examined for their distinctiveness, uniformity and stability. Among the 49 assessed characters, 10 characters were monomorphic, 22 characters were dimorphic and 17 characters were polymorphic indicating their potential for varietal characterization and distinctiveness. No intra-varietal variation was observed for any of the visual characteristics and expression of characters in different varieties remained same for the two consecutive years confirming the uniformity and stability of the varieties for visual characteristics. The present study, highlights the need for and demands of zinc rich rice genotypes in Indira Gandhi Krishi Vishwavidyalaya, Raipur to conserve and protect high value traits for future promotion and utilization. On the basis of grouping characteristics unique morphological profiles could be established for all genotypes. Though, the morphological DUS descriptors establish distinctiveness of some varieties but varieties of similar background could not be discriminated hence some other markers/ descriptors could be thought of for complementing the morphological DUS descriptors for establishing distinctiveness.

Key Words: Biofortification, DUS descriptors, Rice, Zinc fortified lines

Introduction

Micronutrient malnutrition has emerged as one of the important global problems that afflicts billions of people particularly in developing countries. Since human body cannot synthesize micronutrients, it must be made available through diet. Rice (*Oryza sativa*), being the staple food for almost two thirds of the population, plays a pivotal role in Indian economy. Moreover, India ranks first in the world in area of rice cultivation with 42.96 million ha and second in production with 158.75 million tons (Faostat, 2016 http://www.fao.org/faostat/en/#data/QC).

Zinc deficiency is a major cause of stunting among children (Brown *et al.*, 2004). About 165 million children with stunted growth run a risk of compromised cognitive development and physical capability (Black *et al.*, 2013, Wessells *et al.,* 2012). Biofortification, the delivery of micronutrients via staple food crops, has been proposed to complement existing efforts for the alleviation of micronutrient deficiency (Bouis *et al.*, 2011).

Varietal improvement has been carried out towards Zn biofortification in rice. Some indigenous lines with high zinc concentration have been identified at Indira Gandhi Agriculture University, Raipur. There are a number of indigenous rice varieties currently under production whose identity and distinctiveness need to be established by various approaches. Morphological descriptors provide unique identification of cultivated varieties but they not only reflect the genetic constitution of the cultivar but also the environmental interaction within which it is expressed. The basic objective of varietal characterization is to test the occurrence of traits that help in identifying a particular variety. Keeping this in view, the study was taken up with the objective to determine the relative extent of distinctiveness, uniformity and stability of different morphological DUS descriptors in 25 zinc rich rice genotypes for their protection under the PPV&FR Act.

Materials and Methods

The experimental material consisted of twenty five rice genotypes (table 1). The trials were conducted at Rai Mohini Devi College of Agriculture and Research Station, Ambikapur during the *kharif* seasons of 2015 and 2016 in Randomized Block Design with three replications. Each

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Table 1. Name of genotypes along with zinc concentration in polished grain of 25 rice genotypes

S. No.	Genotypes	Zinc (µg/gm)	S. No	Genotypes	Zinc (µg/gm)
1	R-GM-AS-45	19.20	14	R-RHZ-MI-31	21.30
2	R-GM-AS-40	18.20	15	R-RHZ-R56	20.90
3	R-GM-AS-41	18.85	16	R-GM-AS-43	17.70
4	GP-145-70	17.50	17	R-RHZ-GI-56	17.55
5	R-GM-ATN-47	23.60	18	Kharigilas	23.90
6	GP-145-48	25.30	19	Jeeraphool	19.10
7	GP-145-66	18.50	20	Bisni-1	14.30
8	RKVY-77	23.35	21	IET-24780	15.00
9	R-HZ-SM-14	23.70	22	Chittimuthyalu	28.50
10	R-RHZ-IB-13	25.90	23	Kalanamak	21.70
11	R-RHZ-LI-22	22.25	24	Samba Mahsuri	10.10
12	R-RHZ-HI-11	21.10	25	IR-64	20.30
13	R-RHZ-LI-23	21.90			

replication consisted of three rows of 6 m length with 30 cm X 20 cm spacing. The observations were recorded on 49 of the 62 DUS characters at specified stages of crop growth period when characteristics under study had full expression (DRR, 2002). Of the 49 morphological characteristics studied, 40 were visually assessed and 9 were measure. Similarity matrix was generated using the SimQual programme of NTSYS-pc software version 2.02 (Rohlf, 1998). The Shan similarity coefficients were used for cluster analysis and dendrogram was constructed by Unweighted Pair-Group Method with Arithmetic Average (UPGMA) (Mathew et al., 2000). Field view and agromorphological characterization for some traits presented in Fig 2.

Results and Discussion

The various morphological traits recorded among rice genotypes are presented in Table 2. The basal leaf sheath colour for the rice genotypes varied from green to purple. Genotypes R-RHZ-GI-56, Jeeraphool, Bisni-1, ITE-24780, Chittimuthyalu, Kalanamak, Samba Mahsuri, IR-64 had green basal leaf sheath colour, while R-RHZ-GI-56 and Kharigilas showed purple while all remaining genotypes showed green colour for basal leaf sheath. The anthocyanin colouration of leaf was present in genotypes GP-145-48 and Kharigilas while this was absent in rest of the genotypes. The pubescence of leaf blade surface was found to be weak in GP-145-

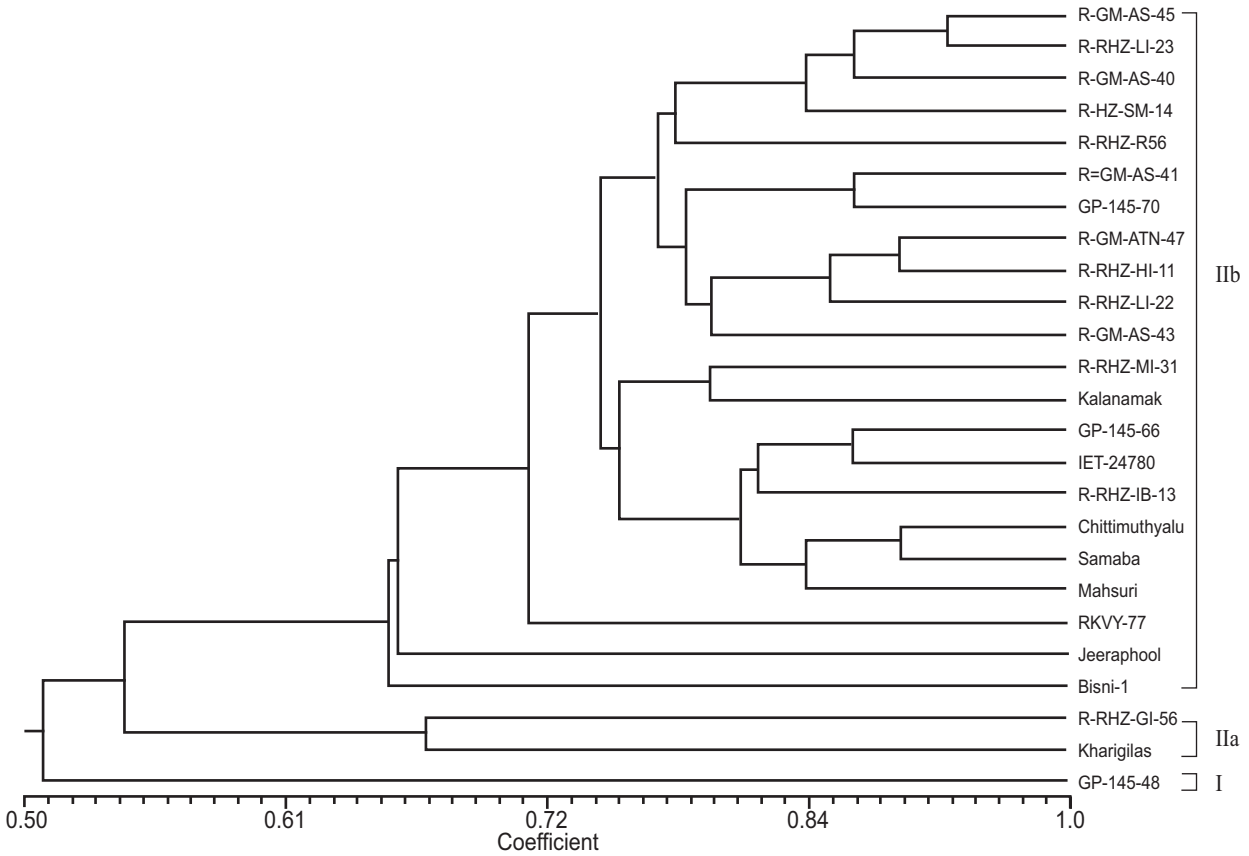

Fig 1. Dendrogram of rice genotypes based on morphological characters

Table 2. Morphological characters of rice genotypes based on DUS descriptor

S. No.	Characters	Genotypes																								
		A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y
1	Coleoptiles colour	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2	Basal leaf sheath colour	1	1	1	1	1	3	1	1	1	1	1	1	1	1	1	1	3	3	1	1	1	1	1	1	1
3	Leaf intensity of green colour	3	7	5	5	7	3	5	5	7	5	5	7	5	5	7	7	3	7	5	5	5	7	5	7	5
4	Leaf anthocyanin colouration	1	1	1	1	1	9	1	1	1	1	1	1	1	1	1	1	1	9	1	1	1	1	1	1	1
5	Leaf distribution of anthocyanin colouration	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
6	Leaf sheath anthocyanin colouration	1	1	1	1	1	9	1	1	1	1	1	1	1	1	1	1	9	9	9	1	1	1	1	1	1
7	Leaf sheath intensity of anthocyanin colouration	1	1	1	1	1	3	1	1	1	1	1	1	1	1	1	1	5	5	3	1	1	1	1	1	1
8	Pubescence of blade	5	5	5	5	5	3	5	3	5	5	3	3	5	5	3	3	5	3	3	3	3	3	3	3	3
9	Leaf auricles	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
10	Leaf auricles colouration	1	1	1	1	1	3	1	1	1	1	1	1	1	1	1	1	3	2	1	1	1	1	1	1	1
11	Leaf collar	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
12	Leaf anthocyanin colouration of collar	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	9	9	1	1	1	1	1	1	1
13	Leaf ligule	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
14	Leaf shape of ligule	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
15	Leaf colour of ligule	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	3	2	2	1	1	1	1	1	1
16	Leaf length of blade	5	5	7	5	5	5	5	7	5	5	5	5	5	5	5	5	5	7	7	5	5	5	5	5	7
17	Leaf width	3	5	3	5	5	7	5	5	3	5	3	5	3	3	3	5	3	3	5	5	5	5	3	3	5
18	Culm attitude	3	5	3	5	5	5	3	7	5	5	5	5	5	5	3	5	5	5	3	5	3	3	5	5	5
19	Time of heading (50%)	3	3	5	5	3	3	5	3	3	5	3	3	3	3	5	5	5	5	3	3	5	5	3	5	3
20	Flag leaf attitude of blade (early observation)	3	3	1	1	1	3	3	1	1	3	1	1	3	3	1	1	3	3	3	5	3	3	3	3	3
21	Spikelet density pubescence of lemma	5	3	5	1	3	1	5	5	5	1	3	3	5	3	5	5	5	7	1	1	1	5	3	3	5
22	Male sterility	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
23	Lemma anthocyanin coloration of keel	1	1	1	1	1	3	1	1	1	1	1	1	1	1	1	1	1	1	1	3	1	1	1	1	1
24	Lemma anthocyanin coloration of area below apex	1	1	1	1	1	5	1	1	1	1	1	1	1	1	1	1	1	1	1	5	1	1	1	1	1
25	Lemma anthocyanin coloration of apex	1	1	1	1	1	5	1	1	1	1	1	1	1	1	1	1	1	1	1	5	1	1	1	1	1
26	Spikelet colour of stigma	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	5	1	1	1	1	1	1	1
27	Stem thickness	3	3	3	3	3	5	3	5	3	3	5	5	5	5	3	5	5	3	3	3	3	5	5	3	3
28	Stem length excluding panicle	1	1	1	5	1	7	9	7	1	1	1	1	3	3	1	3	5	9	9	7	5	1	9	1	1
29	Stem anthocyanin coloration of node	1	1	1	9	9	9	9	9	1	1	1	1	1	1	1	9	9	9	9	9	9	9	9	9	9
30	Stem intensity anthocyanin colouration of nodes	3	3	3	3	5	3	5	5	3	3	3	3	3	3	3	5	5	5	5	3	3	3	5	3	5
31	Stem anthocyanin colouration of internode	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	9	9	1	1	1	1	1	1	1
32	Panicle length of main axis	5	9	5	5	5	5	7	5	7	5	5	5	5	5	5	5	5	5	5	5	7	9	5	5	5
33	Flag leaf attitude of blade (late observation)	5	5	3	3	3	3	3	3	5	3	3	5	5	1	1	5	5	5	5	5	3	3	3	3	3
34	Panicle curvature of main axis	5	3	3	1	3	3	5	1	5	3	3	3	5	1	5	3	3	5	3	5	5	3	3	5	5
35	Panicle per plant	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	5	5	3	3	3	3	3	3	3
36	Spikelet colour of tip lemma	4	4	4	4	3	5	6	3	5	3	4	3	4	3	4	4	3	6	3	3	3	3	6	3	3
37	Lemma and palea colour	6	6	7	7	2	9	7	7	6	6	6	7	6	7	6	6	7	9	7	7	7	7	7	7	7
38	Panicle awn	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
39	Panicle colour of awn (late observation)	4	4	4	4	4	2	9	8	2	2	4	4	4	4	2	2	8	2	2	2	2	2	9	2	2
40	Panicle length of longest awn	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
41	Panicle distribution of awn	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
42	Panicle presence of secondary branches	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
43	Secondary branching	2	2	2	2	2	1	2	2	2	2	2	2	2	2	2	2	2	2	2	1	2	2	2	2	2
44	Panicle attitude of branches	3	3	1	1	1	3	3	1	1	3	1	1	3	3	1	1	3	3	3	5	3	3	3	3	3
45	Panicle exertion	7	7	7	7	7	3	7	7	7	7	7	7	7	3	3	7	7	7	7	7	7	7	7	7	7
46	Time of maturity (days)	5	5	5	5	3	5	3	5	5	3	3	3	5	5	5	5	5	3	3	5	5	5	5	5	5
47	Leaf senescence	7	7	7	7	7	7	7	3	7	7	7	7	7	7	7	7	7	7	7	3	7	7	7	7	7
48	Sterile lemma colour	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
49	1000 grain weight	5	5	5	5	5	3	5	5	5	5	6	5	5	7	7	5	7	7	1	1	5	1	3	1	7

A- R-GM-AS-45, B- R-GM-AS-40, C- R-GM-AS-41, D - GP-145-70, E- R-GM-ATN-47, F- GP-145-48, G- GP-145-66, H- RKVY-77, I- R-HZ-SM-14, J- R-RHZ-IB-13, K- R-RHZ-LI-22, L- R-RHZ-HI-11, M- R-RHZ-LI-23, N- R-RHZ-MI-31, O- R-RHZ-R56, P- R-GM-AS-43, Q- R-RHZ-GI-56, R- Kharigilas, S- Jeeraphool, T- Bisni-1, U- IET-24780, V- Chittimuthyalu, W- Kalanamak, X- Samba Mahsuri, Y- IR-64

Fig.2. Field view along with Morphological observations for different characters

48, RKVY-77, R-RHZ-LI-22, RHZ-HI-11, R-RHZ-R-56, R-GM-AS-43, Jeeraphool, Bisni-1, ITE-24780, Chittimuthyalu, Kalanamak, Samba Mahsuri, IR-64, medium in R-GM-AS-45, R-GM-AS-40, RGM-AS-4, GP-145-70, R-GM-ATN-47, GP-145-66, R-HZ-SM-14, RHZ-IB-13, R-RH Z-II 23, R-RHZ-MI-31, R-RHZ-GI-56, Kharigilas. GP-145-48 and R-RHZ-GI-56 had purple auricles colour Kharigilas light purple and rest of genotypes were colourless. Anthocyanin colouration of collar was found present in genotypes R-RHZ-GI-56 and Kharigilas, while absent in rest of the genotypes. Colour of ligule was light purple in Kharigilas and Jeeraphool while purple in R-RHZ-GI-56 and white in rest of the genotypes. The leaf length of blade was noted medium for R-GM-AS-45, R-GM-AS-40, GP-145-70, R-GM-ATN-47, GP-145-48, GP-145-66, R-HZ-SM-14, RHZ-IB-13, R-RHZ-LI-22, R-RHZ-HI-11, R-RH Z-II 23, R-RHZ-MI-31, R-RHZ-R-56, R-GM-AS-43, R-RHZ-GI-56, Bisni-1, ITE-24780, Chittimuthyalu, Kalanamak and Samba Mahsuri, long for RGM-AS-41, RKVY-77, Kharigilas and Jeeraphool. The leaf blade was medium R-GM-AS-45, RGM-AS-41, R-HZ-SM-14, R-RHZ-LI-22, R-RH Z-II 23, R-RHZ-MI-31, R-RHZ-R-56, R-RHZ-GI-56, Kharigilas, Kalanamak, Samba Mahsuri and broad in rest of the genotypes. The early observation for Culm attitude revealed semi erectness in R-GM-AS-45, RGM-AS-41, GP-145-66, R-RHZ-R-56, Jeeraphool, ITE-24780, Chittimuthyalu, Samba Mahsuri and IR-64, open attitude in genotypes R-GM-AS-40, GP-145-70, R-GM-ATN-47, GP-145-66, R-HZ-SM-14, RHZ-IB-13, R-RHZ-LI-22, R-RHZ-HI-11, R-RH Z-II 23, R-RHZ-MI-31, R-GM-AS-43, R-RHZ-GI-56, Karigilas, Bisni-1 and Kalanamak, and spreading type attitude present in genotypes RKVY-77. The stigma colour was found to be light green in R-RHZ-II-23, and purple in Kharigilas while white in rest of the genotypes. The lemma anthocyanin colouration of keel found to be weak in genotype GP-145-48 and Bisni-1, while absent/ very weak in all genotypes. The stem anthocyanin colouration of internode present in R-RHZ-GI-56 and Kharigilas. The panicle length in main axis measured long panicle length in three genotypes viz., GP-145-66, R-HZ-SM-14 and Bisni-1, and very long panicle present in two genotypes R-GM-AS-40 and ITE-24780. The spikelet colour of tip lemma appeared brown in twelve genotypes R-GM-ATN-47, RKVY-77, RHZ-IB-13, R-RHZ-HI-11, R-RHZ-MI-31, R-RHZ-GI-56, Jeeraphool, Bisni-1, ITE-24780, Chittimuthyalu, Samba Mahsuri, and IR-64, red for R-GM-AS-45, R-GM-AS-40, RGM-AS-41, GP-145-70, R-RHZ-LI-22, R-RH Z-II 23, R-RHZ-R-56 and R-GM-AS-43, purple colour GP-145-48 and R-HZ-SM -14, and black in genotypes GP-145-66, Kharigilas and Kalanamak. The panicle were partially exerted in four genotypes GP-145-48, R-RHZ-MI-31 and R-RHZ-R-56,

and well exerted in rest of the genotypes. Based on 1000 grain weight Jeeraphool, ITE-24780, Chittimuthyalu and Samba Mahsuri had very low test weight while, GP-145-48, R-RHZ-GI-56, Chittimuthyalu, and Kalanamak had low test weight, R-GM-AS-45, R-GM-AS-40, RGM-AS-41, GP-145-70, R-GM-ATN-47, GP-145-66, RKVY-77, R-HZ-SM-14, RHZ-IB-13, R-RHZ-LI-22, R-RHZ-HI-11, R-RH Z-II 23 and R-RHZ-MI-31 had medium test weight. Similar attempts for establishment of distinctiveness have also been made in aromatic rice genotypes (Joshi *et al.*, 2011), released rice varieties (Tiwari *et al.*, 2013) and in clustered rice germplasm (Sahu *et al.*, 2014).

UPGMA Cluster Analysis

The dendrogram generated using UPGMA cluster analysis grouped studied rice genotypes into two major clusters (cluster I and II) Pairwise. Jaccard's similarity coefficient between genotypes ranged from 0.51 to 0.92. The analysis revealed wide genetic variability among the genotypes. Cluster I consisted of only one genotype GP-145-48 (25.30 µg/gm zinc) which indicates their diversity in the rice gene pool. Cluster II was further divided into 2 sub clusters and only 54% similarity were observed. Sub cluster-1, included two genotypes R-RHZ-GI-56 (20.90 µg/gm zinc) and Kharigilas (23.90 µg/gm zinc) with a genetic similarity of more than 67 %. Sub cluster-2 consisted of rest of the genotypes with a genetic similarity ranging from 65 to 92 %. A close association among the genotypes in particular clusters is largely due to common pedigree involved in breeding of the genotypes. The morphological dendrogram generated from similarity or genetic distance matrices has provided an overall pattern of variation as well as the degree of relatedness among genotypes (Tiwari *et al.*, 2013). Early maturing varieties viz, Rasi and Tulasi with a common pedigree in their breeding grouped in a sub cluster with a similarity index of more than 67 % (Ravidra Babu *et al.*, 2015). Similarly, the study on genetic diversity of Venezuelan cultivars by Herrera *et al.* (2008) and diversity of Brazilian rice cultivars by Raimondi *et al.* (2014), demonstrated the narrow genetic base of the cultivars developed in their respective countries which can lead to genetic vulnerability to any emerging stress conditions.

Zinc deficiency is probably the most widespread micronutrient deficiency in cereals. Breeding for enhancing bio-available Zinc in edible portion through increasing the concentration of metal binding protein has been needed. But for initiating any breeding programme, morphological characterization should be the first step along with screening variability for target trait before more profound biochemical or molecular studies are carried out. As a prerequisite for efficient utilization of the germplasm, it must be properly evaluated, characterized and documented so that it could be easily retrieved and used in breeding programme (Elangovan *et al.*, 2013). The present study revealed sufficient genetic variability for most of the trait observed. Zinc rich rice genotypes identified with distinct variation in this study may be used in breeding programme for introgression of zinc content gene or QTLs in the improved varieties. Notably, there were appropriate differences in zinc content suggesting the existence of genetic potential to increase the concentration of zinc in rice grain. Identified cultivar with distinct morphological variation Chittimuthyalu (28.50 µg/gm zinc), R-RHZ-IB-13 (25.90 µg/gm zinc), GP-145-48 (25.30 µg/gm zinc) and R-RHZ-SM-14 (23.70 µg/gm zinc) may be used as zinc donor in future. These unique accessions bearing special trait of interest can be further investigated to understand its inheritance pattern. The morphological dendrogram generated for similarity or genetic distance matrices has provided an overall pattern of variation as well as the degree of relatedness among rice accessions also found by Tiwari *et al.* (2013). In the era of IPR issues, characterization of germplasm and varieties assumes greater importance. The application of morphological markers is the simplest of the methods that could be repeatedly done and effective used for IPR issues (Lyngdoh *et al.*, 2007). However, morphological descriptors alone may not be sufficient for DUS criteria. Hence, some other markers/ descriptors based on DNA (Simple Sequence Repeat) and protein (SDS-PAGE) markers could be considered for complementing the morphological DUS descriptors.

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RESEARCH ARTICLE

Profiling of Groundnut (*Arachis hypogaea* L.) Genotypes for Seed Quality Traits

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The study analyzed seed quality traits in 58 groundnut germplasm accessions of elite breeding material obtained from ICRISAT, Hyderabad along with three checks in 2016-17. A wide variation was obtained in case of hundred kernel weight (HKW), fatty acids and total soluble sugars (TSS). Some of the lines viz. ICGV15001, ICGV15003, ICGV15004, ICGV15036, ICGV15037, ICGV15039, ICGV15041, ICGV15042 and ICGV15050 had oleic acid above 80% which also contributes to high oleic to linoleic acid ratio (O/L ratio) that imparts longer shelf life to the oil. Palmitic and linoleic acid was positively correlated with oil, however, negatively correlated with oleic (r= -0.339*) and O/L ratio (r = -0.335*) which indicates their antagonistic nature. Cluster analysis based on the studied traits, divided 61 genotypes into two main clusters. The differentiation in clusters is based on oleic acid content which was above 80% in lines falling under cluster I and 37-68% in lines under cluster II. The study helped in the identification of lines viz, ICGV15003, ICGV15039 and ICGV15050 with high O/L ratio and comparable oil and TSS content to checks. These genotypes may further be used for studying genetics of quality traits in groundnut breeding programmes.

Key Words: Breeding, Fatty acids, Oil, O/L ratio, Total soluble sugars

Introduction

Groundnut is an important oilseed crop that stands next to soybean in acreage and production in India. The area, production and productivity of groundnut in India during 2015-16 were 5.8 mha, 6.8 mt and 1.18 tonnes/ha respectively (FAO, 2016). It accounts for 45% of the area, 55% of the production of total oilseeds in the country and 43% of total oil production in the country (Aher, 2014). In fact, it plays a significant role in supporting the oilseed economy of India. Almost every part of the crop is useful. Oil is used for cooking purpose, in preparations of soaps, ointments, medicated emulsions, fuel, cosmetics, leather dressings, furniture cream and lubricants, etc; whole kernels for table purpose snacks; cake as a feed for animals and poultry, whereas, shells for making particle boards or as a fuel or filler in cattle feed (Janila *et al.*, 2013). On an average, globally, 50% of total groundnut production is used for oil purpose, 37% for use in confectionery and 12% as seed (Taru *et al.*, 2010). The projected demand of groundnut in Asia alone by 2020 is expected to move 1.6 times higher than its production level in 2000 (Sai *et al.*, 2016). In order to meet this demand, there is need to increase the production of the crop at a much faster rate than the existing one.

Groundnut is a nutritious source of fats, protein, carbohydrates, vitamins and minerals. Groundnut seeds contain 40-50% fats, 20-28% protein and 10-20 % carbohydrates (Belamkar *et al.*, 2011) and also rich source of vitamin E and other minerals for human health including niacin, calcium, phosphorus, magnesium, zinc, iron, riboflavin, thiamine and potassium. High nutritional profile of groundnut has encouraged its consumption and thus, processing in many forms (Mattes *et al.*, 2008). Groundnut and its products may be promoted as nutritional foods to fight energy, protein, and micronutrient malnutrition among the poor.

Since about 50% of total groundnut production in India is used for oil extraction, improvement in oil content and its quality is of major interest to plant breeders and millers. Oil quality of a crop is determined by its fatty acid profile. In groundnut, there are mainly eight fatty acids viz, palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), arachidic (20:0), eicosenoic (20:1), behenic (22:0) and lignoceric (24:0). Among them, oleic acid (18:1), a monounsaturated fatty acid (MUFA) and linoleic acid, a polyunsaturated fatty acid (PUFA) together accounts for 75-80 % and remaining 20% is contributed by rest of the fatty acids, among which palmitic acid (10%) shares the largest proportion

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(Orsavova *et al.*, 2015). As a source of edible oil, the important quality requirements of groundnut are high oil content in seed, high oleic acid and high oleic/ linoleic acid (O/L) ratio. The O/L ratio determines the shelf life of the seed, the higher the ratio the longer the shelf life (Braddock *et al.*, 1995). Oleic acid also lowers the bad cholesterol (LDL) (Kris-Etherton *et al.*, 2001) which is desirable for healthy heart. Higher proportion of oleic acid in oil leads to high smoking point, faster cooking and less absorption of oil (Miller *et al.*, 1987). Groundnut oil is relatively more stable and has longer shelf life than safflower and sunflower oil, which have higher content of PUFAs. Moreover, due to increasing health awareness among the consumers and for decreased tendency towards rancidity of the products imparted by high O/L ratio, the groundnut varieties with low oil content, high O/L ratio, high protein and sugar content are being preferred for confectionery purpose (Dwivedi and Nigam, 1995). Sweetness of the groundnut is a heritable trait (Pattee *et al.*, 2000). It has been also observed that sweetness in groundnut is mainly due to the presence of large amount of free sucrose (Mason *et al.*, 1969). In comparison to abundant information on groundnut protein, oil and fatty acid contents, there are only few reports related on sugars.

The assured irrigation facilities and strong marketing policies have led to expansion in area under rice and wheat; leaving less area under other crops particularly oilseeds and pulses. The availability of other cheap oils like palm oil in India led to severe decline in area under available edible oils like mustard and groundnut. However, rich nutritional profile and mounting demand for table purpose groundnut seeks imperative steps for the revival of this crop. Development of high yielding groundnut varieties coupled with improved quality traits may help in meeting the raising nutritional demands. The present study was meant to evaluate 58 groundnut lines received from ICRISAT, Hyderabad along with three checks for confectionery traits viz., hundred kernel weight (HKW), oil content, fatty acid profile and total soluble sugar (TSS) content so as to identify donor lines for utilization by plant breeders, nutritionists and health advisors to make best use of it.

Materials and Methods

Seed samples of 61 groundnut genotypes were procured from ICRISAT, Hyderabad along with three checks viz, SG99, M522, TG 37A and were sown in *kharif* season during 2016-17. The germplasm was evaluated in alpha design having two replications along with three checks (SG99, M522, TG 37A) and selection of germplasm was based on confectionery traits having high sugar contents, bold seeded and low fatty acids. At maturity seed samples were evaluated for HKW, oil, fatty acid profile, and TSS content. The groundnut seed samples were analyzed for the oil content using Nuclear Magnetic Resonance (MQC23, Oxford Instruments, UK) which is a non-destructive, quick, easy to perform and simple to calibrate. Standard samples containing 5 g seeds of groundnut with known oil content were set to calibrate the instrument. The fatty acid composition was determined by gas chromatography of fatty acid methyl esters. These were prepared as per the procedure developed by (Appelqvist, 1968) and analyzed on Agilent technologies Gas Chromatography model 7820A series equipped with flame ionization detector with CP-Sil 88 (25m × 0.25mm × 0.20mm) FAME column. Temperatures of oven, detector, and injector were maintained at 210, 240, and 230°C, respectively. Two µl of sample was injected at a split rate of 10:1. Individual fatty acids were expressed as percentages of the total fatty acids. The TSS extraction was performed according to an adapted methodology from Garcia *et al.* (2006), using three replicates with 0.2 g of grounded defatted meal and estimation was done colorimetrically by phenol-sulphuric method (Dubois *et al.*, 1956), using glucose (100 µg ml-1) as a standard.

The range, mean, standard error of mean, coefficient of variability for different characters and simple correlation coefficients among quality characters were worked out following standard statistical methods. Cluster analysis was carried out based on HKW and eleven seed quality traits using neighbor-joining method of Paleontological statistics software package for education and data analysis (PAST).

Results and Discussion

The range of variation, mean, standard deviation and coefficient of variation (CV) of HKW, oil, fatty acid composition, O/L ratio and TSS content in 61 germplasm accessions are listed in Table 1. The HKW covers a wide range of 26.00 (ICGV15050) to 102.60 g (ICGV06229). Oil content exhibited low variability and ranged from 48.06% in ICGV15050, ICGV15054 genotypes to 51.04% in ICGV05174 genotype. Sixteen genotypes namely, ICGV05174, ICGV05182,

Table 1. Range, mean and coefficients of variation (CV) for hundred kernel weight (HKW) and different quality parameters in 61 groundnut genotypes

Quality traits	Range	Mean±SE	CV (%)
HKW (g)	26.00-102.60	63.70±2.44	5.42
Oil content (%)	48.06-51.04	49.50±0.62	1.78
Palmitic 16:0 (%)	6.73-14.29	10.69±0.25	3.31
Stearic 18:0 (%)	0.98-4.59	2.01±0.09	6.32
Oleic 18:1 (%)	37.39-84.71	58.90±1.64	3.94
Linoleic 18:2 (%)	1.52-38.17	22.60±1.34	8.38
Arachidic 20:0 (%)	0.74-2.33	1.36±0.04	4.16
Eicosenoic 20:1 (%)	0.61-3.59	1.23±0.07	8.05
Behenic 22:0 (%)	0.52-3.89	2.29±0.08	4.94
Lignoceric 24:0 (%)	0.21-1.9	1.06±0.04	5.35
Oleic/Linoleic ratio	0.97-55.67	7.84±1.77	31.88
Total soluble sugars(%)	4.37-7.22	5.94±0.09	21.49

ICGV05198, ICGV06188, ICGV06216, ICGV06188, TG-51, ICGV199, TG-26, Gangapuri, ICGV06217, ICGV06222, ICGV06214, ICGV86504, ICGV06216, TG37A (check) out of 61 showed more than 50% of oil content (Table 2). Such a high content of oil may affect the flavour and shelf life. For commercial production, the oil content in the groundnut cultivars is generally around 50%, while some germplasm accessions have been reported to contain more than 55% oil (Liao and Holbrook, 2005).The results are within the range of oil content obtained in other studies (Wang *et al.*, 2009; Shokunbi *et al.*, 2012; Chowdhury *et al.*, 2015, Rosalva *et al.*, 2015).

Wide variation was observed in the fatty acid profile of the genotypes under study. Oleic (18:1) and linoleic (18:2) acid together constitute 75-86%, palmitic (16:0) upto 14% and rest of the fatty acids viz, stearic (18:0), arachidic (20:0), eicosenoic (20:1), behenic (22:0), lignoceric (24:0) though in smaller amounts, together constitute upto 7% (Table 2). A similar observation was made by Anderson *et al.* (1998) on comparison of fatty acid profile of six high oleic acid peanut genotypes. The range of fatty acids obtained in the present study was also supported by the observations made by Campos-Mondragón *et al.* (2009) and Chowdhury *et al.* (2015). In recent years, focus has been laid on MUFAs which contains only one double bond which imparts susceptibility towards oxidation and thus prevents tissues from oxidative damage. MUFAs (oleic acid, ω9) are highest in olive oil (67.0%) followed by canola oil (26.9-67.6%) and groundnut oil (43.1%). From health point of view, the presence of oleic acid in oil is considered very useful as it is effective in reducing blood pressure (Teres *et al.*, 2008) and bad cholesterol (LDL) level (O'Byrne *et al.*, 1997). Surprisingly, nine of the genotypes viz. ICGV15001, ICGV15003, ICGV15004, ICGV15036, ICGV15037, ICGV15039, ICGV15041, ICGV15042 and ICGV15050 in the studied material were observed to have oleic acid more than 80% as compared to M-522 (check) (62.80%) which also enhances the O/L ratio upto 55%. Remaining genotypes had O/L ratio varying from 0.97-5.02.Thus, a high O/L ratio (≥10:1) in peanut results in an increased shelf life (upto 10 times) and improved flavor when compared to a normal O/L ratio (~1.5:1) (Isleib *et al.*, 2006; Chamberlin *et al.*, 2014). In USA, Norden *et al.* (1987) for the first time identified groundnut lines F435-2--1 and F435-2--2 with O/L ratio of nearly 40. Since then several high oleic acid groundnut cultivars have been released (Kassa *et al.*, 2009). There has been an increasing demand for high oleic peanuts in the peanut industries and hence focus is laid on the development of varieties with high oleic content. Apart from these nine genotypes, in rest of the genotypes, range of linoleic acid was observed from 13.67-38.17%. The results were in agreement to different studies (Dean *et al.*, 2009; Hassan and Ahmed, 2012). Linoleic acid (18:2) is considered as one of the most important PUFA in human food as it prevents heart vascular diseases (Boelhouwer, 1983).

TSS content is another important trait of groundnut seeds. Higher sweetness is more in demand among the consumers. Of all the genotypes tested from germplasm collection, 31 genotypes showed less than 6% TSS, and remaining 30 accessions showed >6%. Two of the genotypes, namely, Gangapuri and ICGV06216 were found to have TSS above 7%. Gocho (1992) observed around 9% of sucrose content in groundnut seeds. Luo *et al.* (2004) analyzed 13 groundnut cultivars, mainly of Spanish type, and found the sucrose content varied from 5.1 to 5.9%.Wang *et al.* (2007) identified groundnut genotypes with 6.1% sucrose content. Sugar content was higher in groundnut grown in Argentina than that from USA and China (Bett *et al.*, 1994).

Breeding for better quality necessitates deep understanding of relationships among quality traits. Fatty acid biosynthetic pathway involves modifications such as elongation and desaturation. Hence, any modification in the pathway influences the whole fatty acids profile. Present correlation studies revealed significant and positive association of oil with palmitic acid (r = 0.286*) and linoleic acid (r=0.365**), however, negative

Table 2. Hundred kernel weight (HKW) and quality attributes of groundnut genotypes

Genotypes	HKW (g)	Oil (%)	Fatty acid (%)								Total soluble sugars (%)	Stability index (Oleic : Linoleic ratio)
			Palmitic (16:0)	Stearic (18:0)	Oleic (18:1)	Linoleic (18:2)	Arachidic (20:0)	Eicosenoic (20:1)	Behenic (22:0)	Lignoceric (24:0)		
ICGV15001	39.60	49.23	7.87	1.86	83.81	2.07	0.98	1.89	1.93	0.59	4.90	40.48
ICGV15003	41.10	48.80	7.48	1.67	82.49	2.73	1.00	2.11	0.87	0.90	6.59	30.21
ICGV15004	34.80	49.79	7.03	1.78	82.05	2.36	1.08	2.30	0.90	1.28	5.42	34.76
ICGV15036	43.30	49.01	6.73	2.33	82.37	3.03	0.87	2.59	1.67	1.04	5.73	27.18
ICGV15037	41.30	49.02	7.20	1.85	83.97	2.21	1.13	1.58	1.90	1.10	4.84	37.99
ICGV15039	44.70	48.95	6.95	1.51	84.38	1.58	1.25	1.76	2.10	0.80	6.79	53.40
ICGV15041	36.40	48.75	7.72	1.32	84.71	2.11	1.00	1.80	1.52	1.13	5.88	40.14
ICGV15042	41.30	49.21	7.57	1.60	82.29	2.19	1.43	1.98	2.17	0.76	5.37	37.57
ICGV15050	26.00	48.06	8.24	1.21	84.62	1.52	1.30	1.30	2.05	0.97	6.22	55.67
ICGV15054	42.40	48.06	10.39	1.25	56.77	24.49	0.87	2.59	2.65	1.01	5.82	2.31
ICGV15170	92.90	49.83	10.89	1.38	52.13	28.67	1.10	2.11	1.80	1.21	6.58	1.81
ICGV05174	57.10	51.04	11.36	0.98	55.93	27.63	0.97	0.95	1.71	0.48	4.79	2.02
ICGV05182	45.20	50.00	12.30	2.55	56.55	24.16	1.26	1.03	2.09	0.21	5.82	2.34
ICGV05184	47.40	49.52	8.26	1.67	68.73	14.28	1.12	3.59	1.67	0.66	5.39	4.81
ICGV05193	73.20	49.83	12.00	1.10	53.90	28.90	1.00	0.82	1.65	0.60	5.29	1.86
ICGV05198	69.60	50.06	12.50	1.40	55.80	26.90	0.74	1.16	2.03	0.77	6.92	2.07
ICGV05200	88.00	49.00	12.96	1.06	55.10	24.53	1.06	2.13	0.52	0.86	4.97	2.24
ICGV06188	70.70	50.37	10.59	1.71	63.74	20.16	0.93	1.26	2.15	1.16	6.56	3.16
ICGV06189	76.00	49.56	11.82	1.83	59.01	24.23	1.04	0.96	0.94	1.00	5.61	2.43
ICGV06233	51.40	49.83	11.64	1.23	55.50	26.78	1.18	0.83	2.19	0.88	6.52	2.07
ICGV06227	89.50	49.20	10.63	2.50	62.26	22.75	1.19	0.75	1.83	0.60	5.65	2.73
ICGV06211	72.10	49.37	11.10	1.04	63.11	19.87	1.03	0.78	1.96	1.10	6.73	3.17
ICGV06214	47.50	49.75	11.44	1.95	55.41	25.64	1.13	1.03	2.29	1.12	5.42	2.16
ICGV06216	75.60	50.91	11.32	1.26	53.59	29.28	1.03	0.82	1.92	0.78	5.32	1.83
ICGV06229	102.60	48.90	10.08	2.52	62.66	20.15	1.15	0.81	1.90	0.74	6.37	3.10
ICGV06212	80.40	48.94	10.55	2.45	58.83	21.29	1.50	1.00	2.91	1.47	5.28	2.76
ICGV07214	68.80	49.88	12.25	1.80	43.38	36.59	1.12	1.14	2.47	1.24	6.09	1.18
ICGV06760	94.90	48.97	10.23	2.97	55.78	24.42	1.73	0.98	2.78	1.12	5.17	2.28
PM-1	42.00	49.04	14.20	1.81	40.86	36.68	1.32	1.19	2.69	1.24	5.96	1.11
ICGV07359	65.60	48.45	12.35	1.62	49.12	30.32	1.36	1.14	2.68	1.40	6.58	1.62
ICGV06201	68.80	49.04	11.18	1.89	59.28	21.57	1.68	0.98	2.43	0.99	6.67	2.74
ICGV06188	77.80	50.49	10.47	1.10	59.45	21.01	1.25	1.60	3.22	1.90	5.74	2.82
ICGV07758	88.00	48.34	11.00	1.66	56.22	25.04	1.50	0.98	2.59	1.01	4.37	2.24
ICGV91114	44.20	49.78	13.23	2.79	41.38	34.63	1.68	1.04	3.89	1.36	5.24	1.19
ICGV07210	65.90	49.19	13.67	2.51	37.39	38.17	1.92	1.10	3.87	1.37	5.21	0.97
ICGV06227	84.80	48.51	9.65	1.98	66.04	16.82	1.65	0.93	2.14	0.79	6.68	3.92
ICGV07363	97.90	48.70	8.76	1.90	68.63	13.67	1.98	0.97	2.93	1.16	6.70	5.02
ICGV01114	44.80	48.59	13.35	2.20	45.10	33.72	1.42	0.74	2.39	1.08	5.02	1.33
ICGV07768	82.20	49.99	8.46	1.40	66.39	16.83	1.60	1.08	2.85	1.38	5.81	3.94
TG-51	63.60	50.61	12.64	2.45	45.71	34.37	1.04	1.52	1.52	0.75	5.11	1.32
TPG-41	76.70	49.03	10.68	2.91	56.53	23.55	1.59	1.02	2.64	1.08	6.92	2.40
ICGV07362	82.10	49.21	8.94	3.04	63.73	16.39	1.96	1.17	3.39	1.39	6.33	3.88
ICGV95070	66.00	49.24	8.73	1.53	68.02	15.56	1.31	1.29	2.18	1.38	6.69	4.37
ICGV00199	51.10	50.47	10.69	2.66	53.10	26.77	1.51	1.13	2.72	1.42	5.98	1.98
ICGV06211	83.70	49.05	11.41	1.40	50.47	30.49	1.54	0.87	2.76	1.07	6.81	1.65
TG-26	48.90	50.26	14.29	2.21	41.21	37.59	1.35	0.64	1.84	0.88	6.93	1.09
Gangapuri	43.90	50.52	11.38	2.66	45.55	33.50	1.77	0.87	2.94	1.34	7.22	1.35
PM-2	42.00	49.96	14.14	2.96	41.32	36.05	1.66	0.61	2.44	0.83	6.18	1.14
ICGV06217	75.00	50.66	9.63	1.94	61.46	20.82	1.70	0.83	2.41	1.22	5.39	2.95
ICGV06222	76.10	50.00	12.09	1.93	46.36	31.67	1.61	1.08	3.69	1.56	6.36	1.46

Contd.

Genotypes	HKW (g)	Oil (%)	Fatty acid (%)								Total soluble sugars (%)	Stability index (Oleic : Linoleic ratio)
			Palmitic (16:0)	Stearic (18:0)	Oleic (18:1)	Linoleic (18:2)	Arachidic (20:0)	Eicosenoic (20:1)	Behenic (22:0)	Lignoceric (24:0)		
ICGV06214	58.70	50.49	9.43	2.41	61.26	20.82	2.33	0.65	2.83	1.26	6.17	2.94
ICGV06229	93.30	49.14	10.03	1.82	60.21	21.16	1.98	0.75	2.97	1.07	6.14	2.84
ICGV86504	76.70	50.04	11.12	2.29	59.26	21.69	1.33	0.93	2.29	1.09	6.33	2.73
ICGV06233	67.40	48.82	11.12	1.80	50.57	31.08	1.18	1.13	2.05	1.06	6.42	1.62
ICGV06216	72.20	50.60	10.70	1.88	54.17	28.04	1.18	0.84	2.27	0.91	7.16	1.93
VRI-3	36.70	49.99	12.68	4.59	41.99	34.30	1.86	0.86	2.74	0.99	6.19	1.22
ICGV06234	76.10	49.72	11.36	1.74	51.97	28.82	1.31	1.10	2.71	0.98	5.32	1.80
ICGV07217	75.00	48.91	12.25	2.55	48.58	31.02	1.49	1.09	2.69	1.33	4.83	1.56
SG-99(C)	53.40	48.99	9.88	3.43	58.41	21.87	1.78	0.94	2.34	1.36	6.14	2.67
TG37A(C)	45.80	50.60	13.86	1.98	41.53	36.50	1.35	0.87	2.37	1.54	5.04	1.13
M-522(C)	65.80	49.52	9.65	4.02	62.80	18.13	1.58	1.06	1.70	1.05	6.74	3.46

Table 3. Correlation coefficients among traits of 61 groundnut germplasm accessions

Traits	HKW (g)	Oil (%)	Palmitic (%)	Stearic (%)	Oleic (%)	Linoleic (%)	Arachdic (%)	Eicosenoic (%)	Behenic (%)	Lignoceric (%)	TSS (%)
Oil (%)	-0.03										
Palmitic (%)	0.11	0.29*									
Stearic (%)	-0.07	0.04	0.15								
Oleic (%)	-0.20	-0.34**	-0.93	-0.27*							
Linoleic (%)	0.22*	0.36**	0.93	0.21	-0.99						
Arachdic (%)	0.19	-0.04	0.08	0.55	-0.26*	0.19					
Eicosenoic (%)	-0.32**	-0.24	-0.51	-0.28**	0.50	-0.52	-0.45				
Behenic (%)	0.16	0.02	0.26*	0.29*	-0.43	0.38**	0.66	-0.38**			
Lignoceric (%)	0.10	0.02	0.04	0.11	-0.20	0.15	0.39**	-0.09	0.54		
TSS (%)	0.09	-0.001	-0.06	0.08	0.005	-0.002	0.14	-0.18	0.07	0.07	
O/L ratio	-0.52	-0.33**	-0.70	-0.23	0.82	-0.83	-0.26	0.46	-0.34	-0.17	-0.07

HKW: Hundred kernel weight; TSS: Total soluble sugars
* = Significant at 0.05 level; ** = Significant at 0.01 level.

correlation with oleic acid (r = – 0.339*) and O/L ratio (r = – 0.335**). The relationship of saturated fatty acids (palmitic and stearic) was positive and significant with behenic acid (r = 0.258*; r=0.287*) while negative with oleic and eiocosenoic. Linoleic showed positive significant association with behenic acid. O/L ratio showed negative associations with all except oleic and eicosenoic. The findings of the present investigation, in general, were in agreement to the earlier reports (Sarvamangala *et al.*, 2011; Önemli 2012; Azharudheen and Gowda, 2013). A negative relationship between stearic and oleic acid (-0.275*) indicates that one cannot increase much without decrease in other as both are formed from the same source, palmitic acid. Also, the negative association between the two indicates that increased oleic acid results in reduced palmitic and stearic acid which is desirable from health and stability point of view. Among the germplasm accessions, combining several favourable traits i.e. lines with exceptionally high oleic acid (>80%), high O/L ratio (27.18 to 55.67), lowest palmitic (6.73 to 8.24%) could be used in future groundnut quality breeding programmes.

Cluster analysis based on HKW and eleven seed quality traits divided 61 accessions of groundnut into two main clusters (Fig.1). Cluster I consisted of 9 accessions viz, ICGV15001, ICGV15003, ICGV15004, ICGV15036, ICGV15037, ICGV15039, ICGV15041, ICGV15042 and ICGV15050 and these were observed to have less HKW varying from 26.00 (ICGV15050) to 44.70 g (ICGV15039), very high oleic acid more than 80% which also leads to high O/L ratio of upto 55%. O/L ratio plays an important role in maintaining the storage and nutritional quality (and flavor) of peanuts. It is considered as an indicator of oil stability and shelf life index (Campos-Mondragón *et al.*, 2009). The higher the O/L ratio, more stable the oil. Cluster II comprised of 52 accessions and these accessions had oleic acid in the range of 37-68% which leads to low O/L ratio in

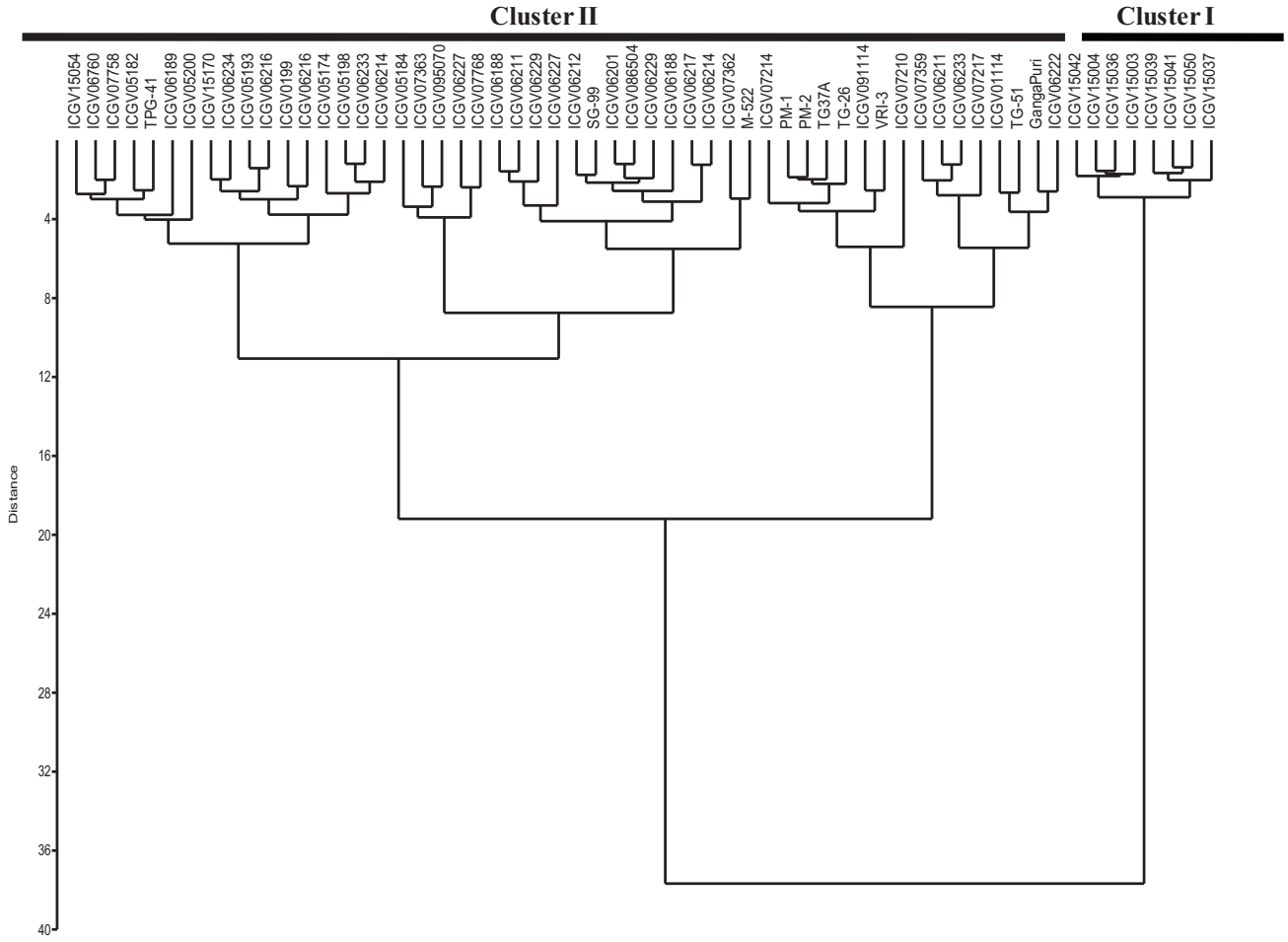

Fig. 1. Cluster analysis based on hundred kernel weight (HKW) and eleven seed quality traits in 61 accessions of groundnut

the range of 0.97-4%. Low O/L ratio indicates that the keeping quality of oil is low. Also, cluster I showed saturated fatty acid composition to be below 10% whereas cluster II had saturated fatty acids in range of 10-16%. Accessions with greater similarity for seed quality traits were placed in the same cluster. Scanty information is available in literature on cluster studies based on quality attributes in groundnut. In addition to this, clustering pattern revealed that the present germplasm has sufficient genetic variability for fatty acid and TSS content which can be used as a donor lines for further genetic improvement.

Conclusion

Groundnut accessions viz., ICGV15003, ICGV15039 and ICGV15050 possessing a combination of high oleic acid (75–80%); high O/L ratio (27.18 to 55.67) and with comparable total soluble sugar content to checks have been identified in the present study which could be helpful in fulfilling the demands of the peanut industries. These lines could be used by the breeders for the development of superior quality lines.

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RESEARCH ARTICLE

Evaluation of China Aster [*Callistephus chinensis* (L.) Nees.] Genotypes for Vegetative, Flowering, Yield and Postharvest Life

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An experiment was carried out with 42 genotypes of China aster to evaluate the performance for vegetative, flowering, yield and postharvest life, in RBD with two replications, during two consecutive years 2015-16 and 2016-17 at Division of Floriculture and Medicinal Crops, ICAR-Indian Institute of Horticultural Research, Bengaluru. Significant differences were recorded for all the traits among 42 genotypes. The genotype IIHRG13 recorded maximum plant height (61.80 cm), duration of flowering (34.40 days) and vase life (9.50 days), while, maximum shelf life (4.42 days) was recorded in IIHRH3. The Phule Ganesh Pink recorded maximum plant spread (42.15 cm) and flower diameter (6.74 cm). Maximum number of leaves per plant (32.35) and number of branches per plant (17.60) was recorded in Phule Ganesh Purple and IIHRE10, respectively. Early flowering was recorded in Matsumoto Red (46.85 days), whereas, it was delayed in Phule Ganesh White (100.15 days). Maximum stalk length was recorded in IIHRCC39 (49.10 cm), however, Arka Poornima recorded highest 100 flower weight. Highest number of flowers per plant was recorded in Local White (65.05), while, flower yield per plant was recorded highest in IIHRC42 (235.21 g). On the basis of overall performance genotypes IIHRCC39, IIHRI69-2, IIHRG13 and IIHRJ3 were found promising for cut flower and IIHECC42 for loose flower.

Key Words: China aster, Evaluation, Genotypes, Vase life, Yield

Introduction

China aster [*Callistephus chinensis* (L.) Nees.], a flowering annual belongs to the family Asteraceae and is a native of China (Navalinskien *et al.*, 2005). The genus Callistephus derives its name from two Greek words 'Kalistos' and 'Stephos' meaning 'most beautiful' and 'a crown', respectively. China aster is commercially grown for cut and loose flower, which are used in flower decoration, preparation of bouquets and garlands. In addition to their cultivation, China aster can be used in landscape gardening as a bedding plant to provide mass aesthetic effect. It is gaining popularity in India, because of its easy cultural practices, array of colours and varied uses (Bhargav *et al.*, 2016). In Karnataka, it is grown in an area of 1531 ha with productivity of 9.05 t/ha (Anonymous, 2014).

The performance of a genotype varies with the region, season and growing conditions (Punetha *et al.*, 2011). Therefore, evaluation of genotypes for quality and yield traits becomes necessary to know their performance in a particular locality. The performance of different genotypes from different climatic conditions has been examined by various workers (Munikrishnappa *et al.*, 2013, Zosiamliana *et al.*, 2013, Chowdhuri *et al.*, 2016 and Rai and Chaudhary, 2016). Considering the importance of the crop, the present investigation was carried to evaluate 42 genotypes for vegetative, flowering, yield and postharvest life under Bengaluru conditions.

Materials and Methods

The present study was conducted at the Division of Floriculture and Medicinal Crops, ICAR-Indian Institute of Horticultural Research, Bengaluru during the two consecutive years 2015-16 and 2016-17. The experimental site was geographically located at 13º58' N Latitude, 78ºE Longitude and at an elevation of 890 m above mean sea level. The soil of experimental plot was red loamy with pH 7.35 and E.C. of 0.26 dSm-1. A total of 42 genotypes including 21 named varieties and 21 stabilized lines were evaluated for vegetative growth, flowering, yield and postharvest life in randomized complete block design with two replications. Twenty plants per replication were planted at a spacing of 30×30 cm under open field conditions. The recommended agronomical practices were adopted to raise the crop.

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Five random plants were selected for recording various observations viz. plant height (cm), number of leaves per plant, plant spread (cm), number of branches per plant, days to first flowering, flower stalk length (cm), flower head diameter (cm), 100 flowers weight (g), number of flowers per plant, weight of flowers/plant (g), duration of flowering (days), vase life (days) and shelf life (days). The analysis of variance was done by the method suggested by Gomez and Gomez (1984).

Results and Discussion

Vegetative Traits

Vegetative growth is usually a good index of plant vigour, which may contribute towards greater productivity. It also serves as a guide to determine the suitable varieties for obtaining maximum yield. The data presented in Table 1 showed significant differences for vegetative traits among the genotypes. The maximum plant height was recorded in IIHRG13 (61.80 cm) followed by IIHRD5 (58.20 cm), while Milady White recorded the lowest (8.20 cm) height. The variation in plant height may be attributed to the genetic makeup of the plant. Chavan *et al.* (2010), Bhargav *et al.* (2016) and Rai and Chaudhary (2016) also reported similar results with respect to plant height in China aster.

Number of leaves was recorded maximum in Phule Ganesh Purple (32.35), which was statistically at par with Phule Ganesh White (32.30), while, minimum number of leaves was recorded in Milady White (9.20). Variation in leaf production among the genotypes are attributed to the genetic character of the individual genotype which has also been reported by Poornima *et al.* (2006) and Chowdhuri *et al.* (2006) in China aster. Plant spread at flowering stage was recorded maximum in Phule Ganesh Violet (42.65 cm), followed by Phule Ganesh Pink (42.15 cm), whereas minimum recorded in Milady White (8.75 cm). The increase in plant spread might be due to spreading branching pattern of the genotypes controlled by genetic constitution. Variation in plant spread across the genotypes has also been reported by Pandey and Rao (2014) and Rai and Chaudhary (2016) in China aster. Number of branches/plant signifies the architecture of plant and number of flowers per plant. The genotype IIHRE10 produced maximum number of branches per plant (17.60), while minimum was recorded in genotype Milady White (6.65).

Flowering Traits

The genotypes showed significant differences for flowering traits (Table 2). The early or late flowering habit of the genotype signifies by number of days taken to first flower opening which helps in regulating the market. Minimum days to first flowering were recorded in Matsumoto Red (46.85) which was statistically at par with Matsumoto Rose (52.05), whereas, Phule Ganesh White (100.15) took maximum days to first flowering which was statistically at par with Phule Ganesh Purple (90.20). Variation for early or late flowering of a genotype seems to be genetically controlled and have also been reported by Kumar and Patil (2003); Khangjarakpam *et al.* (2014); Rai and Chaudhary (2016).

Flower stalk length is an important character, the genotype having long flower stalk is generally preferred as cut flower. Significantly longest flower stalk (49.10 cm) was recorded in IIHRCC39 followed by Local Pink and IIHRI69-2 (47.50 cm), whereas, shortest stalk was recorded in Milady White (4.65 cm). Significant variation for stalk length was reported by Swaroop *et al.* (2004); Zosiamliana (2013) and Rai *et al.* (2016).

The largest flower head was recorded in Phule Ganesh Pink (6.74 cm), which was at par with IIHRE10 (6.58 cm), while minimum was recorded in Matsumoto Yellow (3.54 cm). Variation in flower head diameter might be due to the interaction among the genetic makeup of the genotypes with prevailing environment. Similar inference was drawn by Poornima *et al.* (2006) and Kaushal *et al.* (2014) in China aster and Punetha *et al.* (2011) in chrysanthemum.

Yield Traits

Maximum 100 flower weight was recorded in Arka Poornima (548.25 g), which is mainly due to the heaviest flower of powder-puff type, followed by Phule Ganesh Pink (464.50 g), whereas, minimum was recorded in Milady White (105 g). Similar variation for 100 flower weight has also been reported in chrysanthemum (Punetha *et al.*, 2011).

Number of flowers per plant was significant among the genotypes. Maximum number of flowers per plant was recorded by Local White (65.05), followed by IIHRC42 (61.00), whereas, Milady White (7.35) produced least flowers per plant. The genotypic variation for number of flowers per plant seems to be the interaction effect of

Table 1. Performance of the China aster genotypes for vegetative characters

Genotype	Plant height (cm)	Number of leaves/plant	Plant spread (cm)	Number of branches/plant
1) Arka Kamini	49.20	18.85	21.35	10.65
2) Arka Poornima	48.48	23.50	29.20	10.40
3) Arka Shashank	44.95	20.95	24.83	11.91
4) Arka Violet cushion	51.88	21.15	23.70	11.00
5) Arka Aadya	36.68	18.65	38.98	14.93
6) Arka Archana	39.28	20.35	39.30	16.65
7) Phule Ganesh Pink	52.85	25.15	42.15	12.40
8) Phule Ganesh Purple	53.80	32.35	33.58	14.34
9) Phule Ganesh White	56.63	32.30	31.05	16.05
10) Phule Ganesh Violet	52.75	21.55	42.65	12.68
11) Matsumoto Yellow	32.20	16.58	15.85	10.92
12) Matsumoto White	35.10	18.15	15.73	8.20
13) Matsumoto Rose	28.22	15.05	13.65	9.40
14) Matsumoto Scarlet	32.80	15.25	13.45	9.58
15) Matsumoto red	33.33	17.60	15.83	9.79
16) Matsumoto Pink	35.40	16.53	14.30	8.55
17) Local Pink	57.73	19.75	27.85	14.23
18) Local White	50.90	20.75	25.60	13.25
19) Local violet	39.40	22.45	21.98	11.65
20) Milady Scarlet	13.60	13.05	11.15	9.53
21) Milady White	8.20	9.20	8.75	6.65
22) IIHRD5	58.20	15.25	17.25	9.60
23) IIHRC5	54.25	16.20	18.95	9.91
24) IIHRC42	53.15	15.15	23.35	13.15
25) IIHRCC39	56.35	16.60	24.03	12.25
26) IIHRCC5-1A	50.80	22.25	25.23	12.51
27) IIHRCC31-2	47.20	19.40	21.03	11.11
28) IIHRJ3	55.45	16.45	26.43	13.29
29) IIHRJ22	41.78	18.55	19.98	9.86
30) IIHRI1	53.05	17.20	21.28	10.99
31) IIHRI66	42.58	22.25	23.90	12.33
32) IIHRCC31A	50.50	14.50	23.35	11.80
33) IIHRG13	61.80	17.40	26.70	13.85
34) IIHRI69-2	53.40	22.10	29.78	14.59
35) IIHRD2	50.52	20.40	25.39	12.70
36) IIHRCC19	46.10	21.40	31.20	15.55
37) IIHRJ3-2	53.55	20.90	29.98	14.95
38) IIHRI69	50.30	21.00	24.45	12.35
39) IIHRI16B	46.70	23.55	20.33	10.06
40) IIHRH3	44.43	17.40	24.28	12.09
41) IIHRE10	36.90	21.15	34.75	17.60
42) IIHRC1	50.70	20.80	28.10	13.93
SEm ±	0.66	0.86	0.72	0.30
C.D. (P=0.05)	1.89	2.47	2.06	0.86
C.V. (%)	2.05	6.26	4.14	3.51

genetic makeup and prevailing environment conditions. Similar variation among the genotypes was reported by Khangjarakpam *et al.* (2014) and Chowdhuri *et al.* (2016) in China aster.

Maximum weight of flowers per plant was recorded in IIHRC42 (235.21 g), followed by Arka Poornima (231.62 g), however, Milady White recorded minimum weight of flowers per plant (7.72 g), which was followed by Milady Scarlet (18.70 g). Weight of flowers per plant is an essential character for loose flower (flower with short stalk), as loose flowers are sold on weight basis in flower market. Even though it is controlled by the genetic makeup of the plant, other characters such as weight of an individual flower and number of flowers per plant also play a significant role. The results are supported from the findings of Zosiamliana *et al.* (2013), Tirakannanavar *et al.* (2015) and Rai *et al.* (2016).

Table 2. Performance of the China aster genotypes for flowering, yield and postharvest characters

Genotype	Days for first flowering	Flower stalk length (cm)	Flower diameter (cm)	100 flower weight (g)	Number of flowers/ plant	Weight of flowers/ plant (g)	Duration of flowering (Days)	Vase life (days)	Shelf life (days)
1) Arka Kamini	75.05	34.30	6.34	231.00	41.50	95.88	28.00	8.25	3.29
2) Arka Poornima	82.05	29.30	6.40	548.25	42.25	231.62	32.40	7.40	3.36
3) Arka Shashank	70.95	41.15	4.10	181.50	56.00	101.66	32.25	7.90	3.97
4) Arka Violet cushion	74.75	42.40	5.48	373.50	55.55	207.73	32.40	7.38	3.74
5) Arka Aadya	55.05	34.25	5.06	267.50	41.62	111.28	28.50	6.70	3.69
6) Arka Archana	56.85	37.60	5.56	302.00	58.55	176.80	31.15	6.70	4.32
7) Phule Ganesh Pink	79.80	46.35	6.74	464.50	26.90	124.98	27.80	8.10	3.08
8) Phule Ganesh Purple	90.20	47.40	6.13	407.50	54.50	222.00	25.25	7.20	3.43
9) Phule Ganesh White	100.15	46.85	6.44	447.50	48.85	218.67	22.20	8.15	3.16
10) Phule Ganesh Violet	75.90	44.70	5.65	404.25	47.60	192.45	32.80	6.45	3.52
11) Matsumoto Yellow	63.30	23.15	3.54	184.00	31.00	57.09	25.05	6.35	3.02
12) Matsumoto White	68.15	27.00	3.85	202.50	29.00	58.75	28.50	5.40	2.92
13) Matsumoto Rose	52.05	19.40	3.99	192.00	22.25	42.76	18.55	6.55	3.56
14) Matsumoto Scarlet	59.20	20.90	3.92	190.75	20.80	39.62	17.60	5.85	3.65
15) Matsumoto red	46.85	23.15	3.93	195.50	25.00	48.89	18.75	5.95	3.22
16) Matsumoto Pink	52.95	19.60	3.70	185.50	18.90	35.07	18.75	6.85	3.70
17) Local Pink	67.35	47.50	5.57	315.00	59.08	186.18	23.65	6.60	2.72
18) Local White	65.70	45.35	5.54	277.00	65.05	180.33	22.60	7.30	3.39
19) Local violet	69.05	33.50	4.91	281.50	55.70	156.85	21.15	5.85	2.43
20) Milady Scarlet	54.00	8.15	4.46	177.00	10.55	18.70	19.03	6.70	2.35
21) Milady White	52.47	4.65	4.10	105.00	7.35	7.72	16.58	5.90	2.36
22) IIHRD5	61.75	46.65	5.60	234.50	58.75	137.80	22.60	7.50	3.52
23) IIHRC5	62.50	45.50	5.47	293.00	52.80	154.80	23.45	7.65	3.58
24) IIHRC42	54.25	43.50	5.75	385.50	61.00	235.21	21.15	6.75	3.24
25) IIHRCC39	63.45	49.10	6.23	331.00	35.75	118.28	27.75	6.20	3.41
26) IIHRCC5-1A	69.45	41.55	5.66	347.00	51.50	178.76	27.45	8.50	3.29
27) IIHRCC31-2	57.75	37.10	5.74	224.50	38.30	85.98	21.70	6.45	3.39
28) IIHRJ3	70.35	37.10	5.85	281.50	41.53	116.98	26.90	9.35	4.37
29) IIHRJ22	53.00	31.15	5.20	245.00	27.00	66.19	20.75	5.65	2.74
30) IIHRI1	62.15	36.50	5.35	233.00	42.30	98.62	20.85	7.45	3.65
31) IIHRI66	59.40	37.25	5.66	404.00	42.05	169.94	21.50	6.60	3.22
32) IIHRCC31A	55.45	40.38	5.76	285.00	50.70	144.52	21.45	7.70	3.15
33) IIHRG13	79.55	41.45	5.39	264.00	44.15	116.59	34.40	9.50	3.94
34) IIHRI69-2	68.85	47.50	5.76	390.00	37.55	146.49	22.35	7.45	3.96
35) IIHRD2	66.30	37.30	5.64	254.00	37.30	94.74	25.00	6.95	3.23
36) IIHRCC19	73.50	36.60	6.38	332.50	53.35	177.38	34.20	5.95	2.96
37) IIHRJ3-2	85.40	38.70	5.67	288.00	38.90	112.10	28.05	9.23	3.92
38) IIHRI69	63.20	32.60	6.10	370.50	19.20	71.14	24.50	7.75	3.79
39) IIHRI16B	75.00	35.90	5.39	218.50	37.25	81.33	21.00	7.50	3.58
40) IIHRH3	63.00	38.65	5.95	304.50	52.95	161.26	25.35	6.85	4.42
41) IIHRE10	71.65	27.95	6.58	402.00	34.90	140.22	22.50	5.65	3.84
42) IIHRC1	74.10	37.00	5.98	243.50	43.25	105.31	30.90	6.50	3.71
SEm ±	0.71	0.72	0.07	2.66	0.46	2.06	0.30	0.12	0.08
C.D. (P=0.05)	2.05	2.06	0.19	7.62	1.31	5.90	0.87	0.35	0.22
C.V. (%)	1.51	2.85	1.74	1.29	1.58	2.34	1.71	2.45	3.17

Flowering duration is an important character, which determines the availability of flowers in the market and suitability for bedding purpose. Maximum duration of flowering was recorded in IIHRG13 (34.40 days) which was statistically at par with IIHRCC19 (34.20 days) and Phule Ganesh Violet (33.30 days), whereas, minimum duration of flowering was recorded in Milady White (16.58 days). The variation observed can be attributed to the genetic makeup of the plant. The results are in concurrence with the findings of Zosiamliana *et al.* (2013), Khangjarakpam *et al.* (2014) and Pandey and Rao (2014).

Vase Life and Shelf Life

Vase life and shelf life are two important flower quality traits. The vase life is generally recorded for cut flower

and shelf life for loose flowers, which decides their longevity in flower vase and in the shelf, respectively. Extended vase life was recorded in IIHRG13 (9.50 days), followed by IIHRJ3 (9.35 days), while least vase life was recorded in Matsumoto White (5.40 days). The variation in vase life of different genotypes may be due to the difference in senescencing behavior of individual genotype. The results are in agreement with Rai *et al.* (2016) in China aster and Kumar *et al.* (2012) in gerbera. The maximum shelf life was recorded in IIHRH3 (4.42 days), which was statistically at par with IIHRJ3 (4.37 days), however, it was recorded minimum in Milady Scarlet (2.35 days). Similar results have been reported by Rai *et al.* (2016) and Pandey and Rao (2014) in China aster.

On the basis of pooled data of two years i.e. 2015-16 to 2016-17 for vegetative growth, flowering, yield and postharvest life of 42 genotypes, it can be concluded that the genotypes evaluated, IIHRCC39 and IIHRI69-2 found promising for long stalk length, whereas, IIHRG13 and IIHRJ3 for vase life. The genotypes IIHRCC42 and Arka Poornima were found promising for loose flower yield under Bengaluru agro-climatic conditions.

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RESEARCH ARTICLE

Molecular Characterization of Oilseed *Brassica* Reference Set for Stem Rot Disease

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Present study was conducted on oilseed brassica reference set covering resistance and susceptible genotypes (41 *Brassica juncea*, two *Brassica carinata*, one *Sinapis alba*) to gain the information on extent of genetic diversity using 30 polymorphic Simple Sequence Repeat (SSR) markers. The dendrogram obtained by the UPGMA analysis of 82 polymorphic loci amplified using 30 polymorphic SSR markers, resulted in definite groupings among the reference set. The polymorphic information content (PIC) values varied from 0.23 to 0.67 with an average of 0.53. The highest PIC value was observed for marker named BRMS-054 making it most informative marker. The polymorphic SSR markers identified in the present study can be used for characterizing *Brassica* cultivars, constructing database, tagging genes/QTL governing stem rot tolerance and ultimately in identifying markers for the disease.

Key Words: Brassica, Genetic diversity, Polymorphic information content (PIC), SSR markers, *Sclerotinia* stem rot

Introduction

Rapeseed-mustard group of crops are major source of edible oil, vegetables and condiments. The genus Brassica belongs to the family *Brassicaceae* which include 338 genera and 3,709 species (Warwick *et al.*, 2006). Rapeseed-mustard group of crops comprise seven cultivated Oilseed Brassicas namely, *Brassica napus* L., *B. rapa* L. ssp. yellow sarson, *B. rapa* L. ssp. *brown sarson* and *B. rapa* L. ssp. *toria*, *B. juncea* (L.) Czern. & Coss., *B. carinata* Braun. and *B. nigra* (L.) Koch. The other economically important crop under the family *Brassicaceae* is *Sinapis alba*, which is cultivated as oilseed and fodder crops, particularly on marginal agricultural locations with poor soils or sub-optimal climatic conditions (Duhoon and Koppar, 1998). Modern plant breeding techniques that include intensive selection over prolonged period have drastically reduced the genetic diversity in these crops resulting into increase in the vulnerability of crops to pests and also reduced their ability to respond to changes in climate or agricultural practice (Cowling, 2007; Ananga *et al.*, 2008). However, reduction in genetic diversity caused by this intensive selection can be counterbalanced by introgression of genes from novel germplasm that include exotic lines, wild species and relatives of the crop (Miflin, 2000). These germplasm can be used as valuable source of gene for improvement of crop *Brassicas*. Therefore, attempts have become extremely essential to analyze possible genetic diversity among different species of *Brassicas*.

The analysis of DNA sequence variation is of major importance in genetic studies. In this context, molecular markers are useful tools, and these have greatly enhanced the genetic analysis of crop plants in recent years (Varshney *et al.*, 2005). Molecular marker approach is widely preferred for genetic variability assessment as compared to biochemical and morphological approaches due to their more précised efficiency, reliability and inert behavior to environmental conditions (Mishra *et al.*, 2011). Among the various molecular marker techniques available, simple sequence repeats (SSRs) have been the marker of choice in modern plant breeding because of their reproducibility, multi-allelic nature, co-dominant inheritance, relative abundance and good genome coverage (Gupta and Varshney, 2000).

The present study was undertaken to gain the information on extent of genetic diversity on reference set of oilseed *brassica* covering resistance and susceptible genotypes against Sclerotinia stem rot infestation. Sclerotinia stem rot of rapeseed-mustard, caused by

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the fungus *Sclerotinia sclerotiorum*, is one of the most significant yield-limiting factor (up to 80 percent) and least controllable diseases in oilseed *Brassica* worldwide (Mehta *et al.*, 2010).

Materials and Methods

The present investigation was conducted on reference set of oilseed *brassica* covering resistance and susceptible genotypes (41 *Brassica juncea*, 2 *Brassica carinata*, 1 *Sinapis alba*) to gain information on extent of genetic diversity. The genotypes were selected on the basis of their relative tolerance/susceptibility to stem rot infestation caused by *Sclerotinia sclerotiorum* (Table 1). These 44 genotypes were planted in two rows of

Table 1. Disease Reaction for Sclerotinia stem rot and pedigree information of *Brassica* genotypes

S.No.	Genotypes Name	Origin/Pedigree	Mean Disease Incidence (%)	Disease Reaction
1	NRCYS-5-2	Selection from YS 09/04	35.05	Susceptible
2	EC-597312(JM 6004) (YS)	Australian germplasm	5.6	Moderately Resistant
3	EC-597314(JM 6009)	Australian germplasm	5.0	Moderately Resistant
4	EC-597315(JM 6010)	Australian germplasm	5.9	Moderately Resistant
5	EC-597316(JM 6011)	Australian germplasm	8.3	Moderately Resistant
6	EC-597317(JM 6012)	Australian germplasm	2.3	Resistant
7	EC-597319(JM 6014) (YS)	Australian germplasm	21.4	Moderately Susceptible
8	EC-597320(JM 6015)	Australian germplasm	12.1	Moderately Susceptible
9	EC-597321(JM 6018)	Australian germplasm	18.7	Moderately Susceptible
10	EC-597325(JM 6026) (YS)	Australian germplasm	7.1	Moderately Resistant
11	EC-597328	Chinese germplasm	16.3	Moderately Susceptible
12	EC-597331	Chinese germplasm	9.1	Moderately Resistant
13	EC-597333	Chinese germplasm	11.4	Moderately Susceptible
14	EC-597334	Chinese germplasm	12.1	Moderately Susceptible
15	EC-597341	Chinese germplasm	25.8	Susceptible
16	EC-597343	Chinese germplasm	15.2	Moderately Susceptible
17	EC-597344	Chinese germplasm	18.6	Moderately Susceptible
18	EC-597340	Chinese germplasm	22.9	Moderately Susceptible
19	EC-552573	Australian germplasm	30.6	Susceptible
20	EC-552576	Australian germplasm	22.3	Moderately Susceptible
21	EC-552578	Australian germplasm	37.2	Susceptible
22	EC-552581	Australian germplasm	22.5	Moderately Susceptible
23	EC-552583	Australian germplasm	13.1	Moderately Susceptible
24	EC-552584	Australian germplasm	17.7	Moderately Susceptible
25	RH (0E) 0801	Not available	16.5	Moderately Susceptible
26	RH (0E) 0901	Selection from exotic material	30.9	Susceptible
27	RH (0E) 0902	Selection from exotic material	16.4	Moderately Susceptible
28	RH (0E) 0903	RH(OE)0205 × JN018	19.9	Moderately Susceptible
29	PUSA KRISHMA	Pusa Barani × ZEM 1	21.9	Moderately Susceptible
30	NUDBYJ-10	Eh 1 × MS 21	8.8	Moderately Resistant
31	LES-46	SEJ 8 × LET 18	4.8	Resistant
32	PUSA MUSTARD-21	Pusa bold × ZEM 2	8.3	Moderately Resistant
33	LES-47	Pusa Agarni × LET 14	17.9	Moderately Susceptible
34	RLC-2	QM 4 × Pusa bold	6.9	Moderately Resistant
35	ZEM-1	Australian low erucic line	16.9	Moderately Susceptible
36	ZEM-2	Australian low erucic line	11.5	Moderately Susceptible
37	DRMR-150-35	RH 819 × Pusa Bold	18.6	Moderately Susceptible
38	*Sinapis alba*	*Brassica* species	0	Immune
39	PUSA SWARNIM	HC 4 × Early mutant	1.2	Resistant
40	KIRAN	Selection from HC 5	1.2	Resistant
41	RH-749	RH 781 × RH 9617	12.8	Moderately Susceptible
42	ROHINI	Selection from natural population of Varuna	29.15	Susceptible
43	NAV GOLD	Bio 902 × BM 185-11	10.05	Moderately Susceptible
44	RH-406	RH 9608 × RH 30	9.45	Moderately Resistant

5 m length during *rabi* 2016-17. All the recommended approaches were followed for raising a good crop. The disease pressure was created artificially by inoculating the stems using mycelial suspension of *Sclerotinia sclerotiorum*. The disease scoring was done on the basis of percentage incidence of the disease.

DNA Isolation, Purification and Quantification

For DNA isolation fresh and young leaves (2 g) from healthy plants were collected from each variety. Total genomic DNA was isolated by using CTAB method of Saghai-Maroof *et al.* (1984) with slight modifications. For estimating the quantity and quality of DNA, the isolated DNA samples were loaded on a 0.8% (w/v) agarose gel. Quantification of DNA on gel requires loading a sample DNA of known concentration. DNA samples with high concentration were further diluted in TE buffer to a working concentration of 20 ng/µl and were stored at -20ºC for further use in PCR analysis.

PCR Assay

A total of 110 SSR markers were used to evaluate 44 reference set to assess the genetic diversity among them. PCR amplification was performed in a total volume of 10 µl containing 20 ng template DNA, 20 ng of each primer, 10x standard buffer containing 2.5 mM $MgCl_2$, 200 µM of each dNTP and 1.0 U of Taq DNA polymerase. PCR reactions were carried out in an Eppendorf thermocycler. PCR mastercycler was programmed at 94°C for 5 min for initial denaturation followed by 35 cycles of 45 s denaturation at 94°C, 50 s annealing at 58°C-62ºC and 1 min extension at 72°C with a final extension of 72°C for 7 min. PCR-amplified fragments were electrophoretically separated on 3.5% agarose gel containing 0.01% ethidium bromide, prepared in 1×TAE buffer (Tris-Acetic acid-EDTA). Allele sizes were predicted by comparing with 50 bp DNA ladder (Thermo Scientific). The gel was run for 3 hrs at 80 V. After electrophoresis, the amplification products were visualized in a gel documentation system. (IG/LHR, Syngene, UK).

SSR Analysis and Data Scoring

Amplified fragments of variable sizes were considered as different allele. SSR scoring of gel was done qualitatively from gel photographs by analyzing the amplified fragment. The primers which did not produce amplification were repeated at least two times before discarding them. The clear, instantly recognizable amplicons which migrated to variable distances within the gel were considered as polymorphic, whereas the amplicons which migrated same distance within gel were considered as monomorphic. The binary matrix of 0 (absence) and 1 (presence) for each SSR marker was compiled for amplified products. To compute Jaccard's similarity coefficient (Jaccard, 1908) the binary data matrix was entered into NTSYS-pc version 2.02e programme and analyzed using SIMQUAL. The similarity matrix thus obtained was used in cluster analysis using an unweighted pair-group method with arithmetic averages (UPGMA) given by Sneath and Sokal, 1973. The SHAN clustering algorithm was used to generate dendogram displaying relationships among 44 genotypes. Polymorphic information content (PIC) values for each SSR were calculated using the formula as recommended by Anderson *et al.* (1993).

$$PICj = 1 - \sum_{i=1}^{n} Pij^2$$

where, i = the ith allele of the jth marker,
n = the number of alleles at the jth marker and
p = allele frequency.

Inoculum Preparation and Inoculation of Sclerotinia sclerotiorum

The *S. sclerotiorum* BHP isolate was collected from infected accession available in ICAR-Directorate of Rapeseed Mustard Research, Bharatpur, India. A solo isolate of S. sclerotiorum was taken and sterilization procedure was followed as suggested by Clarkson *et al.* (2003). The potato dextrose broth was used to place half of the sclerotium under sterilized condition (laminar air flow). Subcultures were maintained on PDA at temperature of 25°C and 12 h light/ dark condition under fluorescence. The autoclaved *Sesbania* leaves in glass jar were used to mass multiply the pathogen. The multiplied pathogens were mixed with FYM in soil prior to sowing. The mycelial suspension of the pathogen was prepared in the laboratory on potato dextrose broth. These suspensions were sprayed by the help of automizer at 45 days after sowing. The plants were further inoculated at 60 days of sowing with the pathogen growing on potato dextrose broth and the stem of the plants were tied with the help of parafilm (Li *et al.*, 2007).

Disease Scoring

$$\text{Disease incidence} = \frac{\text{Sum of total of numerical ratings}}{\text{No. of plants examined} \times \text{maximum grade}} \times 100$$

The disease incidence was calculated by using following formula (Phool Chand and Dinesh Rai, 2009). According to intensity of disease, the disease reaction is decided as mentioned below (Table 2)

Table 2. Disease reaction categories with respect to disease intensity

Score	Disease reaction/Grade	Disease intensity (%)
0	Highly Resistant	0
1	Resistant	1-4
2	Moderately Resistant	5-9
3	Moderately Susceptible	10-24
4	Susceptible	25-49
5	Highly Susceptible	>50

Results and Discussion

Disease Data Analysis

The mean disease incidence ranged from 0 to 37.2 percent. Ten out of 44 genotypes had a high disease incidence (>20%), and 34 genotypes had a low disease incidence (<20%). Minimum lesion size was found to be zero whereas the maximum lesion size was recorded 37.8 cm. Stem diameter of 44 genotypes lies in the range of 1.42 cm to 2.46 cm. On the basis of disease severity it was concluded that genotype *Sinapis alba* is highly resistant to stem rot whereas four genotypes namely EC-597317, LES-46, Pusa Swarnim and Kiran showed resistance reaction with disease incidence level ranging from 1.2% to 4.8% as shown in Table 2. Ten genotypes were recorded as moderately resistant with disease incidence ranging from 5.0% to 9.45%, whereas 23 were found to be moderately susceptible according to the grading, disease incidence vary from 10.05% to 22.9%. Maximum disease severity (37.2%) was found among genotype namely EC-552578, whereas none of the genotypes were found to be highly susceptible to Sclerotinia stem rot disease.

SSR Marker Analysis

Among the 110 SSR primers used for polymorphism study, a total of 80 out of 110 SSR markers gave clear amplicons among all the 44 genotypes. A total of 30 SSRs were detected to be polymorphic. All the 30 polymorphic SSR reported in the study provided clear, well-marked and unambiguous amplified fragments which were further taken for genetic diversity evaluation, while rest of the 80 markers did not generate polymorphic banding pattern. The polymorphism percentage revealed in present study is 27.27 percent which is very less as compared with 93.70 percent as reported by Vinu *et al.* (2013) in different set of 44 Indian mustard genotypes. Similarly, Cheng *et al.* (2009) reported 73.4 percent polymorphism in genetic diversity analysis among six *Brassica* genotypes. In present investigation, a total of 82 alleles were amplified from 30 polymorphic markers having 1 to 4 alleles per locus with an average of 2.73 alleles/marker. It is complementary with the results (2.7 allele per locus) as reported by Zhou *et al.* (2006) and Kumawat *et al.* (2015). The average number of allele reported in the present investigation was however, found lesser as reported by 3.53 allele per locus (Thakur *et al.*, 2015), 5.29 allele per marker (Cunmin *et al.*, 2012), 6.6 allele/marker (Yao *et al.*, 2012). The fragment size of amplicons varied from 100 bp to 340 bp.

PIC value is often used to measure the discriminatory power and informativeness of a genetic marker. As suggested by Peng and Lapitan (2005), PIC Value is an important parameter for selecting SSR for germplasm evaluation and gene tagging. Before initiating any research related to genetic diversity assessment by using SSR markers, it is compulsory to select most informative SSRs and this is possible only by estimating PIC value of each SSR marker to be used in the study (Macaulay *et al.*, 2001, Masi *et al.*, 2003, Candida *et al.*, 2006). In this study, the PIC value found to lie between 0.23 (D12) to 0.67 (BRMS-054) with an average of 0.53. The average PIC Value estimated is comparable to 0.57 as calculated by Park J *et al.* (2013) and El-Esawi *et al.* (2016). PIC value observed with marker BRMS-054 was the highest (0.667) indicating it as the most informative and representative marker (Fig. 2). Among all the 110 SSRs used in the study, most of them generated lesser number of bands and lower PIC value which may be due to the reason that these markers are not specific to the species of the genotypes under study. Out of 30 polymorphic markers a total of fifteen markers showed >0.5 PIC value indicates the discriminatory potential and usefulness of these markers for differentiating 44 genotypes under study (Table 3).

Similarity Coefficient and Genetic Relationship Analysis

Jaccard similarity coefficient was found to vary from 0.33 to 0.93 with an average of 0.61. The highest value (92.6%) for genetic similarity was found between EC597316 and EC597317 followed by 89% between EC597315 and EC597316 and also between EC597315 and EC597317. Similar range of genetic variation

Table 3. Detail of PCR amplified product with 30 SSR primers

S. No.	Marker	Forward primer	Reverse primer	Tm (°C)	Range of Amplified product	No.of Polymorphic Bands	PIC Value
1	BRMS-054	GAATCTCTGCAAGAAACAAATG	TTCCTCAGCATCAAGTAACCTC	58	200-250	3	0.667
2	cnu_m621a	GCAGAAGCCTGAGAGTCTGG	AACAAGGCTGAATGCTACCG	55	150-250	3	0.666
3	BJSSR-181	AAACCGGCCTAGCACTCTTC	GGCTTCTCTTCTTGGAGCGT	56	200-300	2	0.660
4	EJU1	GGTGAAAGAGGAAGATTGGT	AGGAGATACAGTTGAAGGGTC	55	180-200	2	0.657
5	BJSSR-473	TCAACATGCTGCTCCTGC	TGGCTCAGGAAGAACAAG	55	200-250	3	0.656
6	BJSSR-125	ACGACGGAGGAGAAACCAAC	CGGACGATACTGAAGGCGAA	55	230-270	3	0.655
7	Ra2-E04	ACACACAACAAACAGCTCGC	AACATCAAACCTCTCGACGG	57	180-300	2	0.654
8	C09	AGCATCAATCTTTTGCTCTGC	TGCACACAAACTCCTTCTCC	55	180-250	3	0.639
9	BJSSR-184	CGTCGATAGCTTCCTCCACC	CTCCCGTGGTCTCTTGTTCC	54	180-230	2	0.624
10	cnu_m593a	TAAGGCAAATTGTTGGGCAT	CCATCTCTCCTTGTCCTCCA	55	300-340	3	0.616
11	Ni2AO7	GGAACCCAACAAGTGAGTCC	AGAGCTTGAGACACATAACACC	55	200-250	3	0.614
12	B02	CGCTGCAATTATACGAAAGC	CCTCATGCTCTCCAAAGACC	55	180-200	3	0.613
13	Ni3-B07	GGAGAAGAGGAAGAAGAAGCC	CGACTTCTAGAGGAACCCCC	55	100-170	3	0.594
14	cnu_m597a	TTGAACCCACGAAAACTTCC	AGAAGGGAGAGAGGTCAGGC	55	180-230	3	0.577
15	PW186	GACGGTGACGACCAATCAGAGCA	GCGTTCGCCATAGACGAGTCCAA	55	120-140	3	0.559
16	E05	CTCGTCTCAGGGATTATGTCG	CAGACAGAGGATAGACCGAACC	58	100-170	3	0.544
17	BRMS-043	GCGATGTTTTTTCTTCAGTGTC	TTAATCCCTACCCACAATTTCC	53	280-320	2	0.524
18	F01	CGTATGTAGAGAGAGAGAGAGAGAGAG	AGAACCGTTGAGGTGCTGTC	58	200-300	3	0.499
19	nia_m042a	CCCATCCCTCCTTGATGATA	GATGGGCATTTCACGACAC	54	250-280	2	0.499
20	ENA28	GGAGTCCGAGCGTTATGAAT	CTTCATCGACCCACCTTGTT	54	150-170	2	0.498
21	Ra3-D02B	CACAGGAAACCGTGGCTAGA	AACCCAACCTCAACGTCTTG	55	350-400	2	0.485
22	cnu_m585a	TTTATCAGTCCGGTTTTGCC	GATGCTCTGAGACACCCAAA	53	100-130	2	0.48
23	cnu_m602a	CATCCACAAGTCCACCAGTC	CGCATTAGACCCTAAAAACCG	56	200-230	2	0.456
24	A09	AAAGGGCGAAGAAGCAGC	TTTCTTCCATTTGACCGACC	53	150-250	2	0.418
25	cnu_m587a	CATCATTGGCTTTGGGAGTT	CGACTGGGAAAGAAAAACACA	52	180-240	2	0.405
26	Ni2AO1	TGCTGCTACAGACAGTGTTGG	AAAGGCTACACACTCATGAAACC	56	230-250	2	0.389
27	ENA20	GATGGAGGAAGAAGACAAGAC	TCTGAACTACCAAAGCCAAC	55	130-150	2	0.357
28	BRMS-033	GCGGAAACGAACACTCCTCCCATGT	CCTCCTTGTGCTTTCCCTGGAGACG	64	150-250	2	0.356
29	nia_m066a	TTGGGAAGAGAGTTGGGTGA	TCGTTTCATATGGGCCTTGT	53	250-300	3	0.256
30	D12	GAGATGAGGATTTGCTTTTGC	ACAGTATGAGAGAGAGAGAGAGAG	55	140-170	2	0.225

has also been reported by Gohel and Mehta (2014), Vinu *et al.* (2013) in Indian mustard. Whereas, the least similarity coefficient was observed in between EC 597321 and ZEM-1 which represents high genetic diversity among these two genotypes. The dendrogram obtained by the UPGMA analysis of 82 polymorphic loci amplified by 30 polymorphic SSR markers resulted in definitive groupings among the studied genotypes, which is being partitioned into two major groups i.e. cluster I and cluster II with a similarity coefficient of 0.53 in the UPGMA dendogram (Fig. 1). The cluster I is further divided into two sub-clusters i.e. cluster I A and cluster I B. The sub-cluster I A and I B showed the genetic similarity of 56%. The sub-cluster I A consists of solitary genotype "NRCYS-5-2" which is found to be a susceptible genotype for stem rot disease in the present study, whereas the cluster I B consists of 37 genotypes. Maximum number of genotypes (30 out of 37) in cluster 1B showed moderately susceptible to moderately resistant disease reaction. Cluster II consists of total 6 genotypes namely LES-47, ZEM-1, ZEM-2, RLC-2, Pusa Swarnim and Kiran. Both the *B. carinata* genotypes viz. Pusa Swarnim and Kiran fall in same cluster and exhibit the genetic similarity of 59% .These genotypes show resistant reaction. However, other genotypes of cluster II are moderately susceptible. Australian low erucic acid

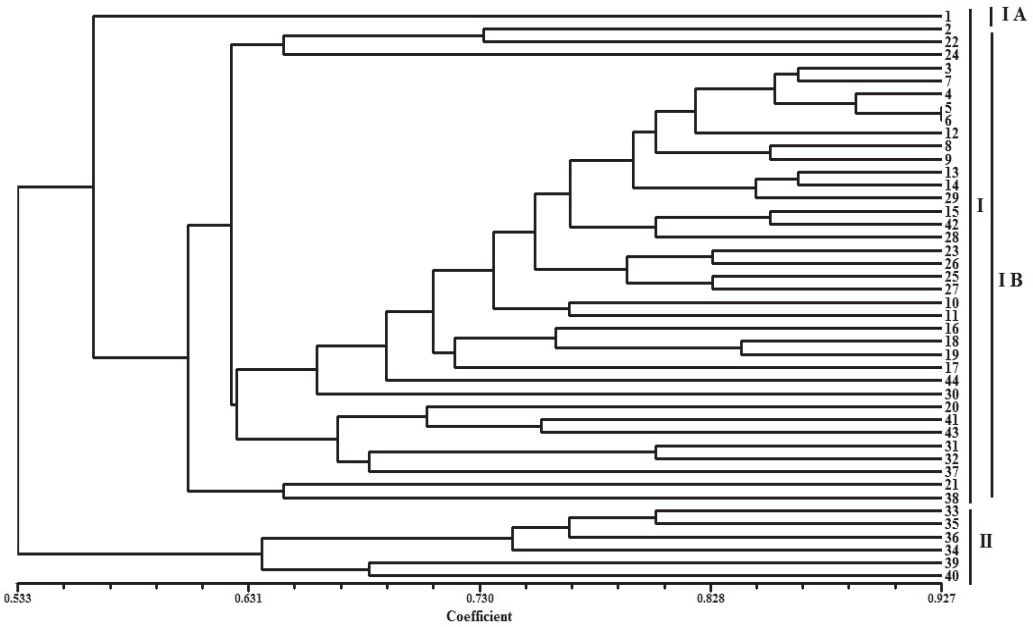

Fig. 1. UPGMA dendrogram showing genetic relationships among 44 genotypes based on SSR markers 1- NRCYS-5-2, 2- EC-597312(JM 6004) (YS), 3- EC-597314(JM 6009), 4- EC-597315(JM 6010), 5-EC-597316(JM 6011), 6- EC-597317(JM 6012), 7- EC-597319(JM 6014) (YS), 8- EC-597320(JM 6015), 9- EC-597321(JM 6018), 10- EC-597325(JM 6026) (YS), 11- EC 597328, 12- EC 597331, 13- EC 597333, 14- EC 597334, 15-EC 597341, 16- EC 597343, 17- EC 597344, 18-EC 597340, 19-EC 552573, 20- EC 552576, 21- EC 552578, 22- EC 552581, 23- EC 552583, 24- EC 552584, 25- RH (0E) 0801, 26- RH (0E) 0901, 27- RH (0E) 0902, 28- RH (0E) 0903, 29- PUSA KRISHMA, 30- NUDBYJ-10, 31- LES-46, 32- PUSA MUSTARD-21, 33- LES-47, 34- RLC-2, 35- ZEM-1, 36- ZEM-2, 37- DRMR-150-35, 38- *Sinapis alba*, 39- PUSA SWARNIM, 40- KIRAN, 41- RH-749, 42- ROHINI, 43- NAV GOLD 44-RH-406

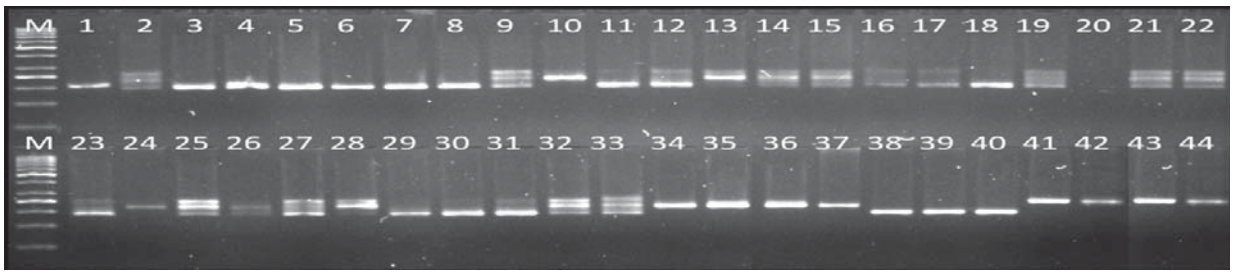

Fig. 2. Polymorphic-SSR marker (BRMS-054) profile of 44 oilseed mustard genotypes
Lane M- 50 bp ladder 1- NRCYS-5-2, 2- EC-597312(JM 6004) (YS), 3- EC-597314(JM 6009), 4- EC-597315(JM 6010), 5-EC-597316(JM 6011), 6- EC-597317(JM 6012), 7- EC-597319(JM 6014) (YS), 8- EC-597320(JM 6015), 9- EC-597321(JM 6018), 10- EC-597325(JM 6026) (YS), 11- EC 597328, 12- EC 597331, 13- EC 597333, 14- EC 597334, 15-EC 597341, 16- EC 597343, 17- EC 597344, 18-EC 597340, 19-EC 552573, 20- EC 552576, 21- EC 552578, 22- EC 552581, 23- EC 552583, 24- EC 552584, 25- RH (0E) 0801, 26- RH (0E) 0901, 27- RH (0E) 0902, 28- RH (0E) 0903, 29- PUSA KRISHMA, 30- NUDBYJ-10, 31- LES-46, 32- PUSA MUSTARD-21, 33- LES-47, 34- RLC-2, 35- ZEM-1, 36- ZEM-2, 37- DRMR-150-35, 38- *Sinapis alba*, 39- PUSA SWARNIM, 40- KIRAN, 41- RH-749, 42- ROHINI, 43- NAV GOLD, 44-RH-406

line i.e. ZEM-1 and ZEM-2 clustered together in same cluster with a genetic similarity of 60%. All Chinese genotypes were grouped in same cluster i.e. cluster I B and most of them are moderately susceptible to the stem rot disease. This shows the effectiveness of SSR markers in identifying the close pedigree relationship in breeding material and can be used in background selection of recurrent parent genome. In present investigation, the clustering of 44 genotypes based on SSR polymorphisms corresponded well to their known origin/pedigree data as well as their response to the Sclerotinia stem rot disease. The polymorphic SSR markers identified in the present study can be used for characterizing *Brassica* cultivars, constructing database, tagging genes/QTL governing

stem rot tolerance and ultimately in identifying markers for the disease.

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REVIEW ARTICLE

Genomic Resources of Plant Abiotic Stress Tolerance: An Overview of Functional and Regulatory Proteins

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Abiotic stress, a complex phenomenon, has been established as a major factor affecting quality and quantity of crop production. Plants have evolved mechanisms to survive abiotic stress conditions and a set of protein families have been identified that work at cellular level to make plants tolerant. Modern genetic engineering and synthetic biology techniques are capable of deploying specific genes to develop crop varieties tolerant to abiotic stress factors. This and the availability of patent protection on the genetic sequences and protocols can make genebanks redundant unless genebanks equip themselves with genomic resources of priority and niche crop species. Here, we review the status of knowledge in 13 protein families, miRNAs and epigenetic regulation known to be involved in plant abiotic response pathways among nine major crops. A comparative analysis is presented of sequence depositions in public databases, research papers and patent protection sought in the area of abiotic stress response pathway in plants. Significance of generation and use of genomic resources for management of plant genetic resources is discussed.

Key Words: Abiotic Stress, Crops, Functional and Regulatory Proteins, Genomic Resources, NCBI Database, Patents

Introduction

Genetic diversity of the world's crop plants is subsiding – both infra-specific and across crop wild relatives (Fu, 2015). Generation and conservation of genomic resources is one of the solutions insuring against genetic erosion. Utilization of genomic resources to add value to genetic resources requires inventorization of genomic resources as much as characterization of germplasm. On one hand, genebanks around the world are carrying out molecular genetic characterization of plant germplasm (Varshney, 2016), and on the other, genetic engineering tools like gene editing have reemphasized the importance of identifying novel genes. Considering possible changes in production environments in future, characterization of genetic resources to identify suitable genotypes, genes and alleles has assumed greater significance. Creation of genomic resources as a step towards "climate ready genebank" requires up-to-date knowledge of genes that have functional and regulatory significance especially those that impart tolerance to abiotic stress.

Abiotic stress – manifested in the form of drought, flooding, salinity, heat and cold, etc. individually and in combination – causes interconnected events of morphological, physiological, biochemical and molecular changes in plants adversely affecting their growth and development. Abiotic stress is a serious constraint to agricultural production and thereby a threat to food security. Plant breeders are joined by agronomists and physiologists to develop crop varieties with enhanced tolerance to various abiotic stress factors. Developing stress-tolerant plants via molecular breeding as well as biotechnological approaches has demonstrated increased crop yield (Mickelbart *et al.*, 2015).

Drought, flooding, salinity, heat and cold can result in osmotic stress (disruption of homeostasis and cellular ion distribution) or oxidative stress (denaturation of functional and structural proteins). Research on decoding molecular basis of plant response to abiotic stress has led to the identification of several stress response genes, pathways and regulatory networks (Jain, 2015). Research in model plants has shown that diverse stress factors activate similar cellular responses including production of stress proteins, up-regulation of anti-oxidants and solute accumulation (Wang *et al.*, 2003). Response to stress in plants is a complex phenomenon and is known

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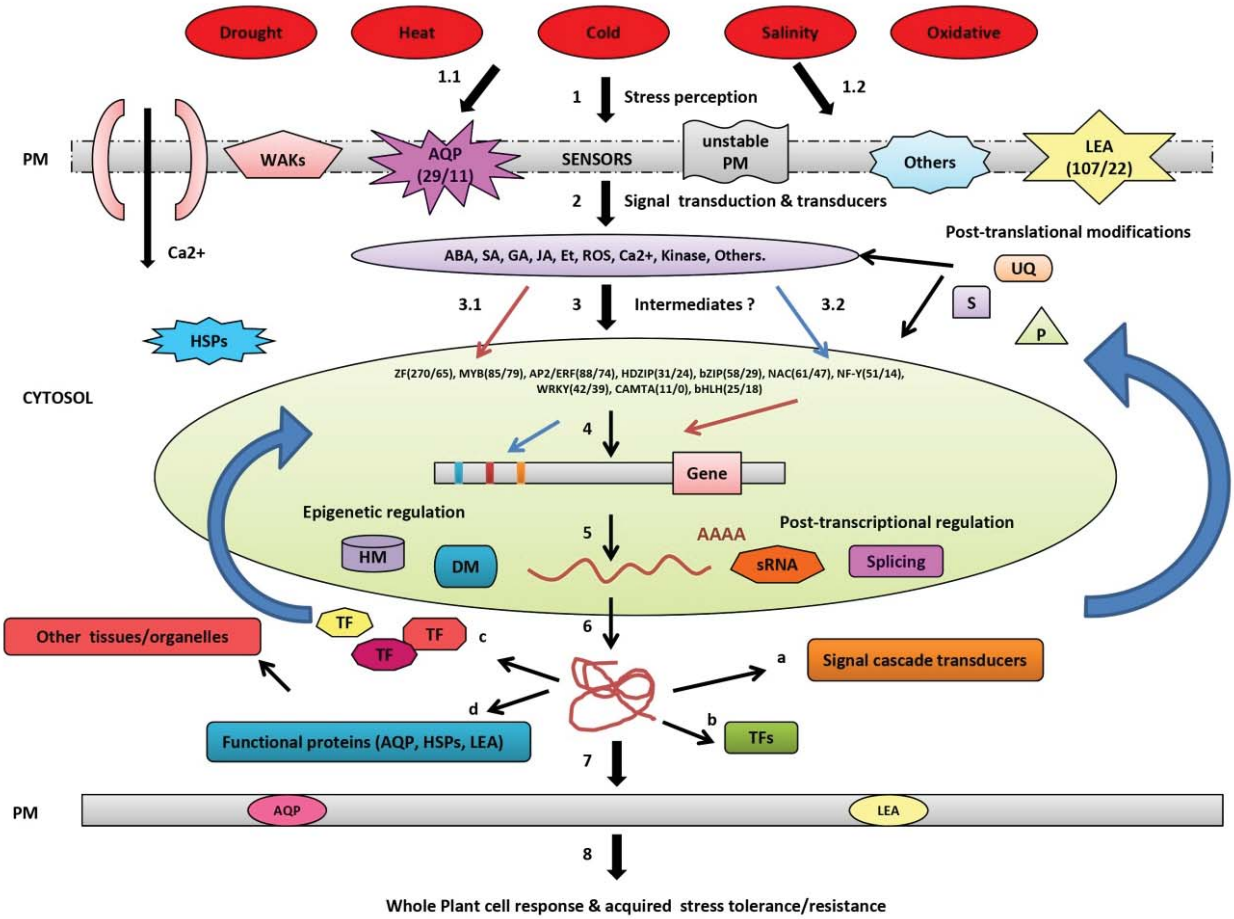

Fig. 1. Schematic presentation of abiotic stress induced changes in plant cell showing role of functional and regulatory proteins. 1. Abiotic (single/multiple) stress signals are perceived by sensors (opening of Ca2+ channels, membrane instability, wall associated kinase (WAK e.g. RLK; receptor like kinase, HK; histidine kinase, COLD1; cold responsive, OSCA1; reduced hyperosmolality-induced calcium increase 1), others (TRP (transient receptor potential channel proteins, G- protein coupled receptors, RBOHD; ROS producing enzyme, Calmodulin binding receptors, CNGCs; cyclic nucleotide-gated channels, GLRs; glutamate receptor-like channels) (Zhu, 2016). 1.1 & 1.2 Functional proteins (AQP, LEA, HSP) become active in PM, Cytosol and other cellular organelles. 2. The stress signals are transduced by various transducers and plant hormones. 3. Various intermediates (unknown) transmit the signal to nucleus. 3.1 Red arrow indicates hormone dependent regulation. 3.2 Blue arrow indicates hormone independent regulation. 4. Single (blue arrow) or couple of Transcription factors (red arrow) bind to =>1 cis-elements of SRG (stress responsive gene). 5. The resulted mRNA undergoes post-transcriptional regulation (sRNA; small RNA, alternative splicing, poly-A tail) and epigenetic regulation (HM; histone methylation, DM; DNA methylation). 6. mRNA is translated into protein (a: involved in signal cascade where post-translational modifications occurs; UQ; ubiquitination, S; sumoylation and P; phosphorylation. b & c: as TF; transcription factors go back to nucleus. d: functional proteins that are directly involved in stress tolerance mechanism). 7. AQP and LEA synthesized goes to PM for instant reaction to stress stimuli. 8. Such downstream complex pathways and proteins cascades results in stress resistance/tolerance plant.

to be regulated by a hub of signal cascades, proteins and hormones (Fig. 1). In the past decade, genomics has been making progress in deciphering pathways underlying stress response mechanism in plants (Langridge and Reynolds, 2015).

Realizing the significance of genomic resources generation, genebanks have taken particular interest in whole genome sequencing projects (Li *et al.*, 2014). As a result, direct characterization and validation of candidate genes in many genera and species has been achieved (Galperin *et al.*, 2017). However, the pace of generation of sequence data and the availability of bioinformatics tools to annotate the sequences in silico can make it challenging to track the information on genes and

proteins and choose the suitable information for designing experiments to characterize trait-specific germplasm at molecular level. The sequences available in open access databases were surveyed for genes known or predicted to be involved in abiotic stress response pathways in plants. NCBI (https://www.ncbi.nlm.nih.gov.in) databases were searched for abiotic stress genes in the model plant *Arabidopsis thaliana*, and eight major crop species including *Triticum aestivum*, *Oryza sativa (*both *japonica* and *indica*), *Zea mays*, *Sorghum bicolor*, *Solanum lycopersicum*, *Solanum tuberosum*, *Brassica napus* and *Glycine max*. These species contribute about 63% of the flowering plant sequences in the NCBI databases and hence our analysis was sufficiently representative. Database search returned 3425 sequences, and those hits described as hypothetical, putative, unpublished, provisional, predicted, under review, uncharacterized and probable types were excluded from further analysis. Only published and characterized sequences (1025) were reviewed and included in the analysis to ensure reliable interpretations. Google patents database (https://patents.google.com) was searched with same set of queries to get information on 457 patent applications and grants on 14 genes/13 protein families analysed in this review.

Cellular location, position in the stress response as well as number of sequence deposits and patents considered for analysis of 14 genes are diagrammatically depicted in Figure 1. The review provides a brief account of each one of these genes, whereas, their details including specific references are provided in supplementary Table. In the first section of the review, brief description of three functional proteins viz. Aquaporins, late embryogenesis abundant like proteins and heat shock proteins, and 10 regulatory proteins including eight eukaryotic transcription factors viz. Basic Helix-Loop-Helix, Nuclear Factor-Y, MYB, Homeodomain leucine zipper, Basic Leucine Zipper, Apetala2/Ethylene Response Factor superfamily, Zinc Finger, Calmodulin Binding Transcription Activator, NAC and WRKY is provided. In addition to the proteins, epigenetic regulation during stress is touched upon. Second section includes a comparative analysis of research output (measured by sequence depositions in public databases and research publications) and intellectual property sought (patent applications) in the area of abiotic stress response pathway in plants. Finally, perspectives for employing genomic resources related to abiotic stress tolerance in screening genebank accessions are presented.

Functional Proteins of Plant Abiotic Stress Pathway

1. AQP (Aquaporin)

Abiotic stress results in tissue dehydration due to imbalance between root water uptake and leaf transpiration. Apart from diffusion, pores existing in the membrane help in rapid flow of water molecules across the cell. AQP, a member of major intrinsic proteins (MIPs) discovered by Maurel *et al.* (1993) in plants and found ubiquitously in all living organisms play crucial role in transport of water molecules across plasma and intracellular membrane. On the basis of sequence homology and distinct sub-cellular localisation, AQP are classified as: plasma membrane (PIPs), tonoplast (TIPs) present in plasma and vacuolar membranes, nodulin26-like (NIPs) in plasma and intracellular membrane of non-leguminous plants and small basic (SIPs) in endoplasmic reticulum. Two decades later, studies in *Physcomitrella patens* found new AQP members: GlpF like intrinsic proteins (GIPs), hybrid intrinsic proteins (HIPs) and uncharacterized X intrinsic major proteins (XIPs) (Maurel *et al.*, 2015).

AQPs undergoes co- and post-translational modifications (Afzal *et al.*, 2016) like phosphorylation and methylation at N- and C- termini, ubiquitination by RMA1, RMA2 and RMA3, and transcription regulation by bZIP24 (repression of expression of PIP2-1 in response to salt stress). As mentioned above, AQPs are essential proteins for maintaining water equilibrium. Surprisingly however, most of the members deposited in sequence database are putative or not characterized so far across crop species including *Arabidopsis*. In Potato sequences are unpublished and no member for sorghum is reported. Amongst characterized sequences most of them are up-regulated by drought, salt, cold, plant hormones like ABA, SA and reactive oxygen species like H2O2. However, PIP is up-regulated by cold stress and down-regulated by GA whereas TIP is down-regulated by cold stress but inducible by drought and salt stress in rice (Sakurai *et al.*, 2005). Notably, validated sequences belong mainly to PIP and TIP families, and role of SIP, NIP, XIP, GIP and HIP in abiotic stress tolerance is yet to be revealed in detail.

2. LEA (Late embryogenesis abundant like protein)

LEA is a major hydrophilic protein providing desiccation tolerance to plants during late stages of seed development.

It was first reported in cotton seeds (Dure *et al.*, 1981). Ubiquitous presence of this evolutionarily ancient protein in vascular and non-vascular plants, anhydrobiotic plants, algae, bacteria and animals makes it essential protein for adaptation during unfavourable conditions (Amara *et al.*, 2014). On the basis of sequence motifs, LEA is categorized into seven groups: LEA1 to LEA7, first three being major LEA groups.

Despite discovered four decades back, only DHN/ LEA2 sequences populate databases and LEA sequences of mustard and sorghum remain unpublished. It is known that different LEA members are induced by specific stress like drought/salt/cold or at best by combination of two stresses. Interestingly, two Arabidopsis LEAs were induced by high light stress and ROS molecules (Weaver *et al.*, 1998; Singh *et al.*, 2005). ABA dependent hormonal regulation was observed in other members. Focus on limited LEA members appears to have underutilized the potential of this protein for its deployment to impart abiotic stress tolerance.

3. HSPs (Heat shock proteins)

HSPs belong to a class of proteins called molecular chaperones, which are vital for protein folding, assembly, translocation, degradation, and stability. HSPs help cellular proteins to achieve correct folding during the stress conditions. As a response to heat stimulus, Heat Shock Factor (HSF) binds to Heat Shock Elements (HSE) containing palindromic sequence (AGAAnnTTCT), causing up-regulation of heat stress genes and synthesis of HSPs (VonKoskull-Döring *et al.*, 2007). HSPs are highly conserved; the diverse members are known to occur in cytoplasm, nucleus, mitochondria, chloroplast and endoplasmic reticulum of cell. HSPs are divided into five major families: HSP70 (DnaK); Chaperonins (GroEL and HSP60); HSP90; HSP100 and small HSP (sHSP)/ HSP20 (Wang *et al.*, 2004). In addition to heat, HSPs are up-regulated by drought, cold, salinity, heavy metal and high light intensity. There are a few examples in which HSPs are down-regulated as in Arabidopsis by ozone stress (Rosano *et al.*, 2011) and in rice by anoxia (Sarkar *et al.*, 2009). Arabidopsis ClpD is induced by senescence but not by heat stress. Most of the HSP research is reported in HSP20 and HSP90. Non-abiotic stress induced HSPs are also known in wheat, sorghum, maize and tomato. Certainly, HSPs are the most researched genes imparting abiotic stress tolerance to crop plants with highest number of sequence deposits in the databases.

Regulatory Proteins

1. bHLH/MYC (basic helix-loop-helix)

bHLH/MYC are the second largest class of TFs present in eukaryotes and are known to be involved in development, biosynthesis, signalling and stress response processes (Castilhos *et al.*, 2014). They were first reported in maize (Ludwig *et al.*, 1989) and consist of 50-60 amino acid bHLH domains with two distinct segments viz. basic and HLH region. bHLH TFs bind to cis-elements such as E-box (5'-CANNTG-3') and/ or G-box (5'-CACGTG-3') present in plant promoters (Carretero-Paulet *et al.*, 2010). bHLH are both induced and repressed by plant hormones like jasmonic acid, abscisic acid and salicylic acid in crops (Abe *et al.*, 1997; Lee *et al.*, 2013); induced by heavy metal stress in Arabidopsis (Theologis *et al.*, 2000); up-regulated by drought, cold, salt and heat stress (Cheng *et al.*, 2011; Gangadhar *et al.*, 2014). Of note, wheat ICE gene is induced specifically by cold stress (Badawi *et al.*, 2008). Our analysis suggested that sequence deposition and validation of bHLH genes in crop plants, other than rice and wheat, are inadequate.

2. NAC [Petunia No Apical Meristem (NAM), Arabidopsis Transcription Activation Factor (ATAF), Cup-shaped cotyledon (CUC)]

NAC is a plant specific TF reported first in Arabidopsis as RD26 (Responsive to dehydration) gene during desiccation (Yamaguchi-Shinozaki *et al.*, 1992). NAC have highly conserved DNA binding domain at N-terminal and structurally disordered diverse transcription regulatory region at C-terminal. They are classified into eight subfamilies (a-h) on the basis of alignment in NAC domain, and each subfamily into distinct subgroups on the basis of diverse C terminal domain (Shen *et al.*, 2009). These TFs undergo regulation at post-transcriptional, post-translational level and by cis-elements such as ABREs (ABA-responsive elements), DREs (Dehydration- responsive elements), jasmonic acid and salicylic acid responsive elements (Shao *et al.*, 2015). NAC is induced by drought, salt, cold, heat, oxidative stress, ozone, plant hormones, chemicals (MMS, PEG) and hypoxia as well as in glucose mediated signals. Few Arabidopsis NACs have been reported to be down-regulated during stress (Kim *et al.*, 2007b; Balazadeh *et al.*, 2010; Li *et al.*, 2013). In addition to academic research NACs have attracted considerable attention for commercial potential.

3. WRKY

WRKY TF belong to the GCM1 like ZF class and was discovered in *Ipomoea batata*, SPF1; *Avena fatua*, ABF1 and ABF2; and *Petroselinum crispum*, WRKY 1, 2, 3 (Ishiguro *et al.*, 1994; Rushton *et al.*, 1996). WRKY are omnipresent in plant kingdom acting as both repressor and inducer of transcription. They have a 60 amino acid WRKYGQK signature domain at N-end and zinc finger motif at C-end. On the basis of phylogenetic analysis they are divided into five monophyletic (except group II) groups, Ia, IIa + IIb, IIc, IId +IIe, and III. The degree of its binding affinity is decided by flanking sequences and core W-box (TTGACT/C). Activity of WRKY genes is influenced by post-translational modification, MAPK mediated regulation, interaction with other TFs and by the co-factor VQ motif (Tripathi *et al.*, 2014).

WRKY TFs are induced by drought, salt, cold, heat, oxidative stress, hormones and chitin as well as in ions-mediated signalling pathway. They maintain redox homeostasis during heavy metal stress stimuli (Hu *et al.*, 2013; Wang *et al.*, 2015). In soybean it is down-regulated during drought stress whereas in mustard it down regulates auxin mediated signalling pathway (Li *et al.*, 2015; Song *et al.*, 2016). Despite identified with several roles, the number of sequence deposits appears relatively less. Interestingly, WRKY sequences of tomato, potato, sorghum and maize in the public database remain predicted/ unpublished, whereas the number patent applications are on the rise.

4. NF-Y (Nuclear factor Y)

NF-Y TFs, belonging to family of CAAT box binding factors, are ubiquitous in higher eukaryotes (Ronchi *et al.*, 1995). It is made of three sub-units: NF-YA (HAP2/ CBF-B), NF-YB (HAP3/CBF-A), NF-YC (HAP5/ CBF-C). In plants, the sub-units coded by about 10 genes explain for diverse function of NF-Y (Petroni *et al.*, 2012). The functional variation is further enhanced by interaction with other TFs as cofactors. Database search showed NF-Y sequence deposits in wheat, maize, mustard and Arabidopsis but surprisingly not of tomato, potato, sorghum, rice and soybean. NF-Y TFs are induced and/or repressed by single stress like salt, drought, cold, high light intensity, and up-regulated by hormone ABA (Warpeha *et al.*, 2007; Liang *et al.*, 2014). Despite having diverse possibilities of functional role in abiotic stress response, number of publications or patents in NF-Y is limited possibly due to lack of critical knowledge regarding their interactions with downstream genes, cis-elements or flanking sequences and grasp of dimerization mechanism.

5. MYB

MYB is the oldest and the largest superfamily of TF discovered in *Zea mays* as MYB- like C1 gene involved in anthocyanin biosynthesis (Paz-Ares *et al.*, 1987). MYB TFs have conserved N-ends containing MYB domain (made up of 1-4 imperfect repeats) but diverse C-ends containing transcription activator/repressor region. On the basis of number and position of repeats MYB is classified into 4 sub-families: 4R-MYB, R1R2R3-MYB, R2R3-MYB and 1R-MYB. The MYB domain forms helix-turn-helix secondary structure to bind with major groove of DNA. MYB TFs are involved in regulation of many genes involved in plant development and balancing redox homeostasis. In turn, MYB proteins undergo epigenetic regulations, post-transcriptional regulation and oxidative suppression (Roy, 2016). They are repressed or induced by nutrient deficiency in different crops (Pareek *et al.*, 2006; Li *et al.*, 2013; Chai *et al.*, 2015). Differential regulation is observed in response to drought, salt, cold, PEG and light stress. It is noteworthy that NCBI databases have uncharacterized sequences of MYB of potato, rice and maize and no records of mustard and sorghum, whereas maximum patents are filed for MYB sequences.

6. HD-Zip (Homeodomain-Zip)

Homeodomain (HD) is a 60 residue motif present in homeobox genes of eukaryotes, first reported in *Zea mays* as KNOTTED1 gene (Vollbrecht *et al.*, 1991). HDs are classified into six families: HD-Zip, PHD, BELL, ZFD, WOX and KNOX. On the basis of gene structure, function, conserved domain and associated conserved motifs, HD-Zip is divided into four subfamilies, HD-Zip I to IV. HD-Zip TFs are induced by drought, salt, ABA, SA and IAA. In rice and mustard they are up-regulated by cold stress and responsive to H2O2 stress (Yu *et al.*, 2005; Zhang *et al.*, 2012). Two members in rice are down-regulated during drought stress (Dai *et al.*, 2008). They are regulated by miRNA 165/166and post-translational modification (Ariel *et al.*, 2007). Number of sequence deposits and publications are low (uncharacterized sequences in wheat and soybean; no sequences of tomato, potato and sorghum), but in relative terms, patent applications are substantial highlighting the immediate commercial potential.

7. bZIP (Basic Leucine Zipper)

bZIP is a large family of regulatory proteins with diverse role during abiotic stress conditions. bZIP consists of two unique regions: a 16-residue basic region with nuclear localized signal and a Leu-rich region made up of heptads of Leu repeats (Landschulz *et al.*, 1988; Izawa *et al.*, 1993). bZIP functions by binding to A-box (TACGTA), C-box (GACGTC) and G-box (CACGTG). In green plants, bZIP has 13 groups (A to L and S) on the basis of basic region and conserved motifs. They undergo various post-translational modifications and epigenetic regulations, and interact with NAC, NF-Y and WRKY (Banerjee and Roychoudhury, 2015). bZIP TFs are regulated by drought, salt, cold, heavy metal, light, oxidative stress (Kang *et al.*, 2010), hormones (Theologis *et al.*, 2000; Garcia *et al.*, 2012) and in response to glucose and chitin (Finkelstein *et al.*, 2005). Number of sequence deposits, publications as well as patents is higher in bZIP than that in HD-Zip.

8. AP2/ERF (APETALA 2/ethylene-responsive element binding factor)

AP2/ERF, a plant specific superfamily of TF, was discovered in APETALA2 of *Arabidopsis* (Jofuku *et al.*, 1994) and EREBP1 of *Nicotiana* (Ohme-Takagi and Shinshi, 1995). They are characterized by a 60 amino acid AP2/ERF domain. AP2/ERF superfamily, one of the biggest gene families, is involved in response to drought, salinity, heat, disease resistance, as well as in flowering control. Based on the number of AP2/ERF domains and the presence of other DNA binding domains, AP2/ERF superfamily includes (1) AP2 (APETALA2), (2) DREB (dehydration-responsive-element-binding), (3) RAV (related to ABI3/VP), (4) ERF (ethylene-responsive-element-binding-factor) and (5) other proteins (Soloist) (Sakuma *et al.*, 2002). Here, we describe DREB and ERF.

8.1 DREB (dehydration responsive element binding protein)

DREB TFs play a critical role in improving the abiotic stress tolerance of plants by regulating many abiotic stress-responsive genes in an ABA-independent manner. First set of CBF1, DREB1A and DREB2A were reported in *Arabidopsis* (Stockinger *et al.*, 1997; Liu *et al.*, 1998). DREB TFs function by binding to dehydration-responsive element A/GCCGAC of cold and dehydration-responsive genes. DREB are divided into 6 sub-groups on the basis of structure characteristic (Sakuma *et al.*, 2002). Although DREB TFs are known as low temperature responsive genes (DREB1 and DREB2 are induced by cold and dehydration, respectively), their expression pattern in some plants is changed in response to different stress conditions. Arabidopsis CBF4 is responsive to drought stress, rice DREB1A is induced in response to salt stress, and *Arabidopsis* DREB1D, Vitis CBF1, Vitis CBF2 and *Vitis* CBF3 are responsive to ABA (Zandkarimi *et al.*, 2015). Our survey showed that DREB has relatively higher number of sequence deposits and patents since it has been possible to engineer stress tolerance in transgenic plants by manipulating the expression of DREBs.

8.2 ERF (ethylene responsive factor)

ERFs act as an important switch in activating or repressing the expression of target genes involved in plant responses to salt and drought stresses by specifically binding to cis-acting elements (Sakuma *et al.*, 2002; Licausi *et al.*, 2013; Thirugnanasambantham *et al.*, 2015). ERFs contain three plant specific transcription repressor motifs (Causier *et al.*, 2012) and interact with MYB, WRKY and undergo post-transcriptional modification like alternative splicing, phosphorylation, and epigenetic regulations (Thirugnanasambantham *et al.*, 2015). Relatively higher number of sequences in the databases and patents indicate that ERFs have been well researched and extensively used in plant genetic engineering.

9. ZF (Zinc Finger)

ZF proteins, with phytohormone or abiotic stress-related cis-elements in their promoter regions, are mainly involved in abiotic stress tolerance in plants. ZF proteins are small proteins having anti-parallel β turn, fingertip and α helix region. Based on the number and order of His and Cys residues binding to Zn ion in the secondary structure, they are divided into nine types; C2H2, C2HC5, CCCH, C4, C8, C6, C4HC3, C3HC4, C2HC (Laity *et al.*, 2001). Control of the ROS level resulting from salt, drought or oxidative stress is critical for abiotic stress tolerance in plants. ZF proteins play critical role in ROS scavenging by regulating the expression of stress-activated genes in plants via ROS signalling (Zhou *et al.*, 2010; Nechushtai *et al.*, 2012). Consequently, ZF proteins confer abiotic stress tolerance by increasing the contents of ABA, proline, soluble sugars or chlorophyll, and reducing the water loss rate. AtTZF, a ZF protein, interacts with MARD1 and RD21A to perform its functions in seed germination responses (Bogamuwa and Jang, 2016). ZF proteins are regulated by stress due to

drought, salt, cold, heat, UV, cation, heavy metal and oxidative state (Barth *et al.*, 2009; Min *et al.*, 2013; Sakai *et al.*, 2013). Few members are involved in cell mediated death and DNA repair under stress conditions (Lin *et al.*, 1999; Epple *et al.*, 2003). ZF proteins have been worked upon very extensively resulting in relatively large number of sequence deposits and patents and more significantly, the highest publications on any abiotic response protein in plants.

10. CAMTA (Calmodulin binding transcription activator)

CAMTA is known to exhibit rapid (<15 min) expression responses to abiotic stress and phytohormones (Yang and Poovaiah, 2002). CAMTA was first reported as negative regulator in *Arabidopsis*, CAMTA3 (AtSR1) (Galon *et al.*, 2008). Conserved domains of CAMTA include NLS N-end, CG-1 domain, TIG domain, Ankyrin repeats and variable IQ motifs at C-end. Spacing between domains is variable but domain organization is conserved amongst proteins (Thompson *et al.*, 1997). CAMTA induced by salt, temperature, light stress and production of H2O2 molecules have been identified in *Arabidopsis* and mustard (Bouche *et al.*, 2002; Reiland *et al.*, 2009). CAMTA is a relatively new family of TF known to be involved in biotic and abiotic stress response and sequences and knowledge are being accumulated.

miRNAs and plant tolerance to abiotic stress

miRNAs are a class of small endogenous RNA molecules of 20 and 24 nucleotides in length. Rice, for instance, has >700 mature miRNAs (http://www.mirbase.org). miRNAs can be conserved across all plants or species specific. Both conserved and species-specific miRNAs may play an important role in plant abiotic stress response (Zhang, 2015). Laboratory experiments have suggested that abiotic stresses induce the aberrant expression of miRNAs. For instance drought induced miR168 in *Arabidopsis* but inhibited in rice. Similarly, salinity stress induced the overexpression of miR156 in *Arabidopsis* but inhibited in maize. Participation of miRNAs became evident by draught induced repression of miR169 expression and whereas stimulation of its target NF-Y subunit A5. miRNAs do not function directly in abiotic stress response. Instead, majority of stress-responsive miRNAs target TFs involved in plant response to abiotic stresses and participate through regulating them. Furthermore, phytohormones regulate miRNA–target gene networks facilitating plant's abiotic stress response. More than a dozen experiments of overexpressing miRNAs exhibited higher tolerance to different abiotic stress factors (Zhang, 2015). Allelic variation in miRNA genes and its role in abiotic stress response remain to be explored and is a great area of work for genebanks.

Epigenetic Regulation during Stress

Epigenetic regulation entails nucleosome assembly/ disassembly, histone variants replacing canonical histones, histone N-terminal modifications (phosphorylation, methylation, acetylation, ubiquitination, and sumoylation), ATP-dependent modifications, DNA methylation and non-coding RNA mediated regulation (Crisp *et al.*, 2016). DNA and histone modifications are known to play a significant, yet undeciphered, role in gene expression and plant development under stress (Viswanathan and Zhu, 2009). Often, the stress-induced epigenetic changes are reset to the basal level once the stress subsides, while some of the changes continue to exist to be carried forward as 'stress memory' and inherited within generation (mitotic) or trans-generational (meiotic). 'Epigenetic stress memory' helps crops cope with subsequent bouts of stress. Histone modification as in drought-induced expression of stress-responsive genes is associated with an increase in H3K4 trimethylation and H3K9 acetylation in *Arabidopsis* (Kim *et al.*, 2008) and DNA hypermethylation was induced by drought stress in pea (Labra *et al.*, 2002). During the time of stress, the plant chromatin undergoes epigenetic (heritable changes) regulations and depending on stability of configuration achieved by chromatin remodelling, the impact of stress can either be short lived or long last as per plant stress memory. Comparative studies on stress-responsive epigenomes and transcriptomes will enhance our understanding of stress adaptation of plants. The adaptive value of stress-induced epigenetic plasticity can be further studied by identifying epigenomic variations among tolerant germplasm.

Comparative Analysis of Research and IPRs in Plant Abiotic Stress Response Genes

The analysis is based on 3425 deposits of 14 gene/protein sequences known to be involved in abiotic stress response in plants; 1025 research publications reporting them; and 457 patent applications (and grants) on their intended commercial use (Fig. 2). It is natural to expect numerical gap between isolation of a gene sequence (resulting in Genbank deposits), characterization and demonstration

of function (resulting in publications) and exploitation of commercial utility (resulting in patent applications). Maximum number of sequence deposits was in HSP (1413) and sequences were from *Arabidopsis*, tomato, rice and potato. Other major sequence deposits were in ZF with 608 sequences from *Arabidopsis*, soybean and tomato, and ERF with 164 sequences mostly of *Arabidopsis*. Interestingly, in case of WRKY and bHLH, soybean had as many sequence deposits as *Arabidopsis* (Fig. 2). Research papers were maximum in ZF (270) followed by HSP (167) and LEA (107). The greatest gap between deposition and publication is in genes coding for HSP (1413 vs. 167) followed by ZF (608 vs. 270). Notably, however, the numerical gap between deposits and publications is not uniform across all crops and all genes studied (Fig. 3). Sequence deposits of HSPs are found in all the plant species analysed unlike the case of other genes where sequences deposited are predominantly of *Arabidopsis*. First-hand information on gene discovery in *Arabidopsis* has, more often than not, resulted in research papers. For instance, in HD-Zip and LEA with only *Arabidopsis* sequence deposits, no gap exists. Total number of deposits of other genes increased because of the heterologous amplification based on *Arabidopsis* sequence information. However, most of these sequences appear not to have novelty or non-availability of mutants for downstream experimentation as in *Arabidopsis*. These sequences are waiting to be exploited in germplasm of crop plants and hence a great opportunity for generation and use of genomic resources. Number of sequence deposits gives an idea about the direction and scope of research in the area of plant abiotic stress. However, number of publications will be produced with sufficient genetic, physiological and biochemical data that needs greater resources like mutants and transgenic testing. It may be noted that sequences of promoters and other cis-elements of these genes are not included in the present analysis but remain a very significant component. Opportunities remain aplenty to identify allelic variation in germplasm and publications thereof.

It was evident that commercial interest of the R&D set up, working in developing abiotic stress resilient crop varieties, lies largely in regulatory genes (Fig. 2) than in structural genes (Aquaporin, LEA and HSP) possibly due to amenability of regulatory genes to genetic engineering. Maximum number of patents is sought in MYB (79) followed by ZF (65).Unlike research publications, where *Arabidopsis* dominates the scene, in patent canvas of abiotic stress tolerance genes, soybean has significant number of patent applications followed by rice seeking protection related to NAC, DREB, bZIP, MYB and ZF (Fig. 4). Commercial interest is evident in DREB (maize and wheat) and AP/ERF and WRKY in maize. A perusal of which countries are entertaining and granting patent protection to abiotic stress tolerance genes of plants gives a conclusive picture (Fig. 5). China, already forged ahead of the USA in research investment and publications, is a clear leader to protect commercial interests with 126 applications and 40 grants. Although USA follows as a distant second with 59 applications and seven grants, it is South Korea, with 19 grants, appears to have recognized the commercial significance of plant abiotic stress tolerance genes in future plant breeding.

India is not in the picture because gene sequences are not patentable under Indian regulation. However, country's preparedness for protecting business and trade of breeding abiotic tolerant crops can be achieved by obtaining IP protection either in USPTO or WIPO. With patenting of plant abiotic stress tolerance genes and uses thereof becoming a norm and availability of advanced genetic engineering and synthetic biology techniques to utilize them in breeding, sovereign ownership of trait-specific genetic resources can become redundant. It is therefore indispensable for genebanks to accelerate generation of genomic resources and their IP protection to ensure 'freedom to operate' in breeding abiotic stress tolerant crop varieties in public institutions.

Perspectives for Employing Genomic Resources related to Abiotic Stress Tolerance in Screening Genebank Accessions

Genomic resources include inter alia DNA markers like SSRs and SNPs singly or as an array in combination with indels and methylation sites; gene and EST sequences; chromosome libraries, QTLs, genetic and physical maps as well as whole genome sequences. Genomic resources have the potential to accelerate trait discovery and aid molecular breeding for enhanced utilization of diverse genetic resources including CWRs. Genomic resources offer a head start to crops with little genetic characterization and evaluation data to develop core and reference sets as well as introgression lines allowing genebanks to enhance their utilization. As a result genebanks around the world have invested in next generation genome sequencing and generation of

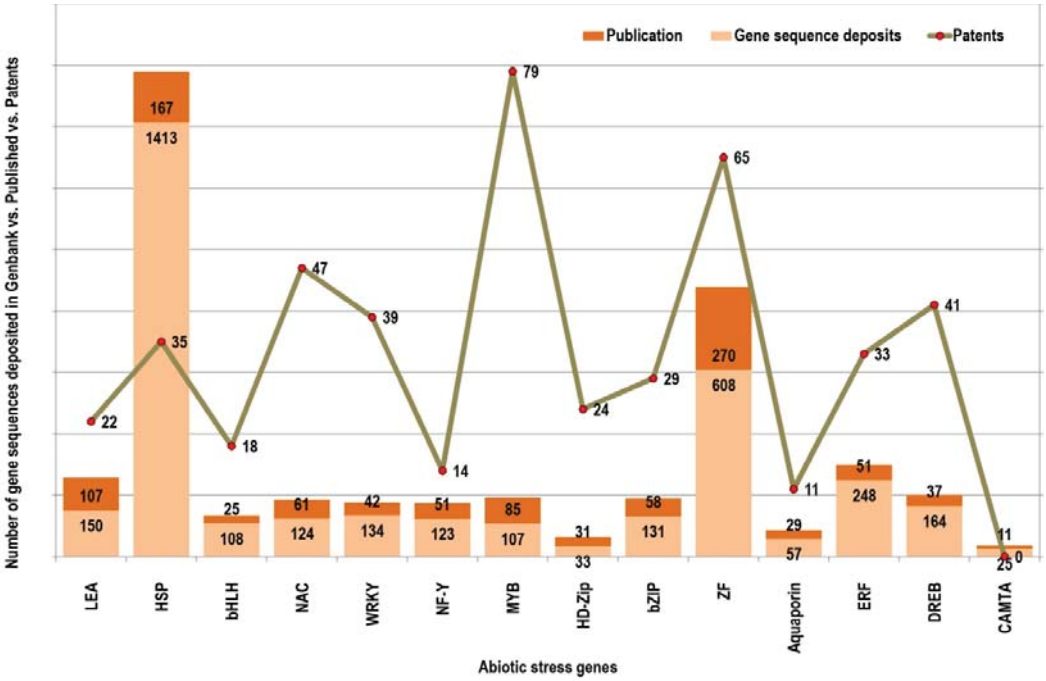

Fig. 2. Comparative analysis of research and IPRs in 14 plant abiotic stress response genes. Data source: Genbank (sequence deposits and publications); Google Patents (patents).

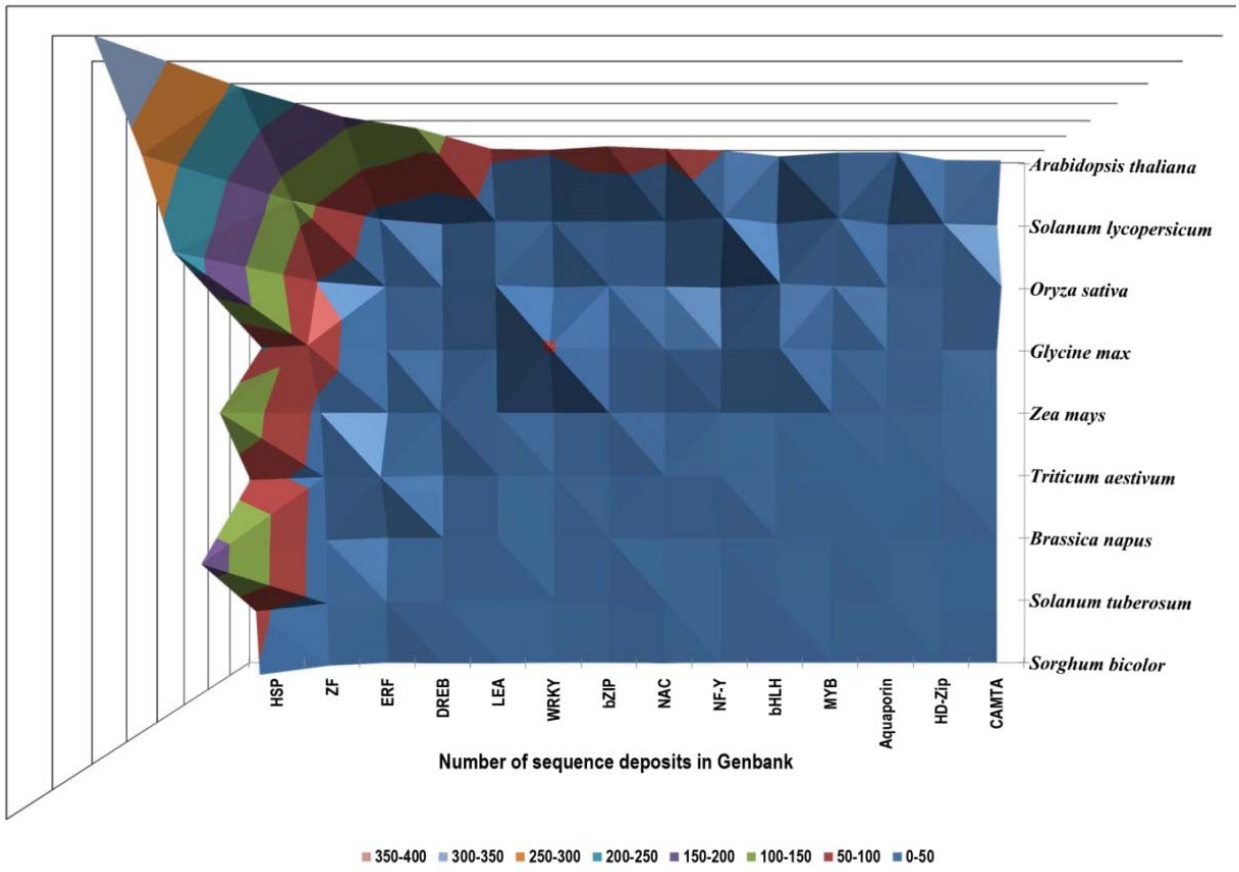

Fig. 3. Sequence deposits of 14 plant abiotic stress response genes in nine species. Data source: Genbank.

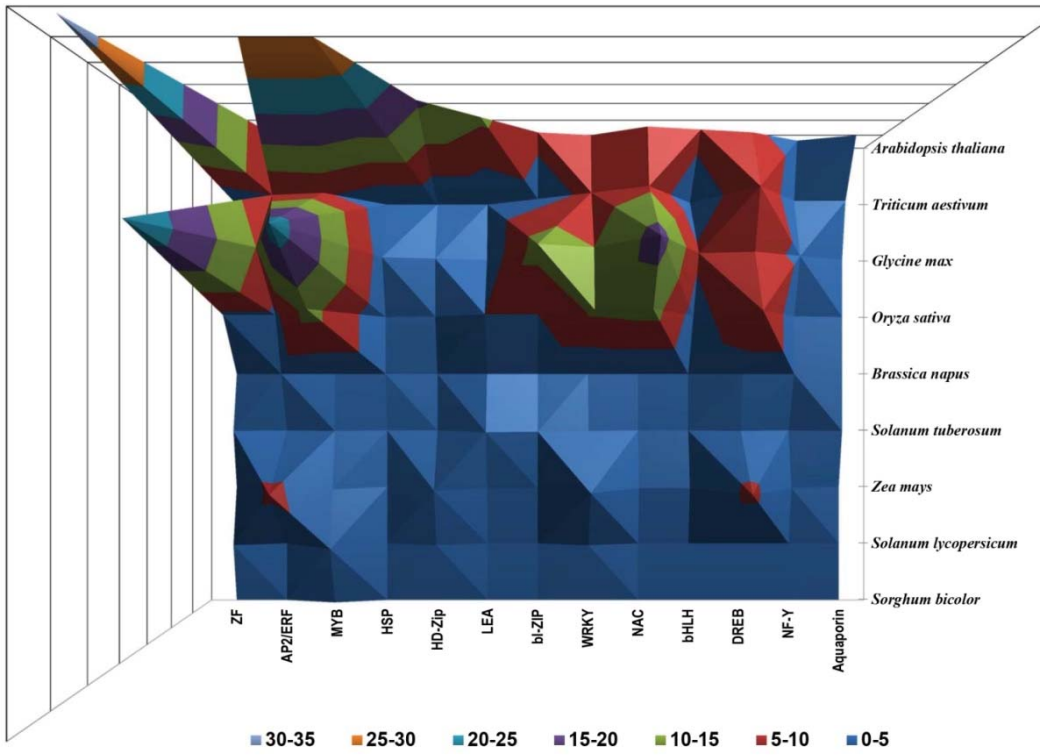

Fig.4. Species wise distribution of patent applications/grants on 13 plant abiotic stress response genes. Data source: Google patents.

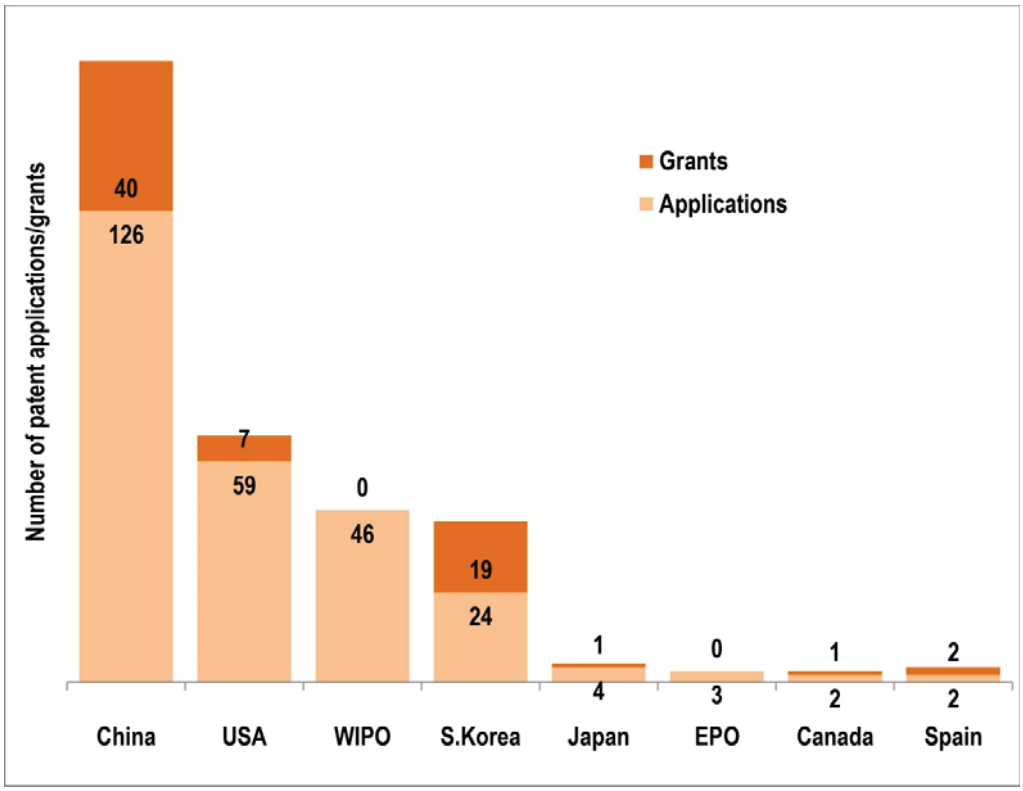

Fig.5. Patent applications/grants related to 13 plant abiotic stress response genes across patent offices. Data source: Google patents.

genomic resources in the public domain (Varshney *et al.*, 2010).

Trait-specific evaluation experiments may fail to identify all the contributing alleles in genebank accessions. It is also possible that genotypes carrying rare alleles are lost during regeneration cycles due to poor adaptation or poor agronomic performance. Genomic resources, on the contrary, allow deploying the specific gene sequences from heterologous sources to mine superior alleles from the germplasm. Screening germplasm for accessions tolerant to a complex trait like tolerance to various forms of abiotic stress requires equipping curators with gene-based or gene-linked markers. This in turn requires genebank researchers to be acquainted with cutting edge knowledge on plant genes known to participate in abiotic stress response pathways. Information provided in this review is expected to guide genebank curators keen on employing genomic resources for screening germplasm against heat and moisture stresses.

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SHORT COMMUNICATION

Characterization of Sorghum (*Sorghum bicolor* (L.) Moench) Germplasm under CRP-Agro Biodiversity

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One thousand sorghum germplasm accessions were evaluated and characterized at SDAU Deesa (Gujarat) under Consortium Research Platform on Agro-biodiversity and majority of accession showed good early vigour, dark green leaves, non-tan type, drooping leaf orientation, white mid rib color, non-senescence, awn less, semi compact ear head, oblong ear head shape, half glum covered, bold and white seeded. The quantitative characters also showed wide variation and the potential germplasm may be utilized for further breeding program.

Key Words: Agro-biodiversity, Correlation, Genetic Variation, Germplasm

Plant genetic resources collection and characterization provides researcher with essential genetic variation to develop new varieties that can significantly impact agricultural systems. Germplasm characterization and documentation are important activity in plant genetic resources management and it eases data retrieval and short listing accessions for the genetic improvement. Germplasm utilization is increased if detailed characterization data is obtained for individual accessions, which should include qualitative and quantitative phenotypic traits, genotypic data, and responses to biotic and abiotic stresses. Detailed characterization data is lacking for more than 50% of the sorghum collections, which emphasizes the need for further data collection. Characterization and evaluation of sorghum accessions are the pre-requisite for the utilization of available diversity in the sorghum crop improvement programme. The study of relationships among quantitative traits is important for assessing the feasibility of joint selection of two or more traits and hence for evaluating the effect of selection for secondary traits on genetic gain for the primary trait under consideration. A positive genetic correlation between two desirable traits makes the job of the plant breeder easy for improving both traits simultaneously. Hence, the present study was under taken by the ICAR-Indian Institute of Millets Research (IIMR), Hyderabad through the All India Coordinated Research Project on Sorghum at Sorghum Research Station, Sardarkrushinagar Dantiwada Agriculture University, Deesa under Consortium Research Platform on Agro-biodiversity to characterize 1000 accessions of sorghum germplasm. The variability present in the germplasm was studied through preliminary characterization for different quantitative and qualitative traits provide essential data to researchers for development of new high yielding varieties and parental lines in sorghum.

The trial was grown in Augmented Block Design (ABD) during *Kharif* 2016 at Sorghum Research Station, Sardarkrushinagar Dantiwada Agricultural University, Deesa, Gujarat (72ºE longitude, 24.5º latitude, 136 m altitude above MSL). These accessions were divided into twenty blocks and each block consisted of 50 accessions with two check varieties viz., CSV 15 and CSV-21F. The soil of the field was sandy in texture with pH value of 7.5 to 8.00 having good physical and chemical properties (Organic Carbon= 0.23, EC dsm- = 0.232, K_2O= 259.9 kg/ha and P_2O_5= 46.2 kg/ha). The experimental unit was a single-row plot of 2.5 m long, spaced at 0.60 m apart. NPK 120:40:00 fertilizers was applied as half basal dose of nitrogen and full dose of phosphorus at the time of sowing and half nitrogen applied after one month of sowing. Plots were thinned down

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after two weeks of crop emergence and plant-to-plant distance of 0.10 m was maintained. The experimental year showed different temperature regimes, humidity, rainfall and sunshine hours during the crop duration. All the other recommended agronomical packages and practices were followed to raise a good crop. Data was collected based on the minimal descriptors released by NBPGR, New Delhi (Mahajan *et al.*, 2000) and the list of sorghum descriptor released by Anonymous (1993). Five representative plants in each accession were tagged for recording the qualitative and quantitative traits. A descriptive statistical analysis and correlation estimate was done for the quantitative characteristics only.

The data of preliminary characterization of 1000 accessions of sorghum germplasm were recorded for 22 agro-morphological characters revealed a wide range of variability in both quantitative and qualitative characters. The range of variability and frequency observed in qualitative characters are given in Table 1. Majority of the accessions showed good early vigour (580 acc.), dark green leaves (457 acc.), non-tan type (956 acc.), drooping leaf orientation (741 acc.), white midrib color (902 acc.), awn-less (353 acc.), non-senescence (309 acc.), semi compact earhead (520 acc.), oblong earhead shape (109 acc.), white (65 acc.), bold seed (109 acc.) and half glume covered seed (147 acc.).

The quantitative characters also showed wide variation in the characterized sorghum germplasm. In the quantitative traits, days to 50% flowering (23.00-100.00 days), total number of leaves (5.00-25.200), leaf length (38.40-145.60 cm), leaf width (3.14-11.62 cm), ear head length (5.94-45.20 cm), ear head width (1.58-22.10 cm), plant height (56.00-368.60 cm), 100-seed weight (1.00-5.27 g), dry-fodder yield (26.00-924.80 g) and grain yield (23.00-100.00 g) showed wider ranges. The preliminary characterization and descriptive statistics revealed that dry-fodder yield, plant height, grain yield, leaf length, days to 50% flowering and ear head length were the most variable characters because they showed higher variance and standard deviation. Earlier reports by Elangovan *et al.* (2009, 2013), Seetharam and Ganesamurthy (2013) and Sujatha and Pushpavalli (2017) have also exhibited the presence of variation for different quantitative characters in sorghum germplasm accessions.

Generally, yield is the complex characters controlled by several components which reflect positive and negative effect on this traits. Thus, to achieve regular enhancement in the yield and component traits, understanding of mechanism of association provides a foundation for formulating suitable breeding approaches for enhancing the yield. On the basis of the present data on grain yield per plant, showed positive and significant correlation with days to 50% flowering (+0.430), total number of leaves (+0.251), leaf length (+0.349), leaf width (+0.428), ear head length (+0.144), ear head width (+0.301), 100-seed weight (+0.311) and dry-fodder yield per plant (+0.021) while plant height (-0.602) showed negative correlation with grain yield which help to identify high seed yield genotypes. High positive and significant correlation was observed between days to 50% flowering with total number of leaves per plant (+0.377), leaf length (+0.590), leaf width (+0.166), plant height (+0.645), grain yield (+0.430) and dry-fodder yield per plant (+0.500) which helps in identifying early genotypes with high biomass.

Table 1. Frequency distribution of sorghum germplasm for different qualitative traits

Characters	Frequency	Characters	Frequency
Early vigour		6. Broom comb	1.00
Poor	46.00	Glum covering	
Good	580.0	1. One fourth	136
Very good	376.00	2. Half covering	147
Leaf color		3. ¾ covering	24
Pale green	148.00	Grain color	
Dull green	395.00	White	65.00
Dark green	457.00	Straw	30.00
Leaf sheath pigmentation		Light brown	46.00
Tan	46.00	Brown	6.00
Non tan	956.00	Reddish brown	9.00
Leaf orientation		Light red	7.00
Erect	259.00	Red	21.00
Drooping	741.00	Dark red	11.00
Leaf mid rib color		Purple	46.00
White	902.00	Black	25.00
Green	76.00	Straw &brown	2.00
Yellow	22.00	Straw & purple	4.00
Ear head compactness		White and red mix	27.00
Compact	29.00	Grain size	
Semi compact	125.00	Small	96.00
Loose	97.00	Medium	94.00
Very loose	25.00	Bold	109.00
Ear head shape		Presence of Awn	
Round	1.00	1. Absent	353
Oblong	109.00	2. Present	30
Ovate	75.00	Stay green color	
Elliptical	28.00	Non senescence	309
Cylindrical	84.00	Senescence	44

The dry-fodder yield was also significant and positively correlated with days to 50% flowering (+0.500), total number of leaves (+0.351), leaf length (+0.545), leaf width (+0.228) and plant height (+0.602). The other component traits were also showed inter-correlated with each other which will also help in selection of suitable sorghum genotypes. Based on the mean some of the accessions showed outstanding performance for different agro-morphological traits viz., early flowering (EC487069 and EC486957 with 23 and 43 days) total number of leaves (EC486946 and EC487120 with < 19.6), leaf length (EC487226 and EC487500 with < 104.4 cm), leaf width (EC487499 and EC487501 with < 10.1 cm), ear-head length (EC487023 and EC487205 with < 42.7 cm), ear-head width (EC486919 and EC487091 with < 14.80 cm), plant height (EC487273 and EC487116 with < 316.2 cm), 100-seed weight (EC486884 and EC487085 with < 4.9 g), dry fodder yield (EC487409 and EC486839 < 696.0 g/ plant) and seed yield (EC486956 and EC486809 with < 139.2g). These accessions with potential for different agro-morphological traits may be utilized in crop improvement program for developing superior varieties and parental lines.

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SHORT COMMUNICATION

Agro Morphological Diversity in Local Rice (*Oryza sativa* L.) Collections from Different Altitudes of Uttarakhand Hills

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Rice (*Oryza sativa* L.) is a major food crop of Uttarakhand hills grown under rainfed upland as well as irrigated ecosystem in different altitudes ranging from 250 m to 2500 m above mean sea level. A huge diversity of this crop exists in this geographical region. Two hundred one hill rice collections from low (≤950 msl), mid (951-1500 msl) and high (≥1501 msl) altitude of Uttarakhand were evaluated and characterized for 12 quantitative characters along with 5 checks during Kharif 2015. The result showed a considerable range of variability for phenological traits viz., days to 50% flowering (71-106 days) and days to maturity (102-130 days) as well as for other important traits and yield components viz., stem length (29-99cm), panicle length (12-26cm), kernel length (4.61-7.41mm), kernel width (1.58-3.13mm), LB ratio (1.13-3.87mm), plant height (48-121cm), grains per panicle (24-178), flag leaf length (16-29.5cm), flag leaf width (1-1.9cm) and grain yield per plant (0.04-7.07g). As per principal component analysis, first four principal components (PC) expressed 70.61% of total variation in which PCI, PCII, PCIII and PCIV accounted for 27.48, 17.90, 16.37 and 8.85% of total variation, respectively. Highly significant positive correlation was observed between PCI with panicle length (0.46), number of grains/plant (0.46), LB ratio (0.36), grain yield/plant (0.29), flag leaf width (0.18), kernel length (0.18); PC II with plant height (0.47), stem length (0.43), Kernel length (0.37), flag leaf length (0.29), grain yield/plant (0.28), number of grains per panicle (0.26), panicle length (0.21), days to maturity (0.17) ; PC III with kernel width (0.57), days to maturity (0.53), days to 50% flowering (0.31), flag leaf width (0.27) and PC IV with flag leaf length (0.66), days to 50% flowering (0.55) and kernel length (0.18). The plotting of first and second principal component axes scores revealed the dispersion among the hill rice accessions and confirmed the existence of variability among these which might have arisen because of diverse agro climatic conditions and altitudinal differences.

Key Words: Altitude, Diversity, Rice (*Oryza sativa* L.)

Introduction

Rice (*Oryza sativa* L.) is the major cereal crop of kharif season accounting for about 31 per cent of the total area under cereals in the state of Uttarakhand. It is the first choice among the existing crops of the season as a source of food, also intimately related with all the religious, cultural and social functions of the life of hill people. The annual rice production of the state is around 6.0 lakh tonnes from an area of about 2.61 lakh hectares (DES, 2016) and nearly half of this area is in the plains and the rest in hills. Owing to the availability of irrigation facilities in plenty, the total rice production in the plains is double that of hills. In Uttarakhand, traditionally spring rice (Chaiti Dhan) cultivation (sown during March end to early April and harvested in the month of September under upland rainfed ecosystem) and Jethi Dhan (sown at onset of monsoon and harvested by end of September/October) are practiced which is confined only to its hill region. Traditional cultivation practices are still followed in this region and despite the extension of improved varieties, local traditional varieties are still grown in the region because of their well adaptability in various agro-climatic conditions (Bisht *et al.*, 2007). Rice cultivation in Uttarakhand hills is still dominated by traditional cultivars like "Thapacheeni, China4, Lal Dhan, Candolia, Thapuli, Chauria, Kusi Pai, Shiaun, Danshau, Jyoli, Rajma Dhan, Nan Dhan, Godi Dhan, Sau Dhan" etc. (Aditya, 2016). Several rice landraces evolved through generations to suit the local conditions of farms, reflect socio-cultural preferences and they are identified by various vernacular names which have been nurtured and conserved by farmers in high-risk environments of hills (Nautiyal *et al.*, 2002; Rana *et al.*, 2009). Traditional farming systems have served to

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preserve the diversity of varieties along with the human knowledge and cultural practices that have shaped this diversity (Bellon, 1991). Rice is cultivated in all the 13 districts of the state in varied agro-ecological niches situated at the low (≤950 msl), mid (951-1500 msl) and high (≥1501 msl) altitude in both irrigated and rainfed conditions. However, the aggressive introduction of modern varieties in the region has resulted in the loss of many valuable landraces particularly those which were grown in lowlands and valleys than rainfed uplands. Besides, fragmented land holdings, changing land use and weather patterns, and shift from subsistence agriculture to commercial agriculture have increased the rate of genetic erosion of valuable rice land races in hills (Rana *et al.*, 2009). The variability occurring in traditional varieties is useful for hill farmers to cope with heterogeneous microclimates and as gene sources in rice improvement programmes (Mehta *et al.*, 2014). The traditional varieties of local rice possess wide range of variability and it is, therefore, pertinent to capture and conserve the genetic diversity existing in the rural and tribal areas to be studied for utilization of these paramount resources in the varietal improvement programmes. The present study was designed with the aim to document the rice diversity in 201 rice accessions collected from all thirteen districts of state of Uttarakhand so that these can be evaluated, documented, utilized and conserved for rice research and development in the future.

Materials and Methods

A total of 201 accessions of rice collected from varied altitudinal range viz., low (≤950 msl), mid (951-1500 msl) and high (≥1501 msl) hills of Uttarakhand were evaluated under mid-hill condition at ICAR-VPKAS experimental farm, Hawalbagh, Almora (29.360N and 79.300E situated at an elevation of 1,250 m above mean sea level) during Kharif 2015 in augmented block design with five checks VL Dhan 85, VL Dhan 65, Vivek Dhan 154, IR 64 and Bali. The checks were randomized evenly in each block. Each germplasm and check was grown in rows of 2 m length and spaced 20 cm apart. All the recommended cultural practices were followed to raise a healthy crop. Observations on principal phenological stages were recorded at 50% of flowering and 80% maturity, respectively on whole plot basis. The remaining quantitative traits viz., stem length (cm), panicle length (cm), kernel length (mm), kernel width (mm), L/B ratio, plant height (cm), grains per panicle (No.), flag leaf length (cm), flag leaf width (cm) and grain yield per plant (g) were recorded on three randomly selected plants in each genotype following standard procedures.

Statistical analysis was performed for 12 quantitative descriptors and range and mean were calculated for each group of the collection and variation within group expressed as coefficient of variation (Zhong and Qualset, 1995). Principal Component Analysis (Rao, 1984) was performed to examine the percentage contribution of each trait to total genetic variation; correlation coefficients between principal component axis scores and the original mean values performed to know traits contributing maximum variability using computer software XL STAT 12 version. First and second principal component axes scores were plotted to aid visualization of origin group differences and to detect morphological variation within collection using SYSTAT 13 version.

Results

In the present study, moderate to high variability was observed for quantitative traits in 201 accessions of rice. Maximum range was observed for quantitative traits (Table 1) viz., grains per panicle (24-178), stem length (29-99cm) and days to 50% flowering (71-106 days) as well as for other important traits and yield components viz., days to maturity (102-130 days), panicle length (12-26cm), kernel length (4.61-7.41mm), kernel width (1.58-3.13mm), LB ratio (1.13-3.87mm), plant height (48-121cm), flag leaf length (16-29.5cm), flag leaf width (1-1.9cm) and grain yield per plant (0.04-7.07g). Coefficient of variability was highest for grain yield per plant (52.52 %) followed by number of grains per panicle (34.07 %) and lowest for days to maturity (5.85%). For traits viz., days to 50% flowering (8.24%), kernel length (9.97%) and days to maturity (5.85%) per cent coefficient of variation was observed less than 10 percent hence, it is suggested that these traits having less variability can be improved through introgression of variability from other sources. The rest of the traits ranged in CV value from 12.04% for kernel width to 52.52% for grain yield per plant. Presence of sufficient amount of variability for traits like stem length, panicle length, kernel width, L/B ratio, plant height, grains per panicle, flag leaf length, flag leaf width and grain yield per plant suggest improvement of these traits through selection. Data on all the traits were subjected to Principal Component Analysis and transformation of quantitative traits into principle component yielded 12 Eigen vectors and Eigen roots. First Eigen vector

Table 1. Genetic diversity in hill rice accessions collected from low, mid and high altitudes of Uttarakhand hills

Trait	Mean	Range	Std. deviation	CV%
Days to 50% flowering	84.48	71-106	6.96	8.24
Stem length (cm)	72.32	29-99	13.85	19.15
Panicle length (cm)	19.37	12-26	2.53	13.07
Kernel length (mm)	5.79	4.61-7.42	0.58	9.97
Kernel width (mm)	2.41	1.58-3.13	0.29	12.04
L/B ratio	2.43	1.13-3.87	0.42	17.36
Days to maturity	115.02	102-130	6.73	5.85
Plant height (cm)	91.77	48-121	14.82	16.15
Grains per panicle (No.)	79.13	24-178	26.96	34.07
Flag leaf length (cm)	22.94	16-29.50	2.87	12.50
Flag leaf width (cm)	1.35	1.0-1.90	0.20	15.14
Grain yield per plant (g)	2.28	0.04-7.07	1.20	52.52

having highest eigen value 3.29 and only first four of the twelve principle component axes (PCA) had given eigen value >1. The first four principle component axes (PCA) together accounted for 70.61% of total variance and PCI, PCII, PCIII and PCIV accounted for 27.48%, 17.90%, 16.37% and 8.85% of total variation respectively. PCA I had significant positive correlation with the traits viz., panicle length (r=0.461), number of grains per panicle (r=0.460), L/B ratio (r=0.365), grain yield per plant (r=0.298), flag leaf width (r=0.185) and kernel length (r=0.180). PCA II mainly defined by plant height (r=0.477), stem length (r=0.439), kernel length (r=0.378), flag leaf length (r=0.295), grain yield per plant (r=0.289) and grains per panicle (r=0.267). PCA III was determined by kernel width (r=0.575), days to maturity (r=0.539), days to 50% flowering (r=0.316) and flag leaf width (r=0.272). Similarly, PCA IV explained by traits viz., flag leaf length (r=0.664), days to 50% flowering (r=0.550) and kernel length (r=0.185). PCA I, II and IV were positively correlated with kernel length whereas, none of the trait was found correlated with all four Principal components (Table 2).

The presence of the broad spectrum of genetic diversity for different key traits of agronomic importance in rice accessions from Uttarakhand hills (Table 3) implies ample opportunity for the genetic improvement of this crop. Accessions namely, IC355825 and IC 355826 were found promising for both the phenological traits days to 50% flowering (<75 days) and days to maturity (< 105 Days); IC 316069 was found promising for traits namely short stem length (90-95cm) and flag leaf length (>28cm) whereas, accession IC 356426 had L/B ratio (>3) as well as flag leaf length (>28cm) and can be utilised as ready use material for the genetic improvement of rice.

Discussion

Local hill rice genotypes showed significant variation for all the agro-morphological traits considered under present investigations. This may be due to

Table 2. Correlation coefficients between the first four principal components (PCI, PCII, PCIII & PCIV) and different agro morphological traits of hill rice accessions

Variables	PC I	PC II	PC III	PC IV	Eigen value	Variability (%)	Cumulative (%)
Days to 50% flowering	0.021	-0.240**	0.316**	0.550**	3.29	27.48	27.48
Stem length (cm)	-0.332**	0.439**	-0.236**	-0.041	2.14	17.90	45.38
Panicle length (cm)	0.461**	0.215**	0.032	-0.198**	1.96	16.37	61.76
Kernel length (mm)	0.180**	0.378**	0.098	0.185**	1.06	8.85	70.61
Kernel width (mm)	-0.045	0.050	0.575**	-0.339**	0.89	7.43	78.04
L/B ratio	0.365**	-0.209**	-0.256**	-0.060	0.84	7.03	85.07
Days to maturity	-0.298**	0.173*	0.539**	-0.164*	0.68	5.74	90.81
Plant height (cm)	-0.285**	0.477**	-0.252**	-0.012	0.57	4.76	95.57
Grains per panicle (No.)	0.460**	0.267**	0.044	-0.145	0.45	3.80	99.37
Flag leaf length (cm)	0.064	0.295**	0.069	0.664**	0.05	0.45	99.82
Flag leaf width (cm)	0.185**	0.120	0.272**	0.115	0.02	0.17	99.99
Grain yield per plant (g)	0.298**	0.289**	0.053	-0.033	.001	0.01	100.0

*, ** Significant at 0.05% and 0.01% probability level, respectively.

different genetic constitution of genotype and can be utilised for improvement of hill rice in future breeding programme. To develop segregating population, genetic distance estimates form the basis for selecting parental combinations with sufficient genetic diversity and for classifying germplasm into heterotic groups for hybrid crop breeding it is necessary and fundamental to understand the magnitude of variability in the population (Nachimuthu *et al.*, 2014). The variability in the rice accessions collected from various parts of the world in respect of plant height, number of tillers, number of grains/panicle, 100 grain weight, days to maturity, panicle shape, awning, husk colour and shattering quality were documented (Rana *et al.*, 2009; Kumar *et al.*, 2010).

The variation within groups expressed as per cent coefficient of variation, gives rough estimate of diversity and variability within groups (Furman *et al.*, 1997).

Genetic variability is of great interest to the plant breeder as it plays a vital role in framing a successful breeding programme (Mehetre *et al.*, 1994).

Principal components axis (PCA) with eigen value >1 were considered in determining the agro-morphological variability in accessions (Kaiser, 1960; Jeffers, 1967).

The biplot (Fig. 1) of PCI and PCII scores distributed the accessions and confirmed the presence of variability among the accessions which could arise due to altitudinal influences as well as differential selection pressure practiced by the hill farmers. However, some overlapping among accessions from low (≤950 msl), mid (951-1500 msl) and high (≥1501 msl) altitude was observed owing to prevailing high levels of stress factors in agro-ecological zones tend to exhibit more homogenous genotypes (Demissie and Bjornstad, 1996).

The indigenous rice varieties may contain a considerable genetic diversity that can serve as a source of germplasm for genetic improvements of cultivated varieties of rice. Diverse landraces traditionally cultivated by farmers and domestication of crops are considered as key natural resources important for maintaining the future to address food and nutritional security issues (Choudhury *et al.*, 2013).

Conclusion

The presence of the broad spectrum of genetic diversity for different key traits in rice accessions from Uttarakhand hills implies ample opportunity for the selection of ready to use material for the genetic improvement of rice. From the present study, accessions viz., IC282814, IC355825, IC355826 can be utilized in breeding programme in order to develop short duration varieties whereas, IC338660, IC419861, IC316065, IC356426, IC393079, IC419862, IC281505 will be helpful for the improvement of grain quality of hill rice.

Table 3. Promising accessions of hill rice for 12 quantitative traits from diverse altitudes of Uttarakhand hills

Quantitative Traits	Promising Accessions	Altitude (m asl)
Days to 50% flowering (<75 days)	IC282814	900
	VASHM-CR-3235	1632
	IC355825	1500
	IC355826	1425
	IC419856	1703
	IC419857	1703
Stem length (90-95cm)	IC393078	1250
	IC282802	1400
	IC316063	200
	IC316067	400
	IC316069	425
	IC356462	850
	IC355830	1300
	IC355831	1200
Panicle length (> 25 cm)	IC281520	1600
	IC356420	875
Kernel length (>7mm)	IC393079	1470
	IC419862	1542
	IC281505	1100
Kernel width (< 2 mm)	IC338660	1700
	IC419861	1542
L/B ratio (>3)	IC316065	1600
	IC356426	875
Days to maturity (<105 days)	IC282814	900
	IC355825	1500
	IC355826	1425
Plant height (115-121cm)	IC281774	1680
	IC282798	1180
	IC316067	400
Grains per panicle (No. >150)	IC419899	1792
	IC338674	1650
	IC337346	1875
Flag leaf length (>28cm)	IC316069	425
	IC356426	875
	IC316066	2200
Flag leaf width (>1.8cm)	IC393067	2000
	IC282846	640
	IC356434	950
Grain yield per plant (>5.5g)	IC419902	1792
	IC281775	1680
	IC393071	1320

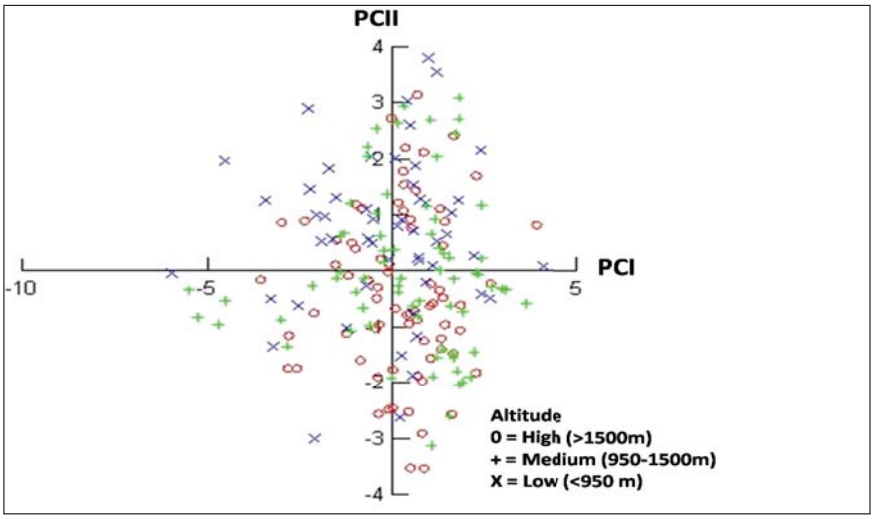

Fig. 1. Scatter plot of 201 hill rice accessions collected from diverse altitudinal range of Uttarakhand hills using PCI and PCII scores (value in parenthesis explains percentage of variation)

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