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SPECIAL ARTICLE

Global Seed Vault: A Pledge to Mankind

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The Svalbard Global Seed Vault was opened in 2008, as a storage facility for duplicates of seed samples that are conserved in gene bank collections. Many gene banks have faced threats that often caused loss of plant genetic resources due to war and conflicts, natural disasters or lack of human or economic resources.

By mid-2019 the Svalbard Global Seed Vault holds today close to one million seed samples that are back-up copies of seeds that are conserved in regular gene banks. The seeds are the property of the depositor gene banks, and the owner gene bank can have the seeds back whenever they might need them.

Key Words: Gene bank, Plant genetic resources, Seed, Svalbard Global Seed Vault

Introduction

Seed gene banks conserve plant genetic resources with the purpose of securing genetic diversity for research, plant breeding and development of agriculture and food production. Genetic diversity in crops is considered to be crucial for future food security, and maximum security for the resources is required. Gene banks face threats and witness incidents that cause loss of plant genetic resources such as war and conflicts, natural disasters or lack of human or economic resources.

To meet the need for securing the resources Norway has built the Svalbard Global Seed Vault as a facility for free of charge conservation of duplicates of seed samples that are stored in gene banks. The Seed Vault was established after significant international support and seed deposits are made in accordance with international agreements and with a depositor agreement between the depositing gene bank and the Norwegian Ministry for Agriculture and Food.

Storing Seeds in the Arctic

The story about the Svalbard Global Seed Vault started already in 1984 when the Nordic Gene Bank put backup samples of the Nordic seed collection in a coal mine in Svalbard. Svalbard is a Norwegian archipelago of islands in the Arctic, not far away from the North Pole. The grounds in Svalbard provide constant permafrost conditions, securing that seeds remain frozen even without artificial cooling.

The Nordic solution gained significant international interest, and the idea of establishing a facility for back up samples of other national and international gene banks in permafrost was launched. After comprehensive

The NBPGR seed box in the Seed Vault shelves between boxes from other countries

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international support Norway presented the offer to build and manage a global seed vault in Svalbard at the FAO Commission on Genetic Resources for Food and Agriculture meeting in Rome, Italy in 2004. The Seed Vault was built and funded by the Norwegian government during 2007 and opened on the 26th of February 2008.

The Nordic Genetic Resource Centre (NordGen) was involved in the planning process and has been responsible for management and operation since the start. Thanks to significant joint efforts and financial support from the Global Crop Diversity Trust, many gene banks prepared and shipped seed samples for depositing at the Seed Vault already for the opening in 2008. Thanks to these efforts, 320,549 seed accessions from 22 gene banks were deposited in the Seed Vault already during the first year of operation.

Today, more than 11 years after its opening, the establishment of the Seed Vault has proven to be an unconditional success. Now, in 2019, 76 gene banks located in 58 different countries have deposited security seed samples in the Seed Vault. The depositor gene banks are international agricultural research centres, national and regional gene banks and gene bank collections held by universities, breeding and research institutes and NGO's. More than one million seeds samples have been deposited in the Seed Vault.

The Facility

The Seed Vault facility is unlike the Nordic security storage in the coal mine, constructed in virgin solid rock without any coal layers. The facility consists of a tunnel of about 100 m, a fore hall and three seed storage chambers, each with the capacity to store around 1.5 million seed samples. The Seed Vault is located 130 m above sea level, which is above worst case climate change scenario for sea level rise.

Artificial cooling secures that the temperature in the seed storage is maintained at -18^{o}C, which is in accordance with the FAO Gene Bank Standards. Permafrost in the soils secure that seeds stay frozen even if the cooling system should fail.

Conserving seeds in the Seed Vault is a free of charge service. All gene banks making their genetic resources available for research, breeding and education are invited to conserve backup copies of their seed collections in the Seed Vault. Seeds are stored in sealed seed boxes and only the owner gene banks can get access to the seeds.

The Seed Vault is owned by the Norwegian government and the management is the responsibility of NordGen. Funding is secured through a three party agreement between the Ministry, Crop Trust and NordGen.

The operation of the Seed Vault is overseen by an International Advisory Panel reporting to the Ministry. The Svalbard Global Seed Vault is considered to be a vital part of the global system for conservation and use of plant genetic resources, as stated by the International Treaty for Plant Genetic Resources for Food and Agriculture. (ITPGRFA 2017).

Cooperation NBPGR and NordGen

As having one of the world's largest national gene banks, NBPGR is in particular invited to deposit security copies of seed samples in the Seed Vault. By the end of 2018, NordGen Director and Seed Vault Coordinator visited the NBPGR headquarter and Gene Bank in Delhi. The Nordic visitors were informed about the comprehensive National Gene Bank in India holding about 438,000 seed accessions, in addition to germplasm cryopreserved and conserved *in vitro*.

NBPGR Director Kuldeep Singh and NordGen Director Lise Lykke Steffensen met at Dehli in November 2019

Cooperation between the NBPGR in Delhi and the Svalbard Global Seed Vault was established already in 2008, when NBPGR signed the Seed Vault depositor agreement with the Norwegian Ministry of Agriculture and Food. The first seed deposit was made in 2014 and so far, the gene bank has in total deposited 225

seed samples of pigeon pea (*Cajanus cajan*), rice (*Oryza sativa*) and sorghum (*Sorghum bicolor*). Plans for conserving security samples of the 26,000 NBPGR accessions that are registered in the Multilateral System (MLS) of the Plant Treaty in the Seed Vault have been included in NBPGR and NordGen discussions.

Seed Vault coordinator Åsmund Asdal putting the seed packages from NBPGR into a standard box before inserting in the seed storage

About NordGen

The Nordic Genetic Resource Center is a Nordic organization dedicated to the safeguarding and sustainable use of genetic diversity within crop plants, farm animals and forest trees. The Nordic countries have been co-operating for more than 30 years on conservation of genetic resources, and NordGen was established in January 2008 as a result of a merger between Nordic Gene Bank, the Nordic Gene Bank Farm Animals and the Nordic Council for Forest Reproductive Material. NordGen is mainly financed by the Nordic Council of Ministers. The Nordic Gene bank for plants was established back in 1979.

NordGen is a Nordic knowledge centre and its primary task is to contribute to securing the broad diversity of genetic resources linked to food and agriculture. This is done through conservation and sustainable use, solid documentation and information work and through international cooperation and agreements.

NordGen is today one of few regional gene banks in the world, covering gene bank activities for neighbouring countries in a geographical region. The SADC Plant Genetic Resources Centre (SPGRC) gene bank in Lusaka, Zambia, is the other major regional gene bank. SPGRC is facilitating conservation of seeds and other regional cooperation carried out at national centres for plant genetic resources in 16 countries in southern Africa. NordGen and SPGRC have through decades cooperated on regional gene bank developments and projects.

The Nordic seed collection conserved at NordGen consists of 35,000 unique accessions representing 530 different crop species and wild plant species related to crops.

NordGen is managing the so called *Public Private Partnership for Pre-Breeding* (PPP) project which is funded by the Nordic countries and gathers research and plant breeding companies in the countries in joint efforts for improving plant genetic material for agriculture in the region. The PPP project has sub-projects for apples, barley and rye-grass which are important crops for the Nordic agriculture and horticulture. The project is an important link connecting gene bank material to practical plant breeding and agriculture.

NordGen distributes every year between 8,000 and 12,000 seed samples to users in the region and abroad, within research, breeding and training, but also to farmers and hobby growers that want to cultivate this unique material.

Seed Vault Deposits

NordGen is aiming at securing its entire seed collection in Svalbard Global Seed Vault. At the moment more than 70% of the seed collection has been duplicated in the Seed Vault.

After nearly twelve years of operation, the total number of deposited seed samples in the Seed Vault has reached 1,078,673. The major part, about two thirds, has been deposited by international agricultural research centres (CGIAR institutions). Four of these have deposited more than 100,000 samples each: CIMMYT (International Maize and Wheat Improvement Center) in Mexico, IRRI (International Rice Research Institute) in The Philippines, ICRISAT (International Crop Research Institute for the Semi-Arid Tropics) in India and ICARDA (International Institute for Agricultural Research in Dry Areas), which until recently had its genebank in Syria.

The largest depositors among national genebanks are the USA, Germany, Canada, Australia, the Netherlands, South Korea and Switzerland. Genebanks in several developing countries have deposited seeds as well; Mali,

Nigeria, Sudan, Uganda, Zambia, Burundi, North-Korea, Myanmar and Pakistan, among others.

The Seed Vault now holds samples of about 5,000 different species. Rice and wheat are represented by more than 150,000 seed samples each. Further, 15 major cereal, vegetable and forage crops are represented by more than ten thousand seed samples.

So far, only one institute has requested seeds to be returned. This took place in the autumn 2015, when ICARDA, which until then had its headquarter in Aleppo, Syria, lost access to its gene bank due to the ongoing conflict in Syria, and needed seeds from Svalbard to establish new functional genebanks at ICARDA units in Lebanon and Morocco. Seeds from the Seed Vault were shipped back on three occasions, and have been sown and multiplied at these sites. Since the spring of 2017, ICARDA has already re-deposited new seeds in the Vault on four occasions. Withdrawals and redeposits of seeds by ICARDA imply that the current number of seed samples secured in the Vault by September 2019 is 962,186.

Open Invitation

Gene banks are invited to ship seeds to Svalbard on three or four regular Seed Vault opening occasions every year. Every year since 2008 between 12 and 29 institutes have deposited seeds. Many of these have deposited seed several times, as part of a comprehensive program for securing their seed collections. NordGen maintains a web portal database where transparent data about all deposited seed samples, depositor institutes, countries of origin etc. is displayed, i.e. the Seed Portal at https://www.nordgen.org/sgsv/.

The main conditions for depositing seeds in the Seed Vault imply that the depositing gene bank shall conserve the original and primary seed sample in its own long-term gene bank. Only orthodox seeds of crops and their wild relatives can be accepted for storage in the Seed Vault. The genetic material should be available for breeding and research in accordance with applicable international law. Storing seed samples in the Seed Vault is free of charge, and the seeds remain the property of the depositor. The seeds are returned to the depositor on request if the accessions are lost or inaccessible from their own or from cooperating gene banks' repositories. Only the depositing institution can obtain access to the seeds it has deposited in the Vault.

Ever since the Seed Vault opened it has been a great attraction. It has drawn attention to the issues on conservation and sustainable utilization of plant genetic resources. Influential politicians and policy makers have regularly paid visits to Svalbard and the Seed Vault and there has been a continuous great interest from many of the world's leading media to tell the story about the global undertaking of conservation and utilization of crops and their wild relatives.

The Seed Vault is now probably Norway's internationally most well-known building and it has been an iconic symbol of the importance of taking care of plant genetic resources. The ultimate goal is that all unique plant genetic material conserved in gene banks is secured and copied in the Svalbard Global Seed Vault. The long term security of deposited seeds is guaranteed for by the Government of Norway.

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SPECIAL ARTICLE

Phyto-diversity and its Conservation Assessment in Permafrost Condition at Extreme Altitude of Trans-Himalaya in Leh-Ladakh, India

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Efforts have been made to identify the suitable natural condition for long-medium term storage of horticultural crops and rare threatened species of the region, seeds cost effectivity, by comparing different natural storage conditions including controlled chamber maintained temperature at -18ºC and the other at ambient natural conditions at different locations i.e., permafrost are Chang-La, situated at the height of 17,600 ft. in trans-Himalayan region with subzero temperature for most part of the year, Leh location where temperature also remains at-subzero for half the year and Chandigarh having a tropical climate. The 16 important horticulture crops and four medicinal plants representing the dicotyledonous seeds were assessed for different storage conditions for their germination percentage and longevity. The germination of stored seeds was monitored after five years. The results showed that all the accessions were able to maintain their seed germination percentage very close to their original values in the two locations i.e., Chang-La and Leh. Suggesting that some horticulture crops and rare medicinally important species could be stored *in-situ* for long and medium-term conservation at natural low temperature at Leh and at extreme altitude 17,600 ft above sea level permafrost facility at Chang-La.

Key Words: Chang-La, Leh, Conservation, Horticulture, Germplasm, Locations, Permafrost.

Introduction

Conservation of seeds ex-situ underpins many of the recovery efforts being undertaken to help preserve rare threatened and poorly known plant species. The international standard for long-term storage for seed conservation is at -18ºC, with seed moisture content between 3 and 7% depending on the species (Genebank Standards, 1994). These storage conditions apply only to seeds of orthodox species (Roberts, 1973), which are able to survive desiccation to such low moisture levels.

Storage of plant genetic resources (PGR) at large scale as per a standard protocol would be a very expensive and more over malfunctioning of such facilities results in loss of valuable PGR, but still maintenance of such storage conditions is essential for future security of food and nutrition. As far as expense and technical problems of such facilities is concerned, experiments were carried out by Singh *et al*. (2008) on storage of seeds at natural permafrost conditions in Himalayan region for long-term storage to facilitate cost effective conservation of plant genetic resources (PGR). In the present study, experiments were conducted in the natural permafrost location at Chang-La in Ladakh trans-Himalayan region situated extreme north of India (Jammu and Kashmir), 90 km from Leh town at the elevation of 17,600 feet above mean sea level where ambient temperature remain (-12.49±7.86 ºC), in a cold desert conditions especially at higher altitudes where temperature remain sub-zero and low relative humidity for most part of the year except in summer months. By taking an advantage of such natural low temperature, a storage facilities has been built for long-term storage of seeds, including control where the facility is given a low temperature of -18 ºC (L-I) following standard protocol for long-term storage and other locations built at ambient conditions: Chang-La (L-II), Leh (L-III) and Chandigarh (L-IV) conditions.

Materials and Methods

Seed samples of 20 representative accessions from horticultural crops and medicinal plants were taken. Seed material was processed as per the established genebank standards (IBPGR, 1994) i.e., checked for physical purity, germination percentage and dried to a moisture level ranging between 5 to 7 per cent depending on the type of crop/species. 500 seeds were kept in each packets in three replication and stored at different locations i.e., L-I, L-II, L-III and L-IV. The

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packets were vacuum sealed in aluminium doubled layered sheets. The aluminium packets containing the seed material were kept in perforated plastic baskets and stored for five years. The seed materials were retrieved for monitoring of germination after five year storage, the seed quality (germination percentage) was monitored following ISTA rules (Anonymous, 1999) for standard germination test, with a modification of using three replications of 100 seeds each. The initial seed germination test was conducted after cleaning and drying the accession within two-three months after receipt of the sample in the genebank. The initial germination tests were done by following genebank standards with modification of 100 seeds (Genebank Standards, 1994).

In the present study, out of 20 species representing the dicotyledonous seeds 16 are horticulture crops and 4 are medicinal plants. Sample collection and their storage was completed in 2011 from field of Defence Institute of High Altitude Research, Leh and from the wild, including French bean var. Pusa Parvati, chickpea var. Amazing, okra var. Varsa, cucumber var. Pusa Sanyog, parsley var. Petra, brinjal var. Mukta, tomato var. Sultan, coriander var. Caribi, Summer squash var. Pusa alankaar, water melon var. Ayashi tamato, vegetable mustard var ARU black, carrot var. Nantes, celery var. triumph, true potato seeds, cabbage var. Gonzals, sunflower, brinjal var. Mukta, *Verbascum thapsus, Hypericum perforatum, Inula racemosa, Achillea millefolium*. The local taxa were identified by flora of Ladakh (Polunin and Stainton, 1989; Kachroo, 1997). All seeds were cleaned and dried to avoid weed seeds, pests and associated diseases. To achieved the moisture level of 3-7%, the seeds were dried at 10-15% relative humidity at 10-15% ºC, following (FAO, 2014).

Longevity of the seeds was calculated following standards of ICAR-National Bureau of Plant Genetic Resource (ICAR-NBPGR) on the basis of critical value of 85% viability (Trapa *et al.,* 2012; Sharrock *et al.,* 1998) (Table 3).

All the samples were analysed for ANOVA using SPSS version 17. The level of significance was tested at 5% among locations on the basis of seed performance for germinations in all species (Table 2).

Results and Discussion

Significant variations in seed quality were recorded in location L-I, L-II, and L-III when compared to initial values, except for Chandigarh during the five years storage of among vegetable crops and medicinal plants presented as per ANOVA estimation for each locations on the basis of different groups of plant germination percentage P=0.05. Details of accessions used, seed moisture content at the time of seed storage and mean germination percentage registered in seed samples of different accessions, at different locations over five years are presented in Table 2.

A total of 19 accessions were stored in different conditions, out of which 16 accessions were of major horticulture crops and 4 were of medicinal plants. The monitoring of seed germination in accessions of these crops showed the following trend during their storage. Out of 16 horticulture crops, cabbage, celery, cucumber, tomato, chick pea, parsley maintained the seed quality near to their original values in the three locations except at Chandigarh. Vegetable mustard: the stored accession at different locations showed initially increase in germination percentage up to 5%, in L-1 and L-II and then showed decreases in germination percentage to 51% when compare to initial germination. Carrot: the accession stored showed increased 4% germination at L-I and decreased to 1%, 9% at L-II and L-III respectively. True potato seeds: The stored seeds showed increase in germination by 15% from original value at L-I and L-II, 10% at L-III. French bean: accessions showed decrease in germination by 37%, 70% and 89% at L-I, L-II and L-III respectively from the original value. Water melon: accessions showed drastic increase in germination from 63% to 99%, 99%, 98% in L-I, L-II and L-III respectively. Summer squash: accessions showed 100% germination at L-I, L-II and L-III, an increase of 4% in germination from the original value. Sunflower: accessions showed very poor initial germination and increase by 12% in L-I and gradual decrease by 21% and 23% in L-I and L-II, respectively. Brinjal: accessions stored showed increase in germination percentage from 96% to 99%, 98%, 98% at L-I, LII and L-III respectively. Coriander: accessions showed increase from 71% to 90%, 90%, 88% at L-I, L-II and L-III respectively. Medicinal plants: *Inula racemosa* showed significant increase in germination percentage from 48% to 99%, 99%, 97% at L-I, L-II and L-III respectively. *Hypericum perforatum* showed slight increase from 41% to 49%, 47%, 47% at L-I, L-II and L-III respectively. *Achillea millefolium* showed drastic increase from 58% to 93%, 89%, 81% at L-I, L-II and L-III respectively and last accession *Verbascum thapsus* also showed drastic increase from 42% to 87% at L-I, L-II and L-III (Fig 2).

Table 1. Temperature and relative humidity at various locations surveyed for identification of site for zero energy based conservation 2011

Months	Changla RF Temperature (ºC)	Changla NRF Temperature (ºC)		Leh Temperature (ºC)		Chandigarh Temperature (ºC)	
		Min	Max	Min	Max	Min	Max
January	-18	-25.4	-22.2	-17	-5	12.8	18.9
February	-18	-22.0	-19.1	-15	-1	14.8	21.0
March	-18	-20.1	-15.7	-13	0	19.4	26.0
April	-18	-14.7	-10.2	-9	16	26.7	34.6
May	-18	-11.7	-6.7	9	31	31.1	38.8
June	-18	-6.2	-2.0	13	32	33.0	39.6
July	-18	-0.8	4.2	14	33	30.5	34.9
August	-18	0.3	4.5	8	29	28.8	32.9
September	-18	-4.5	-0.9	7	27	28.5	33.4
October	-18	-14.2	-10.9	-7	14	24.9	32.0
November	-18	-15.3	-11.3	-11	12	19.0	26.4
December	-18	-15.3	-11.7	-20	-6	14.1	20.7

Table 2. Seed germination at five year intervals in seed samples of various crops stored at different locations in 2011-2016

S. No.	Horticulture crops	Moisture Content (%)	Initial germination %	Avg germination % of Changla RF P=0.04	Avg germination % of Changla NRF P=0.02	Avg germination % of DIHAR Leh P=0.02	Avg germination % of DIHAR Chandigarh P=0.46
1	Cabbage	4.9	98.67	97.33	95.67	94.17	31.33
2	Vegetable mustard	4.1	92	97.33	97.33	96	41.33
3	Carrot	4.4	81.33	85.33	80	72	42
4	Celery	5.46	82	81.33	78.67	77.33	51.12
5	True potato seed	7.4	82.5	97.33	97.33	92	24
6	French beans	6.4	100	62.67	30.12	20.23	10.67
7	Cucumber	5	92	91	90.67	90.67	22.33
8	Water melon	4.35	62.67	99.33	99.33	98	21.33
9	Summer squash	6.3	96	100	100	100	32.5
10	Okra	5.7	85.33	97.33	91	96.33	21.67
11	Tomato	5.86	96	92.67	89.33	87	21.12
12	Sunflower	4.2	53.33	64.67	32	29.67	27.33
13	Chick pea	7.5	91	90	85	84.67	39.33
14	Brinjal	5.8	96	98.67	98.33	98.33	22.67
15	Coriander	7.8	70.6	89.67	89.67	87.67	21.67
16	Parsley	5.4	76	74	72.67	71.65	26
Medicinal Plants							
1	*Inula racemosa*	5.8	48	98.5	98.67	97.42	37.33
2	*Hypericum perforatum*	6	41	49.33	46.67	46.53	10.27
3	*Achillea millefolium*	5.6	58	93.33	89.33	81.33	32.67
4	*Verbascum thapsus*	6.9	42	87	86.67	86.67	33.33

As expected all the accessions showed drastic decreases in germination percentage at L-IV, Table 2 (Fig 1). It has been suggested that there would be degradation of alleles of germplasm after it reached upto 85% of critical viability (Trapa *et al.,* 2012; Sharrock *et al.,* 1998) (Table 3). It is therefore important to regenerate the base sample when it reached to its critical value. We evaluated the longevity of the each samples stored at different locations, accordingly in refrigerated module the longevity of the celery is highest 112 years (yrs), cucumber 75 yrs, chickpea 75 yrs, cabbage 56 yrs, parsley 38 yrs and tomato 23 yrs till they reach to critical value at 85% deterioration. In outside natural module the maximum survivability is of carrot, cucumber 56, followed by cabbage 25, parsley, celery 20, chickpea 13 and tomato 11 years. In Leh condition the highest

Table 3. Longevity of the seeds at different locations.

Name of crop	Test treatments											
	Initial Germination % 2011	Chang-la inside module			Chang-La outside module			DIHAR Leh			DIHAR, Dett. Chandigarh		
		Germmination % 2016	Deterioration % in 05 yrs	Longevity @ 85%	Germ. % 2016	Deterioration % in 05 yrs	Longevity @ 85%	Germination % 2016	Deterioration % in 05 yrs	Longevity @ 85%	Germ. % 2016	Deterioration % in 05 yrs	Longevity @ 85%
Cabbage	98.7	97.33	1.34	56	95.7	3	25	94.2	4.5	16.7	31.3	67.3	1.1
Vegetable mustard	92	97.33	ND	ND	97.3	ND	ND	96	ND	ND	41.3	50.7	1.5
Carrot	81.3	85.33	ND	ND	80	1.3	56.4	72	9.3	8.03	42	39.3	1.9
Celery	82	81.3	0.67	111.9	78.7	3.8	19.6	77.3	5.2	14.5	51.1	24	3.1
True potato seed	82.5	97.33	ND	ND	97.3	ND	ND	92	ND	ND	24	58.5	1.3
French beans	100	62.67	37.33	2.00	30.1	69.9	1.07	20.3	79.8	0.94	10.7	89.3	0.8
Cucumber	92	91	1	75	90.7	1.3	56.4	81.3	10.7	7	22.3	69.7	1.1
Water melon	62.7	99.33	ND	ND	99.3	ND	ND	98	ND	ND	21.3	41.3	1.8
Summer squash	96	100	ND	ND	100	ND	ND	100	ND	ND	32.5	63.5	1.8
Okra	85.3	97.33	ND	ND	91	ND	ND	96.3	ND	ND	21.7	75.7	0.9
Tomato	96	92.7	3.33	22.5	89.3	6.7	11.2	87	9	8.3	21.1	74.9	1.0
Sunflower	53.3	64.7	ND	ND	32	21.3	0.0	29.7	23.6	4.7	27.3	25.9	2.5
Chick pea	91	90	1	75	85	6	12.5	84.7	6.3	11.8	39.3	51.7	1.5
Brinjal	96	98.7	ND	ND	98.3	ND	ND	98.3	ND	ND	22.7	73.3	1.0
Coriander	70.6	89.7	ND	ND	89.7	ND	ND	87.7	ND	ND	21.7	48.9	1.5
Parsley	76	74	2	37.5	72.7	3.3	22.4	71.7	4.3	17.24	26	50	1.5
Inula racemosa	48	98.5	ND	ND	98.7	ND	ND	97.4	ND	ND	37.3	10.7	7.03
Hypericum perforatum	41	49.3	ND	ND	46.67	ND	ND	46.5	ND	ND	10.3	30.7	2.4
Achillea millefolium	58	93.3	ND	ND	89.3	ND	ND	81.3	ND	ND	32.7	25.3	2.9
Verbascum thapsus	42	87	ND	ND	86.7	ND	ND	86.7	ND	ND	33.3	8.7	8.7

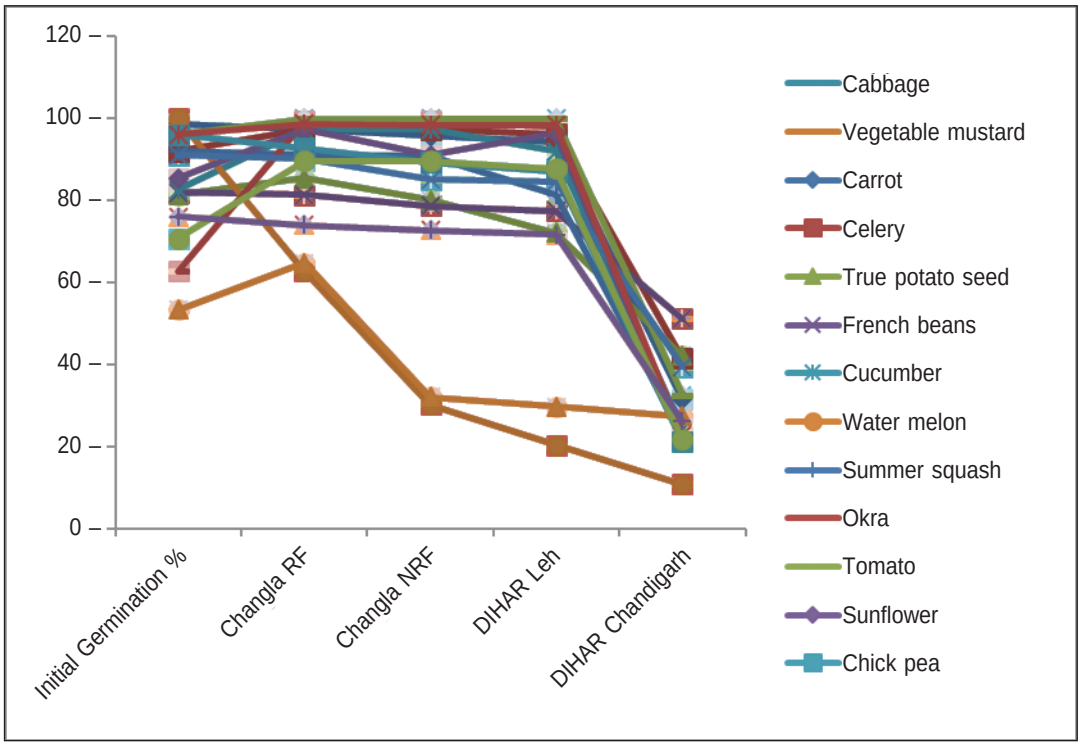

Fig 1. Seed germination of different vegetable crops stored under different storage conditions including controlled at -18 °C, Chang-la, Leh and Chandigarh.

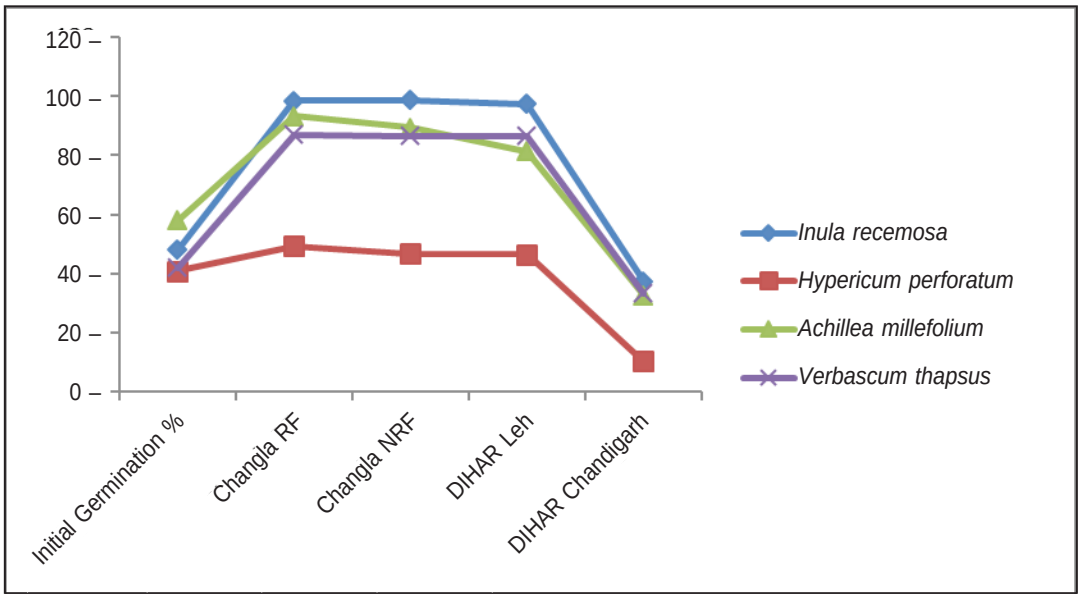

Fig 2. Seed germination of medicinal plants stored under different storage conditions including controlled at -18 °C, Chang-la, Leh and Chandigarh.

survivability was recorded in cucumber 56, followed by parsley 17 yrs, cabbage 17 yrs, chickpea 12 yrs, carrot 8 yrs and tomato 8 yrs and at Chandigarh longevity of all the seed were recorded very low except for *Verbascum thapsus* (Table 3). Some crops such as true potato seeds, water melon, summer squash, okra, brinjal, coriander and medicinal plants shows more or less increase in germination percentage in L-I, L-II and L-III from

their initial values, therefore they were not determine (ND) for the longevity, the increase in germination of the crops may be due to the physiological dormancy of the seeds, therefore the longevity of these crops will be analyse after another five years of storage. However, all the samples were drastically decrease in longevity in L-IV. Vegetable mustard showed increase in germination from initial value in L-I and L-II and gradual decrease, crops such as carrot and sunflower showed increase in germination percentage only in controlled module and observed considerably decreases in germination in L-II, L-II and L-IV (Table 3).

The study was conducted to explore the feasibility of long-term storage of orthodox seeds under natural low temperature conditions in the region, to facilitate cost-effective conservation of plant genetic resources. The critical evaluation of meteorological data using (tiny tag data logger) (Table 1), indicated that Chang-la pass situated at the height of 17,380 on a way to Pangong lake is the most appropriate place with sub-zero temperatures (–25^{o} to 0.4 ºC) most of the year, with mean temperature of (-12 ºC). Monitoring of the seed quality (germination percentage) in 16 and 4 different plants, belonging to horticulture crop and medicinal plants group respectively, stored under the four locations, three natural conditions, prevailing at Chang-La, Leh and Chandigarh and the controlled maintained-18 ^{o}C. Storage, starting from year 2011 to 2016 registered various trends in maintaining the seed quality between the accessions of the crop and wild species. This could either because of the genotypic differences among accessions or due to differences in physiological maturity of the seed materials, at the beginning of the seed storage. Most crop species are able to maintain the seed quality near to their initial values or increase in germination percentage from the initial value. The results were similar to those of the seed material stored under artificial low temperature conditions of -18 ºC in refrigerated chambers at Chang-la. Thereby, suggesting that in general all the crops could be stored under the natural low temperature conditions (permafrost) of Himalayan Region with similar level of success. However, differences may occur, mostly due to the differences in quality of seed samples at the start of seed storage or may be due to fluctuation of temperature in natural conditions. According to the longevity of each sample it may be recommended that, celery, parsley, cabbage, cucumber, and carrot can be stored at natural permafrost condition at Chang-la for long term storage at 20-56 years without any energy inputs. Tomato and parsley can be preserved for medium-term at natural low temperature at Leh conditions for 8-12 years. Some sensitive seeds can be stored in the refrigerated condition by utilization of small amount of energy at Chang-la.

Among various crops, the parsley, cabbage, cucumber, carrot, tomato and parsley depicted a consistent trend in maintaining the seed quality, very near to their original values at two natural locations, L-I and L-II. Vegetables which includes mustard, carrot, true potato seeds, water melon, summer squash, okra, brinjal and coriander and medicinal plants in fact showed a sharp increase in the germination, which perhaps continued to increase in subsequent monitoring. This may be because of variations in the physiological maturity or due to physiological dormancy of the seeds. On the other hand, French bean and sunflower, registered low seed germination during storage and showed decreases in germination in L-II L-III and almost nil in L-IV (Fig. 1).

Most of the vegetables, as solanceous, cucurbitaceous, cole crops, tuber crops, including medicinal plants depicted a consistent behavior in maintaining the seed quality in natural low temperature in L-I and L-II closer to their original values and showed almost similar trend with seeds stored in controlled chamber at -18 ^{o}C during the five years of conservation with an exception sunflower and French bean which need further evaluation.

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REVIEW ARTICLE

Characterization of Farmers' Varieties of Potato (*Solanum tuberosum* L.) of Cooch Behar District of West Bengal

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Three Farmers' Varieties of potato from Cooch Behar district of West Bengal viz., Pakri alu, Badami alu and Cooch Behar Local-1 have been identified. They are being cultivated by the local farmers since long. For registration under Protection of Plant Varieties and Farmers' Right Authority (PPV&FRA), New Delhi, these varieties were characterized following the "Guidelines for Conduct of Test for Distinctiveness, Uniformity and Stability on Potato (*Solanum tuberosum* L.)" published by PPV&FRA (2009). Twenty seven qualitative and eleven quantitative characters have been used to compare the distinctiveness of these cultivars. Significant variability was observed for plant height, number of matured tubers per plant, tuber length, tuber diameter, tuber weight and tuber yield. However, days to emergence, number of branches per plant, leaf length and leaf width showed insignificant differences among the Farmers' Varieties. The plant height ranged from 29.92 cm to 63.90 cm. Maturity of tuber was earliest (92.34 days) in Pakri alu followed by Badami alu (94.58 days) and Cooch Behar Local-1 (95.50 days). Highest yield was recorded in Cooch Behar Local-1 (16.57 t/ha) followed by Badami alu (15.85 t/ha) and Pakri alu (15.03 t/ha).

Key Words: Distinctiveness, Farmers' Varieties, Potato, PPV&FRA

Potato (*Solanum tuberosum* L.) is one of the most important food crops of India as well as of many other countries of the world. It produces more calories and protein per unit area with minimum time and water than most of the major food crops (Upadhya, 1995). West Bengal ranks second in potato production after Uttar Pradesh. The total production of potato in West Bengal was 13391240 MT (Food Processing Industries Survey, West Bengal–Potato, 2012-13) in the year 2010-11. It is estimated that 61.47% of the total potato production is used for table purpose, 21% as seed, 0.5% processed and only about 0.03% are exported while about 17% are lost during post harvest handling, marketing and storage. The quality is the most important for export of potato. The Farmers' Varieties (FVs), namely Badami alu, Pakri alu and Cooch Behar Local-1 (popularly known as *Deshi Alu*) of West Bengal satisfy the export quality. Besides export, these local cultivars fetch much higher price, about 2.0-2.5 fold higher in the local market as compared to the improved varieties.

Potato cultivars are easily distinguished on the basis of morphological traits. Phenotypic characterization in potato is done by assessing morphological variations in the flower, leaf and tuber characteristics (Huaman, 1991). Morphological characterization has been used for various purposes including identification of duplicates, studies of genetic diversity pattern and correlation with characteristics of agronomic importance (CIAT, 1993). The unique diversity in FVs of potato from West Bengal is well recognized for significant traits like taste, size and colour. Distinct characters of the farmers' varieties have ample importance during registration under PPV&FR Act (2001). Considering the importance of these facts, three farmers' varieties of Cooch Behar district have been characterized following the "Guidelines for the Conduct of Test for Distinctiveness, Uniformity and Stability on Potato (*Solanum tuberosum* L.)" of PPV&FRA (2009).

The material consists of three FVs of potato, namely Badami alu (PPV&FRA Reg. No. 2015/1744), Pakri alu (PPV&FRA Reg. No. 2015/1745) and Cooch Behar Local-1 (REG/2016/911). Multi-location experiments were conducted in the farmers' fields at Kayakhata (Salsalabari, Alipurduar district), Salmari (Hatiduara, Cooch Behar district) and Petlanepra (Sitalkuchi, Cooch

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Behar district) during *Rabi* 2014-15 and 2015-16. Sprouted tubers of each genotype were planted in rows with five rows for each genotype in each replication in randomized block design with eight replications. Standard agronomic practices compatible to the humid sub-tropic of Terai Zone (Roy, 2015) were adopted to ensure good crop growth. Data on different morphological characters were recorded on individual plant basis from 10 randomly selected plants in each replication for each genotype. Statistical analyses of data were conducted with absolute values, using cultivar as variables. The data were subjected to standard statistical methods of analysis of variance (ANOVA) using AgRes Statistical Software, (c) 1994 Pascal Intl Software Solutions, Version 3.01 and significant differences were compared by LSD.

Badami Alu

It is the most popular Farmers' Variety (FV) of potato in the northern part of West Bengal. The shape of the tuber is reniform and colour is reddish purple. The shape mimics the shape of ground nut (Fig. 1A), so locally it is known as *Badami Alu*. Predominant colour of the light sprout is pink and the shape is cylindrical (Fig. 1A). Foliage structure of the plant is semi-compact with hollow and angular cross section of the stem. Predominant stem colour is purple. Flesh colour of tuber is creamy with a predominant dark pink coloured ring along the cross section of tuber (Fig. 1B). Other salient features of this FV as per the descriptor as outlined by (PPV&FRA, 2009) has been given in Table 1.

Pakri Alu

Pakri alu also a popular FV of Cooch Behar, Jalpaiguri and Alipurduar districts of West Bengal. However, the area of production is much less than Badami alu and Cooch Behar Local-1. Cultivation of this FV is mainly concentrated in Sitalkuchi Sub-Division of Cooch Behar District. The shape of the tuber is round (Fig. 1C) and colour is whitish cream. The skin colour is not uniform, patches of dark and light whitish cream on the skin surface, so it is locally known as *Pakri*. Predominant colour of the light sprout is pink (Fig. 1C), shape is cylindrical. Foliage structure of the plant is semi-compact with hollow and round cross section of the stem. Predominant stem colour is purple. The flesh colour of tuber is creamy (Fig. 1D). Other details of this FV as per the descriptor as outlined by (PPV&FRA, 2009) has been given in Table 1.

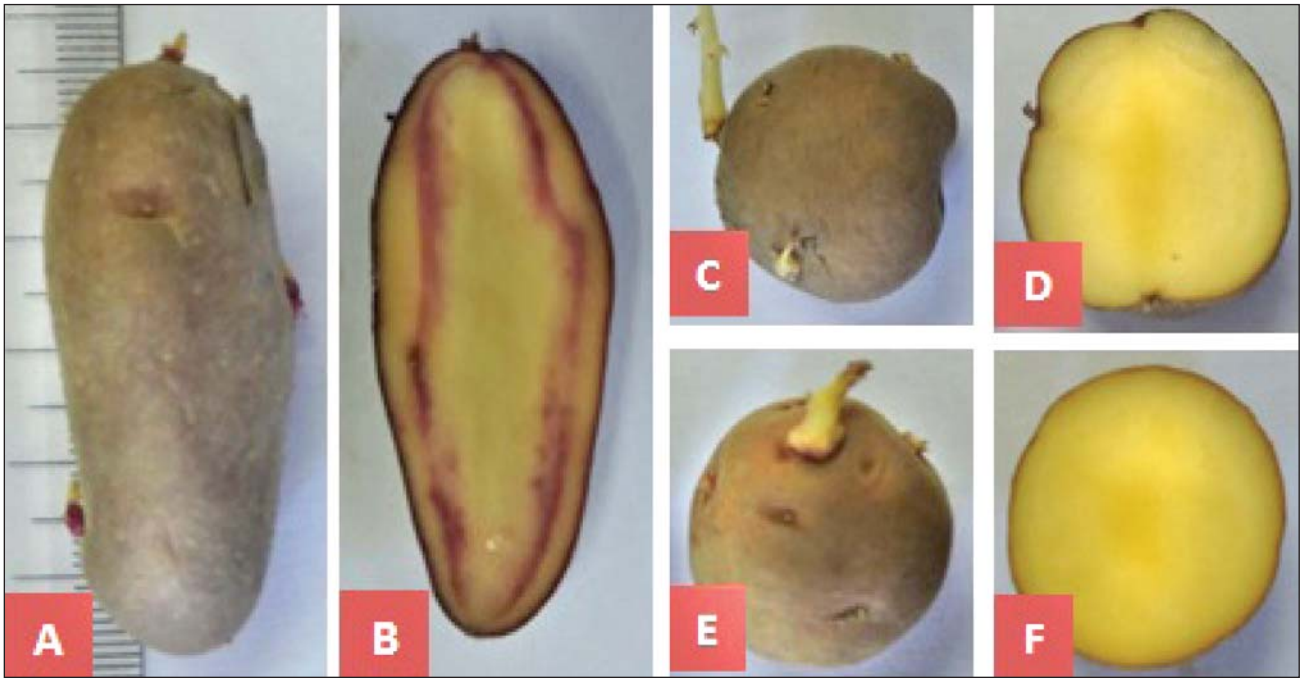

Fig. 1. Different qualitative characters of FVs of potato. A) Sprouting tubers of Badami alu; B) Cross section of tubers of Badami alu; C) Sprouting tubers of Cooch Behar Local-1 alu; D) Cross section of tubers of Cooch Behar Local-1 alu; E) Sprouting tubers of Pakri alu; F) Cross section of tubers of Pakri alu.

Table 1. Qualitative characteristics of Famers' Varieties of potato of Cooch Behar district of West Bengal based on the "Guidelines for

Conduct of Test for Distinctiveness, Uniformity and Stability on Potato (*Solanum tuberosum* L.)" published by PPV&FRA (2009)

Characteristics	Farmers' Varieties		
	Badami alu	Pakri alu	Cooch Behar Local-1
Light sprout: Predominant colour	Pink	Pink	Pink
Light sprout: Shape	Cylindrical	Cylindrical	Cylindrical
Light sprout: Intensity of anthocyanin colouration at base of sprout	Medium	Light	Light
Light sprout: Intensity of anthocyanin colouration at sprout tip	Medium	Medium	Medium
Light sprout: Pubescence base	Absence	Absent	Absent
Plant: Foliage structure	Semi-compact	Semi-compact	Semi-compact
Stem: Solidity	Hollow	Hollow	Solid
Stem: Cross section	Angular	Round	Angular
Stem: Predominant colour	Purple	Purple	Purple
Stem: Secondary colouration	Purple	Purple	Purple
Stem: Distribution of secondary colour	Through lightly scattered	Through lightly scattered	Through lightly scattered
Leaf: Structure	Intermediate	Intermediate	Intermediate
Leaf: Anthocyanin colouration of rachis	Absent	Absent	Present
Leaf: Anthocyanin colouration of midrib	Absent	Absent	Absent
Leaf: Leaflet (lateral) shape	Ovate	Ovate	Ovate
Leaflet: Waviness of margin	Weak	Weak	Weak
Leaflet: Glossiness of upper side	Weak	Weak	Weak
Leaflet: Pubescence of blade at apical rosette	Present	Present	Present
Flower: Anthocyanin colouration of bud	*	*	*
Tuber: Predominant skin color	Reddish purple	Whitish cream#	Pink
Tuber: Secondary skin colour	Red	Purple	Whitish cream
Tuber: Skin type	Smooth	Smooth	Smooth
Tuber: Shape	Reniform	Round	Round
Tuber: Depth of eye	Medium deep	Medium deep	Medium deep
Tuber: Predominant colour of flesh	Creamy	Creamy	Creamy
Tube: Secondary colour of flesh	Reddish purple	Creamy	Creamy
Tuber: Distribution of secondary colour of flesh	Vascular ring	Vascular ring	Vascular ring

*Till date, no flowering has been observed by the farmers

Whitish cream colour is not uniform, it is scattered with whitish cream and pink

Cooch Behar Local-1

This is also another popular FV of potato of northern part of West Bengal. This cultivar is also known as *Deshi Alu* (PPV&FRA Registration No. REG/2016/911). The size of the tubers of this cultivar is larger than the Pakri alu. It is being cultivated evenly in Cooch Behar, Alipurduar and Jalpaiguri districts. The taste of this FV variety is unique, so it fetches higher price in local market as compared to the modern high yielding varieties. The shape of the tuber is round (Fig. 1E) and colour is pink. Predominant colour of the light sprout is pink and the shape is cylindrical (Fig. 1E). Foliage structure of the plant is semi-compact with solid and angular cross section of the stem. Predominant stem colour is purple. The flesh colour of tuber is creamy (Fig. 1F). Other details of this FV as per the descriptor as outlined by (PPV&FRA, 2009) has been given in Table 1.

Plant height, number of matured tubers per plant, days to maturity, tuber length, tuber diameter, tuber weight and tuber yield showed significant variation among the varieties (Table 2). However, days to emergence, number of branches per plant, leaf length and leaf width exhibited insignificant variation among the cultivars. The number of tubers varied from 36.00 to 42.45 per plant. Highest number of tuber per plant was reported for Badami alu and minimum number of tubers per plant was observed for Pakri alu. However, the tuber weight was highest in Pakri (125.77 g) which is at par with the tuber weight of Cooch Behar Local-1 (125.37 g). Lowest tuber weight was for Badami alu (9.47 g). However, the tuber yield of Badami alu (15.85 t/ha) is higher than the Pakri alu (15.03) which may be due to higher number of tubers per plant in Badami alu (42.45) as compared to Pakri alu (36.00). Tuber yield is a complex character associated with many interrelated

Table 2. Mean values of quantitative characteristics of Famers' Varieties of potato of Cooch Behar district of West Bengal

Characters/varieties	Badami alu			Pakri alu			Cooch Behar Local-1		
	Mean values		State*	Mean values		State*	Mean values		State*
Days to emergence	20.77	a	-	20.34	a		20.50	a	-
Plant height (cm)	33.53	b	Small	29.92	c	Small	63.90	a	Medium
No. of branches/plant	8.93	a	-	9.13	a		9.14	a	-
Leaf length (cm)	4.40	a	Small	4.25	a	Small	4.36	a	Small
Leaf width (cm)	2.80	a	Narrow	2.82	a	Narrow	2.95	a	Narrow
Number of matured tubers/plant	42.45	a	-	36.00	c		38.96	b	-
Days to maturity	94.58	b	Medium	92.34	a	Medium	95.50	c	Medium
Tuber length (cm)	40.95	a	-	20.00	c	-	23.13	b	-
Tuber diameter (cm)	15.69	b	-	28.84	a	-	29.01	a	-
Tuber weight (g)	9.47	b	-	125.77	a	-	125.37	a	-
Tuber yield (t/ha)	15.85	b	-	15.03	c	-	16.57	a	-

Values bearing same letter in the row are not significantly different at P = 0.01 of LSD

*As per the Guidelines for the Conduct of Test for Distinctiveness, Uniformity and Stability on potato ((*Solanum tuberosum* L.), published by Protection of Plant Varieties and Farmers' Right Authority (2009), New Delhi.

components. Highest tuber yield was recorded for Cooch Behar Local-1 (16.57 t/ha). High yield with good quality is the most important objective in potato breeding (Fekadu *et al*., 2013). Farmers' varieties of potato are promising sources of desirable agricultural traits (Bamberg and del Rio, 2005). Primitive forms of cultivated potato and their wild relatives provide a rich, unique, and diverse source of genetic variation, which could be a source of variation for potato breeding (Bamberg and del Rio, 2005; D'hoop *et al*., 2008; Jansky, 2010).

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RESEARCH ARTICLE

Importance of Seed Traits in Processing Germplasm Collections: A Case Study of Chickpea Genetic Resources at ICRISAT

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Seed traits like color, shape, size, weight, surface projections and texture determine the type and quality of storage containers, drying behavior, and germination testing and packaging procedures for germplasm conservation. We studied the seed traits on 65 chickpea germplasm accessions and observed significant differences across traits and association with other related parameters. Seed moisture content differed significantly with seed coat color, surface texture and shape. Large Kabuli types and seeds with smooth surface texture have better drying features; however, had deleterious effects on seed quality. Hard-seededness was strongly associated with moisture content, seed types, color and seed coat thickness. Desi type chickpeas were superior to Kabuli and intermediate types with respect to seed germination. The results provide a basis for identifying chickpea types requiring additional care during seed processing and identifying germplasm with desirable seed traits for utilization in research.

Key Words: Chickpea; Drying; Germplasm; Seed germination; Seed trait

Introduction

Management of *ex situ* germplasm collections require innovative decisions on operating conditions, seed processing and storage behavior of seeds. Several researchers have described the importance of seed traits during processing, germination testing and storage. In chickpea (*Cicer arietinum* (L.), apart from utilization point of view, parameters like the seed size, seed coat thickness, shape and surface texture are important since they influence the seed handling procedures in genebanks. The chickpeas are broadly classified into desi (angular seed), Kabuli (owl's head seed shape) and intermediate (pea shaped) types based on morpho-agronomic characteristics including seed traits. However, irrespective of these categories, an array of genotypes with varying degree of seed coat thickness and color are found because of introgression of Kabuli germplasm into desi germplasm and vice versa (Ugale and Bahl, 1983; and Gaur *et al*., 2010). Desi and Kabuli types differ in their seed coat and crude fiber contents and the seed coat thickness of desi types is approximately three times than those of Kabuli types (Singh *et al*., 1981). Kabuli seeds have a thinner seed coat due to thinner palisade and parenchyma layers of desi types which contained fewer pectic polysaccharides and less protein, while palisade layers were rigid and extensively thickened (Wood *et al*., 2011). Dark colored seeds have thick seed coat and vice versa, and the protein content is significantly correlated with seed color and seed size (Govil, 1980). Chavan *et al*. (1986) related the crude fiber content with seed coat content and reported higher fiber content in desi than Kabuli type cultivars. Seed weight and volume are positively correlated to fat and carbohydrate content and negatively correlated to fiber content of chickpea seeds (Singh *et al.*, 1992).

Seeds with pigmented seed coats have a longer storage life, and chickpeas with dark seed coats store better than lighter-colored varieties (Gvozdeva and Zhukova, 1971) and pale-seeded chickpeas are shorter lived than those with thicker, harder coats (Van der Maesen, 1984). Seed length, width, thickness, geometric mean diameter, sphericity and porosity of chickpea seeds increase with increase in the moisture content and true density and bulk density reduce with increase in the moisture content (Mohsenin, 1986). Dutta *et al.* (1988) reported changes in seed surface area, shape,

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bulk density, kernel density and porosity with changing moisture contents. Smaller chickpea seeds have more surface to volume ratio and emerge better under sub-optimum seedbed moisture conditions (Padma Sri, 1998). However, majority of workers have used fewer numbers of accessions (cultivars) representing smaller diversity for seed traits.

The ICRISAT Genebank, Patancheru, India, currently conserve 20,764 accessions of chickpea from 61 countries as *ex situ* collection. These accessions are seed producing and the seeds have orthodox storage behaviour. Representative seed samples of these accessions are conserved as active collection (medium-term storage) in standard aluminum cans at 4 °C and 30% relative humidity) and as base collection (long-term storage) in vacuum packed laminated aluminum foil pouches at -20°C, and <7% seed moisture content and >90% seed viability), as per the recommended genebank standards (FAO/IPGRI, 2014). The collection represents wide diversity for important morpho-agronomic characters including seed traits and the operational procedures for managing the collection have been described (Upadhyaya and Gowda, 2009). The findings of Wood *et al*. (2011) also provide information on differences in the processing behavior between the major chickpea seed types. Grouping germplasm accessions based on seed traits assists in efficient management of the collection. Considering these factors, experiments were conducted on 65 chickpea germplasm accessions representing variation for traits related to seed and their influence on post-harvest processing and seed quality.

Materials and Methods

Sixty-five accessions representing desi, Kabuli and intermediate types from the world collection maintained in the genebank were randomly selected and used in this study (Fig. 1). Freshly harvested seed samples of the selected accessions from the 2010-11 post-rainy season at ICRISAT, Patancheru, India were hand-threshed and cleaned samples were stored under short-term storage conditions (25°C and 40% RH) to attain equilibrium moisture content (emc).

Observations on seed color, shape, surface texture and 100-seed weight were recorded following the Descriptors for Chickpea (IBPGR/ICRISAT/ICARDA, 1993). The moisture content of equilibrated seed samples was estimated on fresh weight basis (w.b.) following oven-dry method (ISTA, 1993). Sample seeds weighing exactly 200 g were kept in clean muslin cloth bags in a seed drying cabinet maintaining a constant temperature of 15°C and 15% RH (Cromarty *et al*., 1982). Seed samples were weighed at two day intervals to record changes in moisture contents and

Fig. 1. Variation for seed color, shape, surface texture and size of chickpea germplasm accessions used in the study.

seed drying continued until the sample weights in all entries stabilized indicating emc under this environment (Sastry *et al*., 2003).

Seed moisture content is an important component affecting seed quality and longevity. Under all storage conditions, the moisture content of seeds comes to equilibrium with the relative humidity of the surrounding atmosphere. Over a wide range of conditions, the longevity of seeds increases in a quantifiable way with decrease in moisture content (Roberts, 1973). The objective of drying in this study is to study the influence of seed traits on emc during drying and the impact of drying on related seed traits including viability. A seed germination test is a widely accepted and direct measure of viability. Germination tests on fresh and dry seed samples of the accessions were carried out following standard procedures (ISTA, 1993) and appropriate dormancy breaking measures (Ellis *et al*., 1985). Hard seeds identified as live and ungerminated at the end of initial testing (seven days) were scarified and the test period was extended to 14 days. Bulk density of seed was calculated by dividing the weight of seed of each sample on its volume, measured by using a graduated cylinder (Ayman *et al*., 2010). The true density was determined by using toluene solution displacement method (Mohsenin, 1986). Bulk density and true density were expressed as g/cc at given seed moisture conditions. The porosity (%) was determined as the percentage of densities of bulk seeds following the methodology of Jha (1999).

The data collected on test weight, moisture content, bulk density, true density and porosity and germination were statistically analyzed as Completely Randomized Design (CRD) using Genstat 15.1 Edition.

Results

Observations on seed traits and physical properties of fresh and dry chickpea samples of 65 accessions are summarized in Table 1. Based on morphological data (ICRISAT, 2017), the test accessions were represented by 18 seed colors. The major seed colors were brown (15), yellow brown (11), beige (9) and 15 other colors represented by 30 accessions. Out of 65 accessions, 46 were desi, 13 Kabuli and 6 intermediate types. Forty-nine accessions had rough seed surface followed by smooth (15) and tuberculated (1) accessions. Twenty different combinations for seed color, shape, surface texture and size are observed among the tested accessions. Tuberculated seed surface is represented by one accession (ICC 6098). The 100- seed weight (fresh seed) of test accessions ranged from 6.8 g (ICC 5590) to 65.0 g (ICC 11183) with a mean of 22.9 g representing wide range of diversity for this trait. There are five accessions with 100-seed weight >40 g; 21 accessions with 20 g to 40 g and 39 accessions with <20 g 100-seed weight. The mean 100-seed weight of desi, Kabuli and intermediate types was 19.3 g, 26.1 g and 22.3 g, respectively. The average 100-seed weight of small, medium, large and very large seed sizes are 12.8 g, 18.2 g, 29.2 g and 50.6 g, respectively. Forty-nine accessions had rough seed surface and medium size, 15 accessions had smooth surface and medium size and one accession had tuberculate surface with a seed weight of 15.9 g.

Seed Moisture Content

The emc of fresh seeds ranged from 10.5% to 11.9% with a mean of 11.1%. The differences in emc were significant among the accessions. Observations on seed moisture contents across seed traits, following drying, are presented in Table 1. The emc in all the accessions remained stable after 30 days of drying indicating no further drying possible in the seed samples. The emc of dry seeds ranged from 4.9% to 6.6% with a mean of 5.6%, a preferred moisture level for conserving chickpea germplasm under long-term conditions. The emc was lowest in ICC 8933, a medium sized intermediate type accession and highest in ICC 15554, a very large sized desi type accession. The 100-seed weight of dry seeds ranged from 5.7 g to 59.8 g with a mean of 19.5 g, indicating an average weight reduction of 3.4 g during drying. The mean seed moisture changes were 4.51%, 0.49%, 0.26%, 0.12% and 0.16% at the end of 6, 12, 18, 24 and 30 days of drying, respectively.

Seed Density and Porosity

Observations on bulk density, true density and porosity of fresh seed and dry chickpea seeds are summarized in Table 2. Bulk density of fresh seeds ranged from 0.62 g/cc to 0.81 g/cc with a mean of 0.71 g/cc. The highest bulk density was observed in ICC 14340 and the lowest in ICC 11883. True density of fresh seed ranged from 1.06 g/cc to 1.40 g/cc with a mean of 1.29 g/cc. Two accessions (ICC 8923 and ICC 1014) with highest true seed density were intermediate types. Porosity of fresh seeds ranged from 31.4% to 52.9% with a mean

Table 1. Summary on seed weight, moisture content and physical properties of fresh and dry seeds of chickpea accessions.

Seed Trait	No. of Acc.	Fresh seed									
		100-SWT (g)		MC (%)		Bulk density		True density		Porosity (%)	
		Range	Mean + SE	Range	Mean + SE	Range	Mean + SE	Range	Mean + SE	Range	Mean + SE
Desi	46	6.8-48.7	19.3 + 9.64	10.5-11.8	11.1 + 0.29	0.69-0.81	0.76 + 0.031	1.06-1.35	1.28 + 0.059	31.4-48.9	40.3 + 3.73
Kabuli	13	13.0-65.0	26.1+ 15.8	10.8-11.9	11.3 + 0.31	0.62-0.80	0.75 + 0.051	1.23-1.39	1.31 + 0.046	36.8-52.9	42.7 + 4.39
Intermediate	6	13.3-30.4	22.3 + 5.76	10.5-11.4	11.0 + 0.36	0.76-0.80	0.78 + 0.014	1.24-1.40	1.32 + 0.068	38.6-44.4	40.7 + 2.43
Small	24	6.8-14.9	12.8 + 2.16	10.6-11.9	11.2 + 0.31	0.70-0.81	0.77 + 0.030	1.06-1.39	1.27 + 0.073	31.4-45.9	38.9 + 3.75
Medium	23	15.0-24.0	18.2 + 3.00	10.5-11.4	11.0 + 0.24	0.69-0.80	0.76 + 0.031	1.21-1.40	1.29 + 0.051	36.3-48.9	40.7 + 3.08
Large	13	25.0-35.2	29.2 + 3.67	10.5-11.7	11.1 + 0.39	0.71-0.78	0.75 + 0.024	1.24-1.40	1.31 + 0.045	38.6-46.8	42.6 + 2.71
Very large	5	40.8-65.0	50.6 + 8.96	11.0-11.7	11.3 + 0.33	0.62-0.73	070 + 0.045	1.29-1.31	1.28 + 0.029	40.8-52.9	45.7 + 4.45
Rough	49	6.8-48.7	19.3 + 9.54	10.5-11.8	11.1 + 0.29	0.70-0.81	0.76 + 0.03	1.06-1.40	1.28 + 0.059	31.4-46.1	40.2 + 3.52
Smooth	15	13.2-65.0	26.6 + 14.21	10.5 -11.9	11.2 + 0.35	0.62-0.80	0.76 + 0.048	1.24-1.40	1.31+ 0.052	37.3-52.9	42.3 + 4.06
Tuberculated	1		15.9		11.1		0.69		1.35		48.9
Overall	65	6.8-65.0	22.9 + 12.41	10.5-11.9	11.1 + 0.31	0.62-0.81	0.71 + 0.19	1.06-1.40	1.29 + 0.059	31.4-52.9	40.8 + 3.84
						Dry seed					
Desi	46	5.7-46.3	18.0 + 9.13	4.9-6.6	5.7 + 0.36	0.72-0.92	0.81 + 0.047	1.24-1.57	1.35 + 0.057	30.1-48.4	39.4 + 3.70
Kabuli	13	11.9-59.8	24.2 + 14.65	5.1-6.0	5.4 + 0.29	0.68-0.86	0.79 + 0.049	1.29-1.49	1.35 + 0.051	35.1-49.0	41.4 + 3.73
Intermediate	6	12.5-27.7	21.0 + 5.46	5.3-5.6	5.4 + 0.11	0.80-0.84	0.82 + 0.014	1.31-1.41	1.35 + 0.034	38.3-43.0	39.7 + 1.77
Small	24	5.7-14.6	11.7 + 2.13	4.9-6.4	5.6 + 0.36	0.79-0.92	0.83 + 0.037	1.24-1.57	1.34 + 0.067	30.1-43.5	37.9 + 3.58
Medium	23	13.4-22.5	17.2 + 2.88	5.1-5.9	5.5 + 0.24	0.72-0.90	0.81 + 0.040	1.27-1.49	1.36 + 0.054	35.2-48.4	40.2 + 3.17
Large	13	22.8-33.3	27.1 + 3.31	5.2-6.3	5.7 + 0.40	0.76-0.84	0.80 + 0.028	1.32-1.39	1.35 + 0.024	38.3-44.9	41.2 + 2.35
Very large	5	38.4-46.3	47.5 + 7.76	5.1-6.6	5.6 + 0.59	0.68-0.78	0.73 + 0.038	1.30-1.33	1.32 + 0.013	39.8-49.0	44.3 + 3.33
Rough	49	5.7-46.3	18.0 + 9.02	4.9-6.6	5.6 + 0.36	0.77-0.92	0.82 + 0.044	1.24-1.57	1.34 + 0.056	30.1-45.3	39.3 + 3.45
Smooth	15	12.2-59.8	24.8 + 13.20	5.1-5.9	5.4 + 0.25	0.68-0.86	0.80 + 0.046	1.30-1.49	1.36 + 0.046	37.1-49.0	41.3 + 3.24
Tuberculated	1		14.7		5.7		0.72		1.40		48.4
Overall	65	5.7-59.8	19.5 + 10.38	4.9-6.6	5.6 + 0.35	0.68-0.92	0.81 + 0.045	1.26-1.57	1.35 + 0.054	30.1-49.0	39.9 + 3.62

Table 2. Summary on germination of fresh and dry seeds of chickpea accessions.

Seed trait	No. of accessions	Observation	Fresh seed		Dry seed		
			Germination (%)	Hard seed (%)	Initial Germination (%)	Hard seed (%)	Final Germination (%)
Desi	46	Range	88-100	0	41-100	0-58	66-100
		Mean (SE+)	97.3 (3.20)		91.3 (13.51)	4.7 (12.59)	95.8 (6.87)
Kabuli	13	Range	87-100	0	79-100	0	79-100
		Mean (SE+)	95.7 (3.97)		93.5 (6.91)	0	93.5 (6.91)
Intermediate	6	Range	77-98	0	69-100	0	69-100
		Mean (SE+)	90.3 (8.45)		88.2 (12.04)		88.2 (12.04)
Rough	49	Range	88-100	0	41-100	0-58	66-100
		Mean (SE+)	97.3 (3.11)		91.7 (13.18)	4.4 (12.25)	96.1 (6.57)
Smooth	15	Range	77-100	0	69-100	0	69-100
		Mean (SE+)	93.0 (6.48)		90.3 (9.45)	0	90.3 (9.45)
Tuberculated	1	Mean	98	0	98	0	98
Small	24	Range	89-100	0	83-100	0-5	83-100
		Mean (SE+)	98.3 (2.42)		97.4 (3.67)	0.3 (1.17)	97.8 (3.64)
Medium	23	Range	86-100	0	66-100	0-13	66-100
		Mean (SE+)	96.0 (4.36)		93.0 (8.20)	0.9 (2.93)	93.9 (8.01)
Large	13	Range	77-100	0	41-100	0-58	69-100
		Mean (SE+)	94.7 (5.92)		81.2 (20.39)	13.0 (21.46)	94.2 (8.63)
Very large	5	Range	93-99	0	73-89	0-12	73-98
		Mean (SE+)	92.8 (5.29)	0	82.2 (6.30)	3.4 (5.27)	85.6 (10.36)
Overall	65	Range	77-100	0	41-100	0-58	66-100
		Mean (SE+)	96.4 (4.46)		91.5(12.27)	3.3 (10.77)	94.7 (7.60)

of 40.8%. Drying seed samples from 11.1% to 5.6% moisture content indicated significant changes in bulk density, true density and porosity. The mean bulk density increased from 0.71 to 0.81 g/cc, true density from 1.29 g/cc to 1.35 g/cc while porosity decreased from 40.8 g/cc to 39.9%.

Fresh and dry Kabuli type accessions had a higher mean porosity of 42.7% and 41.4% respectively, compared to intermediate and desi types. Similarly, fresh and dry seeds with smooth surface had higher mean porosity of 42.3% and 44.3% respectively compared to seeds with rough surface. The highest porosity (49.0%) for dry seeds was observed in ICC 11883, a Kabuli type with largest test seed weight (65.0 g). Kabuli types with smooth seed surface are larger in seed size (both fresh and dry seeds) followed by intermediate and desi types with rough seed surfaces. Mean emc of fresh seeds ranged between 10.5 and 11.9% and emc of fresh and dry seeds was lowest in desi types. Seed bulk density was lowest among very large seeded Kabuli types compared to small seeded desi types with rough seed surface. True density was lowest in desi small seeded accessions with rough seed surface and highest in intermediate types. Porosity was lowest in small seeded accessions and highest with rough seed surfaces. The bulk density of dry seeds was highest in small seeds followed by rough seed surface and intermediate types. Highest true density was observed in desi types with medium seed size and rough seed surfaces and lowest with very large seeds. Chickpea seed types with highest bulk and true densities and porosity are depicted in Fig. 2.

Correlations among physical properties of fresh and dry seeds (data not presented) indicated no significant association between seed size and seed moisture contents. Seed size and bulk densities were negatively associated across seed moistures. However, the association was not significant for true density. Seed porosity was significantly correlated with seed size and true density negatively correlated to bulk density.

Seed Germination

The seed germination of fresh and dry chickpea accessions is presented in Table 2. Observations on germination were recorded after seven days of incubation in fresh seeds while for dry seeds initial recording was done after seven days and final recording after 14 days. Seed germination in fresh samples ranged from 77% to 100% with a mean of 96.4%. The germination was lowest in ICC 8400, a large seeded, intermediate type and smooth seed surfaced accession. Five other accessions had germination less than 90%. The germination levels in 51 accessions ranged from 95% to 100%. In dry seeds, the germination after seven days of incubation ranged from 41% to 100% with a mean of 91.5%. Seeds in several accessions remained healthy and ungerminated indicating the presence of hard seeds (0% to 58%). When the hard seeds were scarified and−tested, the germination increased to 66% to 100% with mean of 94.7%. Lowest germination was recorded in ICC 11321 followed by ICC 8400 (69%) and eight other accessions had germination less than 90% after drying seeds to lower moisture levels.

A summary on seed germination of fresh and dry chickpea accessions across seed traits is presented in Table 2. Fresh seed germination was highest among desi types followed by Kabuli and intermediate types and seeds with rough surface had higher viability compared to seeds with smooth surfaces. A gradual reduction in mean viability was observed as the seed size of accessions increased. In case of dry seeds, the initial germination percentage of desi accessions is

Fig. 2. Chickpea accessions with highest bulk density (left), true density (middle) and porosity (right).

less compared to Kabuli types. This is because of hard seeds occurring in several accessions of desi types (up to 58%).

Discussion

The chickpea accessions in this study represented desi, Kabuli and intermediate types differing in seed color, seed surface texture and 100-seed weight. It is assumed that the moisture content of seeds comes to equilibrium with the relative humidity of the surrounding atmosphere and a narrow range in emc is expected across seed lots in a given species. Seeds of ICC 16948 with beige color, owl's head shape and smooth seed coat recorded highest emc, whereas lowest emc was recorded in two accessions (ICC 506 and ICC 8400) with varying seed color, shape and surface texture including seed weight. The differences in emc of fresh seeds could be attributed to seed traits, especially the seed coat thickness for exchanging moisture with surrounding environment. The mean 100-seed weights (before and after drying) across seed traits were higher in large seeded Kabuli types and seeds with smooth surface, indicating better drying characteristics.

Observations on seed moisture changes (data not presented) under a constant drying environment indicated a three-phase drying of chickpea seeds. In the first phase, considered as rapid drying stage, the loss of moisture is significantly higher compared to the later phases. The mean seed moisture changes at different period indicate rapid drying in the first six days followed by normal (6 to12 days) and slow drying and stable moistures at latter stages. Similar trend was observed under controlled environment drying of different sized chickpea seeds (Sastry *et al*., 2007). Though the initial seed moisture contents remained similar in all accessions, the large seeded Kabuli types and seeds with thin and smooth surface texture lost moisture rapidly resulting in lowest emc compared to seeds with small seeded desi types.

The bulk density and true density of fresh seeds were significant among accessions. Bulk density ranged 0.62 g/cc to 0.81 g/cc with a mean of 0.76 g/cc and highest in ICC 14340 and lowest in ICC 11883, an accession with highest seed weight. Bulk density determines the way the seeds are packed and is useful in determining the size of container needed for a given weight of seed (Gürsoy and Güzel, 2010). Two accessions (ICC 8923 and ICC 1014) having highest true seed density were intermediate types. The minimum and maximum porosity (%) of fresh seeds were observed for very small and largest seeded accessions respectively, indicating strong positive correlation (r = 0.574 for fresh seed and r = 0.530 for dry seed).

Drying seed samples from 11.1% to 5.6% moisture resulted in significant changes in bulk density, true density and porosity. The bulk density and true density of seeds increased while porosity decreased. Bulk density was highest in small sized seeds with angular surface and rough seed coat while it is lowest in large seeded Kabuli type. The relative reduction in seed densities at high moisture content could be attributed to less weight gain due to added moisture in relation to the concomitant volumetric expansion of the seeds. Mohsenin (1986); Konak *et al*. (2002); Amin (2003), Gurhan *et al*. (2009), Nikobin (2009) and Ayman *et al.* (2010), made similar observations under seed moisture regimes of 5% to 35%. A similar observation in the present study even under narrower moisture regimes for fresh and dry seeds signifies the importance of diversity in chickpea seed traits and their influence on physical properties. Seed density is a component of grain yield, positively correlated to seed protein content, and hence selection for increased density could provide an efficient way to improve protein content without affecting seed yield. Measurement of seed density and weight is relatively inexpensive hence it is a low-cost way to identify promising accessions (Hongxia and Joseph, 2002) for utilization in research. Present study resulted in identifying several intermediate type accessions with higher seed densities associated with other useful traits. The ICRISAT chickpea collection is represented by 701 pea shaped accessions (3.5% of entire collection) and it would be worth screening these accessions for physical properties in association with protein content.

Relationships among Seed Size, Shape and Surface

Seed size, shape, and density are important in seed cleaning process (separating seed from undesirable materials). Seed bulk density and porosity affect the resistance to airflow of seeds and are crucial in the development of aeration and drying systems. Seed porosity has significance significant role in selection of seed containers and processing seed samples for conservation especially under vacuum storage. Highly porous seeds require more storage space during packing and chickpea seeds with higher porosity coupled with angular shape and rough seed coat surface demand

additional specifications and care during sealing and storage.

Seed Drying, Germination and Hard Seeds

Fifty accessions maintained germination levels above 90% after drying, indicating good initial seed quality and the typical orthodox nature of chickpea seeds. Drying seeds for base conservation (long-term storage) resulted in development of hard seeds to the extent of 58% in 12 accessions. All these accessions are desi types with angular seed shape and rough seed coat. Large numbers of hard seeds are observed in ICC 1069 (black), ICC 8521 (brownish beige) and ICC 6306 (black) followed by different shades of brown and yellow seed coat color. Seed coat of desi types was 2.72 times and 3 to 25 times thicker than Kabuli types with normal testa and with cracked testa respectively (Yadav and Sharma, 2000). This indicated a strong association of hard-seededness in chickpea with desi types having darker seed color which were characterized by thick seed coats. Rao *et al*. (1990) observed viability loss in intermediate types having cracks on seed coats developed during seed handling and recommended a two-stage drying procedure – an initial slow drying in a cabinet at 15 ºC and 30-40% RH until moisture content reaches 9% or less, followed by drying at 15 ºC and 15% RH to reach desired moisture levels for long-term storage.

Seed coat color is an indicator of mechanical resistance of seeds due to the presence of polymerized phenol, and acts as a chemical defense against microorganisms through soluble phenolic compounds. Kabuli types have very thin seed coats and lack the phenolic compounds, as compared to desi types and are extremely susceptible to a range of seed rotting fungi. The proportion of seed coat was less in large seeded than in small seeded type and damages to seed coat such as cracks, bruises and injuries to cotyledons escape unnoticed during normal processing resulting in poor seed quality. Hard seed coats lessen or alleviate stresses during and after harvest. Weak structure and cracks in the seed coat permit fungal infection, causing seed deterioration (Davenport and Splittstoesser, 1993). Germination of fresh and dry seeds is important from post-harvest seed operations, conservation and utilization point of view. A critical observation of seeds with poor viability before and after drying revealed injuries to seeds with thin seed coat in the form of bruises and ruptures during threshing or cleaning and splitting and cracking (Fig. 3) of seed coat in smooth surface and pea shaped accessions during drying. Yadav and Sharma (1998) observed higher seed coat cracking in Kabuli type chickpeas with large seed size and thin seed coat. Physiological cracking of seed coat in Kabuli types reduces quality of seeds during storage and thin seed coat in Kabuli types opens the avenues for imbibition and pathogenic injuries (Powell *et al*., 1986; Yadav and Sharma, 1998) resulting in reduced germination. Absence of hard seeds in any of the fresh seeds is an important observation in this study and none of the Kabuli or intermediate types had hard seeds as a result of seed drying. This could be attributed to the nature of the seed coat which is considerably thinner in both Kabuli type and intermediate types, as compared to desi type chickpeas. All the accessions with hard seeds have rough seed coat and essentially represent the desi types. Among the seed sizes, hard seeds were significantly high in large sized seeds (up to 58%). Based on extended germination tests of dry seeds desi type accessions with rough seed coat and small seed sized accessions recorded highest viability and such accessions could be safely dried for long-term conservation.

Fig. 3. Chickpea accessions with seed coat injuries to fresh seed (left and middle) and dry seeds (right)

In conclusion, sorting chickpea germplasm based on seed traits results in efficient processing of large number of accessions in genebanks and desi type chickpeas could be safely processed following precautions for hard seeds during germination tests. Kabuli types were prone to seed coat related injuries during processing resulting in poor quality and viability and seeds with pea shape and smooth surface were sensitive to drying necessitating alternate measures for reducing moisture content. Knowledge on seed surface texture, density, and porosity of chickpea seeds contribute to improving processing technologies for bulk seed lots. Pea shaped chickpeas had higher true densities and recording this trait on germplasm accessions is a low-cost way for seed quality evaluation and to identify promising chickpea accessions for further utilization.

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RESEARCH ARTICLE

Morphometric, Physico-chemical and Micronutrient Characterization of Rice (*Oryza sativa* L.) Landraces of Sikkim Himalayas

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Rice landraces diversity of Sikkim consisting of 51 accessions were studied for morphological, physico-chemical and micronutrient diversity. Analysis of characterization data of 50 DUS characters and 16 physico-chemical traits showed considerable variation among the germplasm. While the landraces Redzomu and Taichung were earliest to flower, Doodhkalam, Taichung, Takmaru (LL), Baelbuty and Anandhi have short stem length and 17 other landraces are strongly aromatic. With regards to important quantitative traits maximum test weight was recorded in Anandhi (33.0 g), head rice recovery in Dudheyjuari and Tulasi (70.6%), kernel length in Kalsati (6.70 mm), grain iron (Fe) in Ramsaree (14.6 ppm) and maximum grain zinc (Zn) content in Chinidhan (30.2 ppm). The Pearson Correlation Coefficient for variables revealed associations among morphological and grain quality parameters. Multivariate Analysis indicated seven principal components (PCs) accounting for 80% of the variability in the rice germplasm and the UPGMA Cluster Analysis grouped the germplasm in to five distinct clusters.

Key Words: DUS characters, Multivariate analysis, Northeastern India, Rice, Sikkim

Introduction

Northeastern region (NE) of India is a biodiversity hotspot rich in crop diversity (Myers *et al*., 2000). Globally, the region is considered as one of the hot pockets for rice genetic resources and a potential region with extremely diverse conditions for rice cultivation. Traditional cultivars are a storehouse for a number of desirable alleles which are the source material for tailoring genotypes for improved agronomic performance, resilience to stresses and enhancement of quality traits. For large scale adoption of new improved varieties, the best strategy is to breed for improvement of limiting traits without compromising the farmers' preferred traits. For that knowledge regarding the extent of genetic variation and relationships between genotypes is warranted. In this regard, morphological characterization helps in assessment of existing genetic diversity based on which new varieties were developed from germplasm (Sanni *et al.*, 2012; Chakravorty *et al.*, 2013). Rice grain quality is a major factor for varietal adoption by farmers which varies from region to region and is not universal. Although, this crop is a major source of nutrients to poor and vulnerable society, yet it doesn't contain sufficient levels of iron and zinc to meet the dietary requirements.

Sikkim is one of the Northeastern states nestled in the western part of East Himalayas and has been recently declared as the first organic state of India. Rice is the principal food crop of the region cultivated over varied altitudinal ranges and approximately covered an area of 11.16 (000´ ha) with production and productivity of 20.60 (000´ t) and 1815.74 (kg/ha) respectively during 2013-14 (Anonymous). The state is located on the old caravan route connecting India and Tibet and the local rice cultivars were introduced long time back and have established in the region (Subba, 2011). Moreover, the region has been reported to be the centre of differentiation of *Indica* and *Japonica* rices (Kihara and Katayama, 1963). Rice landraces of the Himalayan region have been studied and reported by various workers (Agnihotri and Palni, 2007; Rana *et al.*, 2009 and Roy *et al*., 2016) however, few reports are available for Sikkim (Kihara and Katayama, 1963 and Kapoor *et al*., 2014). Due to small area of cultivation of landraces in remote and

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far-flung inhabitations, collection of all the local rice germplasm is rather difficult. Rice cultivars being grown in the state are adapted to sub-optimal conditions like low temperature, water stress and low manure/fertilizer inputs and knowledge with respect to their superior traits and genetic potential of these landraces remain unexplored. Grouping of cultivars on the basis of their respective traits unravels their strengths and weaknesses and improvement of these local cultivars for limiting traits through different breeding methodologies can be used effectively after proper characterization. As there are no studies carried out on morphological characterization, quality analysis and micronutrient content of rice landraces of Sikkim, hence, the present study has been undertaken to elucidate morphological variablity and grain quality parameters including micronutrient analysis for proper documentation and utilization of these genetic resources in rice improvement.

Material and Methods

Experimental Material

A total of two explorations were conducted during 2013 to collect germplasm samples of local rice landraces from different rice growing areas in all the four districts i.e East, West, North and South Sikkim from where a total of 51 rice accessions were assembled along with passport data containing place of collection, latitude, longitude, altitude, source of collection and special characters of the cultivar (Table 1). Seedlings of each of the 51 entries were planted on a row length of 3m at spacing of 20 × 20 cm in Augmented Randomized Complete Block Design with four checks namely PD-10, RCPL 123, RCPL 469 and RCPL 473 and raised under organic package of practices under irrigated environment from which five plants/entry were selected for data recording on quantitative and DUS descriptors. The experiment was conducted during *kharif* season of 2013 and 2014 at the experimental farm of ICAR-National Organic Farming Research Institute (NOFRI), Gangtok situated at 27º19᾽0᾽᾽N and 88º36´0´´E at an elevation of 1320 msl.

Data Recording

Data on quantitative traits for plant height (PH), days to heading (FL), number of tillers/plant (NOT), panicle length (PL), days to maturity (MAT), 1000 grain weight (SW) and yield/plant (YP) and on descriptors and states consisting of 51 DUS (Distinctiveness, Uniformity and Stability) characters of rice (PPV&FRA, Govt. of India) were recorded on randomly selected five plants. Also, recorded were grain quality parameters for 16 traits i.e hulling percentage (HL), milling percentage (ML), HRR (head rice recovery,) KL (Kernel length), KB (Kernel breadth), L/B (length breadth ratio), grain type, grain chalkiness, VER (volume expansion ratio), WU (water uptake), KLAC (kernel length after cooking), ER (elongation ratio), ASV (alkali spreading value), AC (amylose content), GC (gel consistency) and aroma as per standard procedures (IRRI, 1976). While the ASV was estimated following the method of Little *et al*., 1958 the GC test by the procedure using Cagampang *et al*., 1973 and the micronutrient analysis (Fe and Zn) by energy dispersive X–ray fluorescence spectrometry (XRF) as per the procedure of Rao *et al*., 2014.

Statistical Analysis

Analysis of Variance for augmented randomized complete block design was computed (Federer, 1976), Pearson product-moment correlation for measuring relationships among 19 traits, principal component analysis for data reduction and for linear transformation of original variables in to new uncorrelated variables following standard procedures (Wiley, 1981) and cluster analysis for grouping rice germplasm using agglomerative UPGMA hierarchical method (Sneath and Sokal, 1973; Panchen, 1992). The software SAS 9.3 was used for statistical analysis of the data recorded (SAS, 2011).

Results and Discussion

Local rice landraces of Sikkim exhibited considerable variation with respect to plant characters, grain quality parameters and iron and zinc content. Analysis of Variance showed significant differences among the cultivars for plant height, panicle length, number of tillers/plant, days to 50% heading, days to maturity, 1000 grain weight and yield/plant. Plant height recorded tallest in *Tulasi* (214.20 cm) while shortest in *Doodhkalam* (66.0 cm). *Bhangeri* was the highest tiller bearing cultivar (19.0) followed by *Attey* (18.0). *Krishnabhog* and *Dhutraj* bear long panicles of 29.20 and 28.0 cm respectively while *Sanoattey*, *Taichung* and *Ramzeera* were short panicle bearing cultivars. Heading started at 88th day in *Red zomu* while it took 131 days for *Kalonunia*. Earliest maturing cultivars were *Redzomu* (121 days), *Taichung* (123 days) and *Kalodhan* (125 days) while *Kalonunia*, *Krishnabhog*, *Brihmphool*, *Khimti* and *Sanokhamti* were last to mature (> 160 days). Highest grain yield per plant recorded in *Japani* (42.54 g/plant), however, *Taichung*, *Kaleybungey*, *Dudheyjauri*, *Pahelodalle* and *Attey* were relatively higher yielding (>30g/plant).

Table 1. Passport data with source and location of rice landraces collected from Sikkim Himalayas

S.No	Landrace	Place of collection	Latitude	Longitude	Altitude
1	Red Zomu	Passingdang, North Sikkim	N 27°33.186'	E 088°28.586'	4882 ft
2	Nepal Dhan	Lower Lingthem, North Sikkim	N 27°31.660'	E 088°30.787'	3519 ft
3	Zomu	Pentong, North Sikkim	N 27°29.258'	E 088°28.913'	5770 ft
4	Takmaru	Pentong, North Sikkim	N 27°29.258'	E 088°28.913'	5770 ft
5	Takmaru LL	Sahgyong, North Sikkim	N 27°24.529'	E 088°33.316'	5341 ft
6	Zokub	Lower Lingthem, North Sikkim	N 27°31.603'	E 088°30.383'	4139 ft
7	Chini Dhan	Upper Lingthem, North Sikkim	N 27°31.381'	E 088°29.970'	4944 ft
8	Taichung	Ramthang, North Sikkim	N 27°24.648'	E 088°33.407'	5469 ft
9	Dharmali	Ramthang, North Sikkim	N 27°24.529'	E 088°33.316'	5341 ft
10	Kalo Dhan	Sahgyong, North Sikkim	N 27°29.258'	E 088°28.913'	5770ft
11	Pahelo Dalle	Yangthang, West Sikkim	N 27°17.366'	E 088°14.015'	4924 ft
12	Tauli	Burmoik (Sumbok), West Sikkim	N 27°15.784'	E 088°15.087'	3675 ft
13	Brihmphool	Toyang, West Sikkim	N 27°16.637'	E 088°14.088'	3438 ft
14	Dudhey Juari	Hee Yangthang, West Sikkim	N 27°16.837'	E 088°14.529'	4769 ft
15	Sijali	Rato Matey, Thamsing, West Sikkim	N 27°14.923'	E 088°14.460'	4283 ft
16	Bhangeri	Sumbok, West Sikkim	N 27°15.559'	E 088°14.435'	3741 ft
17	Ram Saree	Sasbote, West Sikkim	N 27°08.889'	E 088° 12.650'	1559 ft
18	Dhutraj	5th Mile, Budang, West Sikkim	N 27°07.988'	E 088°13.109'	1814 ft
19	Ram Bhog	East Sikkim	–	–	–
20	Japani	Malbasey, West Sikkim	N 27°08.863'	E 088°12.657'	2387 ft
21	Kalsati	Toyang(GPU, Yangthang), West Sikkim	N 27°16.838'	E 088°14.529'	3195 ft
22	Lal Bacchi	5th Mile (Budang), West Sikkim	N 27°07.988'	E 088°13.109'	1814 ft
23	Musuli	South Sikkim	–	–	–
24	Katti	8th Mile (Budang), West Sikkim	N 27°08.889'	E 088°12.650'	2390 ft
25	Marsee	East Sikkim	–	–	–
26	Kataka	Upper Tharpu, West Sikkim	N 27°08.848'	E 088°11.433'	3153 ft
27	Champey	Toyang(GPU, Yangthang), West Sikkim	N 27°16.838'	E 088°14.529'	3195 ft
28	Doodh Kalam	Gerethang, West Sikkim	N 27°18.185'	E 088°13.802'	4450 ft
39	Kaley Bungay	13th Mile, Sumbog, West Sikkim	N 27°15.361'	E 088°14.557'	4555 ft
30	Khimti	Rinchinpong (Burfok), West Sikkim	N 27°15.873'	E 088°15.732'	2836 ft
31	Timburay	Rungdhu, West Sikkim	N 27°15.781'	E 088°15.086'	2868 ft
32	Phouryal	Lower Martam, West Sikkim	N 27°15.909'	E 088°13.922'	3350 ft
33	Ram Zeera	West Sikkim	–	–	–
34	Sano Khamti	North Sikkim	–	–	–
35	Bael Butty	Barthang, South Sikkim	N 27°15.348'	E 088°15.013'	3014 ft
36	Tulasi	Rungdhu, West Sikkim	N 27°15.827'	E 088°15.149'	2692 ft
37	Chari Masini	Rabitar, South Sikkim	N 27°07.963'	E 088°16.775'	3910 ft
38	Anandhi	South Sikkim	–	–	–
39	Chari Nangrey	South Sikkim	–	–	–
40	Yeidehi	West Sikkim	–	–	–
41	Khamti	Lower Tharpu, West Sikkim	N 27°08.562'	E 088°11.423'	2889 ft
42	Tabrey	Ramthang, North Sikkim	N 27°24.529'	E 088°33.316'	5341 ft
43	Jhapaka	8th Mile Budang, West Sikkim	N 27°08.889'	E 088°12.650'	2390 ft
44	Attey	Namprik, North Sikkim	N 27°33.186'	E 088°28.586'	4882 ft
45	Zornali	East Sikkim	–	–	–
46	Chirakey	East Sikkim	–	–	–
47	Thulo Attey	Kaluk Rasi, West Sikkim	N 27°13.344'	E 088°14.333'	4527 ft
48	Sano Attey	13th Mile, Sumbog, West Sikkim	N 27°15.361'	E 088°14.557'	4555 ft
49	Krishna Bhog	5th Mile Budang, West Sikkim	N 27°08.188'	E 088°13.076'	2151 ft
50	Phool Patta	East Sikkim	–	–	–
51	Kalo Nunia	5th Mile Budang, West Sikkim	N 27°08.188'	E 088°13.076'	2151 ft

Cultivars like *Tulasi*, *Dhutraj*, *Krishnabhog*, *Rambhog*, *Kataka*, *Marsee*, *Tauli*and *Attey* are preferred by local farmers due to their tall stature which yield high biomass. Roy and Sharma (2014) studied 84 rice landraces and reported a cultivar with maximum 1000 seed weight with 32.04 g and also recorded the earliest maturing cultivar with 97 days.

Characterization for DUS descriptors and states

Frequencies for DUS descriptors and states were worked out in rice germplasm and the same have been shown in Table 2. Various basal leaf sheath color appeared such as green, uniform purple, light purple and with purple lines. Leaf anthocyanin coloration was present in *Kalonunia*, *Pahelo Dalle*, *Yeidehi* and *Ramzeera*. Anthocyanin coloration was present on tips in most of the genotypes except in *Rambhog* and *Kalonunia* in which coloration was on margins. Leaf auricles were absent in *Krishnabhog*, *Rambhog, Phoolpatta, Ramzeera* and *Musuli.* Anthocyanin colour of collar appeared in *Timburey* and *Yeidehi*. Flower stigma was yellow in *Rambhog*, purple in *Redzomu*, *Chinidhan*, *Timburey* and *Yeidehi* while white coloured in rest of the cultivars. Panicle number per plant was few (<11) in majority of germplasm. *Kalodhan, Tabrey*, *Chinidhan, Takmaru, Kalonunia* and *Brihmphool* bear awned panicles. 1000 grain weight recorded highest in *Anandhi* (33.0g) however *Zomu, Kalsati, Tabrey, Dudheyjuari, Redzomu*, *Dhutraj, Kataka, Japani, Chirakey* and *Taichung*bear heavy grains (26-30g/1000 grain weight). These can be used as donor parents in hybridization programmes or as recurring parents for improving one or two agronomically inferior traits as most of the landraces lacked characters for high yield. As paddy straw is a limitation in Sikkim, farmers prefer tall cultivars inspite of their low yields as agriculture is basically a subsistence activity and high yield is not a priority for the farmers and indeed breeding local cultivars for high yield without compromising plant height is a challenge for the breeders. Landrace *Attey* is most widely cultivated in the region having good quality grain with tall plant habit but the long maturity duration needs to be reduced. Likewise, varieties *Red zomu* and *Kalodhan* although early maturing are low yielding. *Kalonunia, Brihmphool* and *Krishnabhog* are popular as aromatic rice needs improvement in seed yield and reduction in days to maturity. *Kalsati* having long kernels, medium amylose content and tall habit is a quality rice but needs to be improved for medium maturity duration to best fit in the local cropping system. Genotypes *Krishnabhog, Kalonunia, Brihmphool, Khimti* and *Charinangrey* which are aromatic and have medium slender grains are best choice for market purpose but need improvement for high grain yield and medium maturity duration. Many rice workers evaluated rice genetic resources using DUS characters and mention may be made of Chakravorty and Ghosh (2012) who used 46 traits following DUS tests to characterize 51 rice landraces. Subba Rao et al., (2013) used 43 traits to characterize 65 rice landraces and Tripathy *et al*., (2014) who used 23 characters to characterize 91 early duration landraces.

Grain Quality Parameters

There are no universal quality parameters for rice as it varies from region to region. The variability for grain quality parameters in rice landrace germplasm of Sikkim Himalayas has been depicted in Table 4. Head rice recovery is one of the most important traits from milling point of view which is influenced by a number of factors but chalky grains are more prone to breakage during milling. Maximum head rice recovery was recorded in *Dudhey Juari* and *Tulasi* (70.6%) and the minimum in *Brihmphool* (38.3%). Short bold type grains predominate the rice collections. *Dhood Kalam* and *Kalsati* bear long slender grains while *Krishnabhog, Kalonunia, Brihmphool, Khimti, Charinangrey* and *Musuli* were of medium slender type. Grain chalkiness was absent in *Taichung, Ramsaree, Krishnabhog, Kalonunia*, *Dhood Kalam, Pahelo Dalle* and *Anandhi*. Amylose content strongly influence the cooking and eating characteristics of rice. Rice with high amylose content (25-30%) tends to cook firm and dry, whereas rice with intermediate amylose content (20-25%) is generally soft and sticky whereas with low amylose content (<20%) cooks soft and sticky. Amylose content ranged from 14.9 to 25.87%. Grains of thirty six cultivars contain medium amylose content while thirteen have low amylose content and one contain high amylose. Kernel length after cooking was highest for *Kalsati* followed by *Nepal Dhan* and *Yeidehi*. Strongly scented aroma observed in 17 entries while 10 were medium scented. Rice with gelatinization temperature at the lower end often cooks to a softer texture and retrogrades less than the rice with a gelatinization temperature at the upper end. Gel consistency measures the tendency of the cooked rice to harden on cooling. *Red zomu*, *Kalo Dhan*, *Taichung, Pahelo Dalle, Phouryal*, *Timburey, Thulo attey* and *Anandhi* had soft gel consistency

Table 2. Frequencies for DUS descriptors and states recorded on rice landraces from Sikkim Himalayas

S. No.	Descriptor	Descriptor state (Frequency %)
1.	Coleoptile colour	Colourless (100%)
2.	Basal leaf sheath colour	Green (84%): purple lines (6%): uniform purple (2 %): light purple (8%)
3.	Leaf intensity of green color	Medium (94%): light (6%)
4.	Leaf anthocyanin coloration	Present (8%): absent (92%)
5.	Leaf: distribution of anthocyanin coloration	On margins (4%): on tips (96%)
6.	Leaf sheath anthocyanin coloration	Present (12%): absent (88%)
7.	Leaf sheath : intensity of anthocyanin coloration	Medium (4%): weak (6%): very weak (90%)
8.	Leaf pubescence of leaf blade	Weak (36%): absent (24%): medium (30%): strong (10%)
9.	Leaf auricles	Absent (10%): present (90%)
10.	Leaf anthocyanin coloration of auricles	Colorless (56%): light purple (44%)
11.	Leaf collar	Present (100%)
12.	Leaf anthocyanin coloration of collar	Present (4%): absent (96%)
13.	Leaf ligule	Present (100%)
14.	Shape of ligule	Split (100%)
15.	Leaf: colour of ligule	Light purple (6%): white (94%)
16.	Leaf length of blade	Medium (16%): long (84%)
17.	Leaf width of blade	Narrow (26%): medium (74%)
18.	Culm attitude	Semi-erect (30%): open (2 %): erect (68%)
19.	Time of heading (50% of plants with panicles)	Early (8%): late (40%): medium (52%)
20.	Flag leaf attitude (early observation)	Erect (98%): erect (2%)
21.	Spikelet: density of pubescence of lemma	Strong (22%): very strong (2%): weak (10%): medium (66%)
22.	Male sterility	Absent (100%)
23.	Lemma: anthocyanin coloration of keel	Very strong (2%): strong (2%): weak (4%): absent (92%)
24.	Lemma: anthocyanin coloration of area below apex	Very strong (2%): strong (10%): weak (4%): absent (84%)
25.	Lemma: anthocyanin coloration of apex	Very strong (8%): strong (12%): weak (4%): absent (76%)
26.	Spikelet color of stigma	Purple (8%): yellow (2%): white (90%)
27.	Stem thickness	Thick (24%): thin (4%): medium (72%)
28.	Stem length	Very short (6%): short (4%): medium (42%): long (32%): very long (16%)
29.	Stem anthocyanin colouration of nodes	Present (4%): absent (96%)
30.	Stem anthocyanin colouration on internodes	Absent (100%)
31.	Panicle: length of main axis	Long (32%): medium (68%)
32.	Flag leaf attitude of blade(late observation)	Erect (100%)
33.	Panicle: curvature of main axis	Straight (12%): semi-straight (8%): semi-erect (4%): deflexed (76%)
34.	Panicle number per plant	Medium (2%): few (98%)
35.	Spikelet: colour of tip of lemma	Black (12%): purple (10%): yellow (6%): brown (72%)
36.	Lemma and palea colour	Straw (36%): brown furrows on straw (16%): brown tawny (32%): purple black (8%): brown spots on straw (8%)
37.	Panicle awns	Absent (88%): present (12%)
38.	Panicle: presence of secondary branching	Present (100%)
39.	Panicle secondary branching	Strong (64%): weak (36%)
40.	Panicle attitude of branches	Erect to semierect (22%): semierect (70%): erect (2%): semierect to spreading (6%)
41.	Panicle exertion	Mostly exerted (6%): well exerted (94%)
42.	Time maturity	Medium (8%): late (54%): very late (38%)
43.	Leaf senescence	Late (50%): medium (50%)
44.	Sterile lemma colour	Red (10%): purple (12%): straw (78%)
45.	Grain: weight of 1000 fully developed grains	Low (42%): high (6%): medium (52%)
46.	Grain length	Very short (84%): short (16%)
47.	Grain width	Narrow (6%): medium (56%): long (38%)
48.	Grain shape	Short bold (72%): medium slender (12%): long bold (12%): long slender (4%)
49.	Content of amylose	Low (26%): medium (74%)
50.	Gelatinization temperature	Low (94%): medium (6%)
51.	Aroma	Strongly scented (34%): medium scented (22%): non scented (44%)

Table 3. Promising rice landraces identified against important traits

	Character	Cultivars
Time of heading	Early (71 -90 days)	Red zomu, Tabrey, Zomu, Taichung
Stem length	Very short (< 91 cm)	Doodh Kalam, Taichung, Takmaru (lowland)
	Short (91-110 cm)	Bael Buty, Anandhi
1000 grain weight	Very high (> 30g)	Anandhi
	High (26-30 g)	Zomu, Kalsati, Tabrey, Dudhey Juari, Red zomu, Dhutraj, Japani, kataka, Chirakey, Taichung
Stem thickness	Thick (> 0.55 cm)	Krishnabhog, Kataka, Rambhog, Japani, Lal bacchi, Sano attey, Kalsati, Khimti, Ramzeera, Zornalli, Sano khamti
Panicle length	Long (26-30 cm)	Krishnabhog, Tulasi, Dhutraj, Rambhog, Zornalli, Sijali, Tauli, Takmaru lowland, Dudhey juari, Attey, Chirakey, Bhangeri, Japani, Phool patta, Ramsaree, Kataka, Chini dhan, Kaley bungey, Champey, Brihmphool
Panicle: curvature of main axis	Straight	Champey, Khimti, Zomu, Jhapaka, Khamti, Katti,
Kernel length	Long (> 6.60 mm)	Kalsati
	Medium (5.50 mm–6.60 mm)	Doodh kalam, Dhutraj, Japani, Charimasini, Phouryal, Champey, Zornalli, Nepal dhan, Khimti, Anandhi, Kataka, Zomu, Lal bacchi
Maturity	Medium (121-140 days)	Red zomu, Taichung, Kalodhan and Tabrey
Aroma	Strongly scented	Kalo dhan, Tabrey, Ramsaree, Krishnabhog, Rambhog, Kalonunia, Lal bacchi, Chari masini, Pahelo dalle, Tauli, Brihmphool, Kalsati, Khimti, Anandhi, Phool patta, Chari nangrey, Ramzeera

(61-100), this is a preferred character as the cooked rice has high degree of tenderness. The time required for cooking milled rice is determined by gelatinization temperature (GT). GT of milled rice is determined by evaluating the alkali spreading value. It correlates positively with the time required to cook rice. High alkali spreading value recorded for most of the cultivars which denotes low gelatinization temperature (55^{o} to 69 ºC). Consumers prefer length wise elongation of rice kernels and is influenced by factors like type of genotype, water uptake, amylose content, gelatinization temperature, aging and water uptake. KLAC varies from 7.7 mm in *Kaleybungey* to 15.5 mm in *Kalsati.* However elongation ratio recorded highest for *Takmaru* (lowland type) 5.2 mm. Values of volume expansion ratio were high in *Sijali* and *Dhutraj* (5.6 each) while low in *Tauli, Lal Bacchi* and *Attey* (4.0). It is a positive character for lower income group farmers for whom quantity is important, however more the volume expansion ratio, less will be energy content per unit volume. Rice with low amylose content which cooks sticky are preferred in Manipur, Nagaland and Mizoram and some parts of Arunachal Pradesh while short slender to medium slender with intermediate amylose content are preferred in Assam, Tripura and Sikkim. Short and bold grains with intermediate amylose content which become fluffy on cooking are mostly preferred in Sikkim. Subudhi *et al.* (2012) reported that HRR (%) in rice germplasm varied from 43.5% to 68.0% while kernel length after cooking from 7.9 to 12.5 mm in 41 rice varieties. Bajpai *et al.* (2012) reported elongation ratio of 1.60-2.33 mm in Kalanamak rice collections. Verma *et al.* (2015) found variation from 2.0-24.5% in amylose content while grain length after cooking varied from 5.5-12.7 mm. Devi *et al.* (2008) studied 15 important indigenous rice genotypes from different rice ecosystems of Northeastern India of which majority of rice genotypes were bold and amylose content varied from 2.3-24.5% while Devi *et al.* (2012) studied rice quality parameters in 18 indigenous rice cultivars of Tripura of which majority of them have low amylose content (< 20%). Aromatic rices have been popular in the state due to their quality traits and premium price in local market. A considerable variation in aromatic rice signifies ample scope of their improvement for reaping more economic benefits. Due to the complex inheritance of rice grain quality traits (Srivastava *et al*., 2012) initial improvement can be made through pureline breeding through local selection and purification (Singh *et al*., 2000). However, pedigree selection, backcross and convergent breeding can be employed for isolating superior segregants. Genetic enhancement employing pedigree method along with molecular markers were also employed for improvement of plant stature, aroma and grain quality traits (Patnaik *et al*., 2015).

Iron and Zinc content

Iron and Zinc content ranged from 6.4 to 14.6 ppm and 13.4 to 30.2 ppm, respectively (Table 5). Highest

Table 4. Variability for grain quality parameters in rice landraces from Sikkim Himalayas

S No.	Landrace	Hulling (%)	Milling (%)	HRR (%)	KL (mm)	KB (mm)	L/B (mm)	Grain type	Grain Chalk	VER	WU	KLAC	ER	ASV	AC	GC	Aroma
1	Red Zomu	78.2	61.2	39.2	5.11	2.89	1.76	SB	VOC	4.1	270	9.8	1.91	5.0	20.26	64	NS
2	Kalo Dhan	82.4	67.5	64.9	5.01	2.54	1.97	SB	OC	4.8	280	9.8	1.95	7.0	22.02	67	SS
3	Zokub	78.7	70.7	70.4	3.73	2.42	1.54	SB	OC	4.2	275	12.0	3.21	7.0	17.77	22	NS
4	Tabrey	80.4	68.4	60.8	4.82	2.69	1.79	SB	VOC	4.6	265	11.0	2.28	7.0	21.13	50	SS
5	ChiniDhan	78.0	67.5	61.7	5.43	2.29	2.37	SB	OC	5.4	260	11.1	2.04	7.0	22.99	45	NS
6	Nepal Dhan	78.0	67.9	62.8	6.01	2.07	2.90	LB	VOC	4.8	370	14.6	2.42	7.0	17.95	43	MS
7	Zomu	76.2	65.0	59.2	5.62	2.63	2.13	SB	VOC	4.2	265	10.7	1.90	4.0	22.88	58	NS
8	Takmaru	76.2	65.0	65.0	4.32	2.65	1.63	SB	OC	4.5	220	8.1	1.87	6.0	21.88	50	NS
9	Takmaru (Lowland type)	79.2	69.3	65.8	2.26	2.50	1.70	SB	OC	4.6	275	11.8	5.22	7.0	24.55	48	NS
10	Taichung	80.8	71.3	68.6	4.56	2.91	1.56	SB	A	4.6	310	8.6	1.88	7.0	17.21	68	NS
11	Jhapaka	78.4	63.7	56.7	5.11	2.36	2.16	SB	OC	5.0	240	10.3	2.01	7.0	23.20	56	NS
12	Khamti	77.6	66.5	60.1	5.19	2.39	2.17	SB	OC	4.8	320	11.8	2.00	7.0	21.23	47	NS
13	Katti	77.2	66.6	66.2	4.12	2.38	1.73	SB	OC	4.1	200	11.6	2.81	7.0	21.53	47	MS
14	Ram Saree	79.7	70.6	70.2	3.82	2.50	1.52	SB	A	4.6	200	8.7	2.27	7.0	20.53	22	SS
15	Dhut Raj	78.0	68.7	65.6	6.51	2.26	2.88	LB	OC	5.6	300	14.2	2.18	7.0	18.94	52	MS
16	Krishnabhog	78.7	67.0	64.5	5.41	2.08	2.60	MS	A	4.6	120	14.2	2.62	6.0	22.46	60	SS
17	Marsee	76.7	67.3	64.3	4.81	2.57	1.87	SB	VOC	4.8	195	10.8	2.24	6.0	18.97	55	NS
18	Kataka	79.6	68.9	66.6	5.69	2.34	2.37	SB	VOC	5.4	290	10.5	1.84	7.0	21.23	22	NS
19	Rambhog	79.2	70.9	70.4	4.39	2.45	1.79	SB	OC	4.2	300	10.6	2.41	7.0	17.68	22	SS
20	Japani	74.8	69.2	66.4	6.33	2.20	2.87	LB	OC	4.8	260	13.4	2.11	7.0	19.27	42	MS
21	Kalonunia	77.7	67.2	63.2	5.46	2.10	2.60	MS	A	5.0	200	12.5	2.28	6.0	21.61	43	SS
22	Lal Bacchi	78.9	67.2	61.2	5.51	2.23	2.47	SB	VOC	4.0	295	12.8	2.32	7.0	20.76	22	SS
23	Chari Masini	79.8	69.2	65.6	6.31	2.22	2.84	LB	VOC	4.4	260	13.8	2.18	7.0	20.97	51	SS
24	Champey	69.8	60.2	53.8	6.02	2.02	2.98	LB	VOC	4.1	305	13.8	2.29	7.0	16.48	45	NS
25	Dhood Kalam	80.0	69.0	62.4	6.57	2.10	3.12	LS	OC	4.8	290	13.9	2.11	6.0	21.26	35	NS
26	Pahelo Dalle	77.8	68.0	66.1	5.17	2.12	2.43	SB	A	4.5	215	14.1	2.72	4.0	14.90	77	SS
27	Tauli	81.3	69.7	63.5	4.87	2.60	1.87	SB	VOC	4.0	305	11.9	2.44	7.0	23.90	58	SS
28	Brihmphool	78.3	60.6	38.3	5.19	1.99	2.60	MS	VOC	4.8	300	11.1	2.13	7.0	23.40	43	SS
29	Dudhey Juari	81.6	71.0	70.6	4.99	2.68	1.86	SB	VOC	5.5	310	11.1	2.22	7.0	22.90	52	NS
30	Kaley Bungey	78.8	68.8	67.9	3.89	2.53	1.53	SB	VOC	4.6	280	7.7	1.97	6.0	20.97	46	MS
31	Bael Butty	80.1	69.0	59.6	5.14	2.34	2.19	SB	OC	4.3	230	11.5	2.23	7.0	21.47	58	NS
32	Sano Attey	80.0	69.4	68.9	3.85	2.33	1.65	SB	VOC	4.6	225	8.8	2.28	7.0	20.70	60	NS
33	Kalsati	76.2	67.8	64.8	6.70	2.17	3.08	LS	VOC	4.6	295	15.5	2.31	6.0	17.68	51	SS
34	Phouryal	79.7	71.3	68.7	6.19	2.26	2.73	LB	VOC	5.4	335	13.8	2.22	7.0	18.24	61	MS
35	Timburey	79.4	65.4	64.7	4.09	2.73	1.49	SB	VOC	4.6	335	8.6	2.10	7.0	23.11	63	MS
36	Thulo Attey	79.5	69.3	65.7	4.75	2.55	1.86	SB	VOC	4.1	315	10.6	2.23	6.0	22.70	61	MS
37	Tulasi	80.9	71.3	70.6	4.21	2.65	1.58	SB	VOC	4.2	300	9.6	2.28	7.0	22.79	50	MS
38	Sijali	78.8	72.9	65.7	4.99	2.73	1.82	SB	VOC	5.6	305	11.2	2.24	7.0	25.87	55	NS
39	Khimti	77.6	66.5	60.1	5.90	2.14	2.75	MS	OC	4.8	320	11.8	2.00	6.0	17.62	53	SS
40	Bhangeri	76.3	65.4	64.8	4.60	2.57	1.78	SB	VOC	4.3	310	10.6	2.30	6.0	20.73	44	NS
41	Anandhi	79.4	65.5	57.8	5.87	2.82	2.08	SB	F/A	4.1	260	12.6	2.14	7.0	18.06	77	SS
42	Phool Patta	77.3	67.3	64.6	4.67	2.51	1.86	SB	VOC	4.2	395	11.7	2.50	7.0	22.82	54	SS
43	Chari Nangrey	78.9	68.8	64.5	5.45	1.93	2.82	MS	VOC	4.6	340	13.8	2.53	7.0	22.29	49	SS
44	Yeidhei	79.0	69.7	66.8	5.39	2.44	2.20	SB	OC	4.6	285	14.3	2.65	7.0	23.46	53	NS
45	Ramzeera	78.0	70.8	70.3	3.91	2.42	1.61	SB	VOC	4.6	305	8.0	2.04	7.0	23.49	54	SS
46	Musuli	78.7	67.3	61.1	5.36	1.94	2.76	MS	VOC	5.5	320	12.5	2.33	7.0	22.61	56	MS
47	Zornalli	79.4	68.8	61.6	6.02	2.27	2.65	LB	OC	4.6	320	13.5	2.24	6.0	21.64	55	NS
48	Chirakey	79.8	69.4	68.8	4.48	2.90	1.54	SB	VOC	4.5	230	9.1	2.03	5.0	20.76	52	NS
49	Sano Khamti	77.0	66.5	60.1	5.10	2.36	2.16	SB	OC	4.5	310	10.7	2.00	7.0	23.05	57	MS
50	Attey	81.8	71.4	68.0	4.65	2.56	1.81	SB	VOC	4.0	275	9.5	2.04	7.0	24.64	58	NS
51	Dharmali	82.0	69.6	63.2	5.88	2.55	2.30	SB	VOC	4.6	300	11.6	1.97	7.0	23.14	50	NS

* SB: Short bold; LB: long bold; MS: medium slender; LB: long bold; LS: long slender; VOC: very occasional; OC: occasional; A: absent; NS: non-scented; SS: strongly scented; MS: medium scented

Table 5. Variability for Iron and Zinc content (ppm) in rice landraces from Sikkim Himalayas

S.No	Landrace	Fe	Zn	S.No	Landrace	Fe	Zn
1	Red Zomu	12.7	25.3	27	Tauli	10.5	21.9
2	Kalo Dhan	9.7	18.0	28	Brihmphool	9.5	21.7
3	Zokub	9.6	20.5	29	DudheyJuari	10.1	19.8
4	Tabrey	8.7	21.5	30	KaleyBungey	10.4	21.0
5	ChiniDhan	10.1	30.2	31	BaelButty	8.8	23.5
6	Nepal Dhan	9.6	20	32	Sano Attey	10.8	22.3
7	Zomu	11.1	25.6	33	Kalsati	6.4	18.9
8	Takmaru	11.4	23.9	34	Phouryal	9.5	19.9
9	Takmaru (Lowland type)	9.1	20.4	35	Timburey	10.6	21.3
10	Taichung	12.1	26.8	36	Thulo Attey	10.1	21.9
11	Jhapaka	10.3	26.7	37	Tulasi	11.3	23.1
12	Khamti	8.0	19.1	38	Sijali	10.8	25.1
13	Katti	12.5	25.5	39	Khimti	10.5	20.6
14	Ram Saree	14.6	21.3	40	Bhangeri	10.8	16.2
15	Dhut Raj	9.9	16.1	41	Anandhi	11.1	19.8
16	Krishnabhog	10.2	13.6	42	Phool Patta	12.5	16.4
17	Marsee	12.7	20.5	43	Chari Nangrey	13.3	15.3
18	Kataka	8.7	22.6	44	Yeidhei	9.2	22.5
19	Rambhog	8.0	14.7	45	Ramzeera	11.6	16.9
20	Japani	9.6	18.1	46	Musuli	12.5	18.4
21	Kalonunia	8.2	13.4	47	Zornalli	9.8	20.2
22	Lal Bacchi	8.4	18.8	48	Chirakey	12.2	26.9
23	Chari Masini	8.6	21.9	49	Sano Khamti	10.4	27.3
24	Champey	7.9	18.0	50	Attey	11.2	23.4
25	Dhood Kalam	8.2	16.8	51	Dharmali	12.3	20.8
26	Pahelo Dalle	9.4	15.4				

iron concentration in grains recorded for *Ramsaree* (14.6 ppm) followed by *Chari Nangrey* (13.3 ppm) while highest zinc content in *Chini Dhan* (30.2 ppm) followed by *Sano Khamti* (27.3 ppm). Grain iron and zinc content of a genotype depends on various factors such as the nature of grain, soil properties, time of harvest, phloem sap loading and unloading rates and genotype × environment interaction. Anuradha *et al*., (2012) analysed brown rice samples of 126 accessions and reported grain iron and zinc concentration ranging from 6.2 ppm -71.7 ppm and 2.6-67.3 ppm, respectively while iron content of 6.6-16.7 ug/g and zinc of 7.1 to 32.4 µg/g in 192 brown rice accessions (Nachimuthu *et al*., 2014). Biofortification programmes like Harvest plus and All India Coordinated Rice Improvement Project (AICRIP) have fixed 24 ppm as target for zinc in well polished rice grain and varieties having ≥24 ppm zinc content are promoted without compromising yield as well as grain quality. Mostly consumers prefer polished rice and percentage losses of iron and zinc during polishing were observed to be as high as 60 to 70% and 20 to 40%, respectively. Considering the above, nine landraces appeared to be promising for zinc content in polished rice, however, their performance in multiple locations within this region needs to be verified through a separate study. Iron content is low in brown rice which might be due to the soil properties as farming in Sikkim is low input and hardly any soil amendment is done.

Correlation Coefficients and Association

Pearson Correlation Coefficient revealed associations among 19 variables (Table 6). Plant height showed positive significant correlation with panicle length (r=0.504). Days to maturity correlated positively with days to heading (r=0.740) along with low degree of association with kernel length, VER and KLAC. Yield/plant, seed weight and kernel breadth were negatively correlated with days to maturity. Kernel length (r=0.531), kernel breadth (r=0.278), KLAC (r=0.361) and gel consistency were positively correlated with 1000 seed weight. Kernel length showed positive correlation with KLAC while negative association with kernel breadth, elongation ratio and amylose content. Kernel breadth and KLAC were strongly negatively associated (r=-0.644). Head rice recovery showed high significant positive correlation (r=0.80) with milling percentage while negative correlation with kernel length and L/B ratio. Amylose content did not showed high correlation with any of the traits, however moderately positively correlated with hulling percent (r=0.26) and negatively correlated with kernel length (r=-0.29), L/B (r=-0.27) and KLAC (r=-0.32). ASV showed moderate positive correlation (r=0.26) with water uptake and negative correlation with gel consistency. Sinha and Mishra (2013) reported high correlation of 50% heading with maturity time and stem length and also of grain length and grain weight with kernel length and kernel weight respectively. Sharma *et al.* (2012) reported highest correlation between days to flowering and days to maturity, positive and significant correlation among grain weight, width and length.

PCA and Cluster Analysis

Multivariate analytical techniques are important tools for classifying the germplasm and analyzing genetic relationships among breeding material (Mohammadi and Prasanna, 2003). These statistical algorithms are useful in delineating variability in germplasm and reducing the complex data of number of variables for meaningful inferences. Eigen values of the correlation matrix showed

Table 6. Pearson Correlation Coefficient for 19 characters recorded on rice landraces from Sikkim Himalayas

	PH	FL	NOT	PL	MAT	YP	SW	KL	KB	VER	WU	KLAC	ER	ASV	AC	GC	HL	ML	HRR
PH		0.236	0.136	0.504*	0.165	-0.059	-0.173	-0.089	0.115	0.066	-0.024	-0.193	-0.215	0.142	0.210	-0.106	0.143	0.227	0.226
FL			0.071	0.045	0.740*	-0.075	-0.393*	0.021	-0.378*	0.122	-0.107	0.071	-0.018	0.371*	-0.003	-0.306*	-0.264	-0.070	0.122
NOT				0.187	0.057	0.277	-0.153	-0.009	-0.056	0.008	0.085	-0.032	-0.046	0.024	0.310*	0.033	-0.104	0.018	0.008
PL					0.062	0.107	-0.015	-0.056	0.039	0.097	-0.013	0.056	0.162	-0.011	0.209	-0.291*	0.045	0.138	0.102
MAT						-0.336*	-0.303*	0.293*	-0.562*	0.288*	-0.129	0.334*	-0.049	0.134	0.049	-0.220	-0.350*	-0.113	-0.019
YP							0.090	-0.118	0.141	-0.050	0.262	-0.033	0.146	0.072	-0.117	0.130	0.084	0.293	0.290
SW								0.531*	0.278*	0.096	0.183	0.361*	-0.148	-0.164	-0.177	0.329*	0.018	-0.110	-0.196
KL									-0.474*	0.275	0.162	0.695*	-0.460*	-0.107	-0.318*	0.067	-0.241	-0.196	-0.284*
KB										-0.245	-0.029	-0.644*	-0.112	-0.107	0.184	0.266	0.332*	0.178	0.147
VER											0.094	0.133	-0.134	0.194	0.113	-0.026	0.065	0.172	0.104
WU												0.111	-0.069	0.312*	0.045	-0.034	-0.003	0.048	-0.025
KLAC													0.261	0.011	-0.304*	-0.022	-0.269	-0.059	-0.114
ER														0.118	0.081	-0.122	0.025	0.173	0.155
ASV															0.228	-0.268	0.187	0.229	0.182
AC																0.044	0.317*	0.093	-0.019
GC																	0.129	-0.119	-0.143
HL																		0.595*	0.264
ML																			0.791*
HRR																			

* values denote significane at 5% level

Table 7. Eigen values of the correlation matrix deduced for 16 variables

	Eigen Values of the Correlation Matrix			
	Eigen Value	Difference	Proportion	Cumulative
1	3.06512862	0.36522576	0.1916	0.1916
2	2.69990286	0.95432561	0.1687	0.3603
3	1.74557725	0.13762996	0.1091	0.4694
4	1.60794729	0.28967657	0.1005	0.5699
5	1.31827071	0.16953303	0.0824	0.6523
6	1.14873768	0.08329421	0.0718	0.7241
7	1.06544348	0.25759360	0.0666	0.7907
8	0.80784987	0.06251302	0.0505	0.8412
9	0.74533685	0.18909656	0.0466	0.8878
10	0.55624028	0.09837642	0.0348	0.9225
11	0.45786386	0.12613647	0.0286	0.9511
12	0.33172739	0.10092477	0.0207	0.9719
13	0.23080263	0.09281185	0.0144	0.9863
14	0.13799077	0.07636523	0.0086	0.9949
15	0.06162554	0.04207063	0.0039	0.9988
16	0.01955492		0.0012	1.0000

that seven principal components (PCs) have Eigen value > 1 which account for approximately 80% of the variability which indicate the contribution of many traits with higher level of correlation to explain the genetic variability in the rice collections (Table 7). First five PCs were employed for cluster analysis using UPGMA clustering which divided rice accessions in to five clusters (Fig. 1) and the mean values of the clusters have been shown in Table 8. Accessions of cluster I have lowest mean days to heading and maturity along with lowest yield per plant while cluster II members have highest days to heading and maturity. Cluster III comprised of entries having shortest plant height, heaviest grains, longest kernel length and kernel length after elongation while cluster IV have tallest plants, highest number of tillers, longest panicle length, highest water uptake and highest grain yield per plant. Entries of cluster V have lowest 1000 grain weight, short panicle length, shortest kernel length and highest elongation ratio. Cultivars in cluster IV like *Attey* and *Tulasi* are preferred due to tall height having good straw yield and relatively high grain yield but prone to lodging and take longer duration to mature. Hybridization of entries in cluster IV with elite high yielding medium to early duration lines/varieties may result in isolating superior segregants. Earliness is a preferable trait of cluster I, however improvement in grain yield can be made by crossing with high yielding varieties which perform better under organic conditions

Table 8. Mean values of 16 characters for five clusters grouped based on UPGMA

Character	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V
Plant height (cm)	135.00	133.84	121.33	149.25	125.83
Days to 50% heading	99.25	116.50	111.55	109.75	113.85
Number of tillers	9.30	11.36	9.76	13.90	9.68
Panicle length (cm)	25.04	25.02	24.95	26.50	24.71
Days to maturity	142.13	164.00	155.09	147.25	154.00
Yield per plant (g)	12.61	14.33	20.38	27.03	17.62
1000 grain weight (g)	24.93	22.74	25.88	22.82	20.44
Kernel length (mm)	4.87	5.34	6.19	4.66	4.29
Kernel breadth (mm)	2.68	2.25	2.24	2.57	2.45
Volume expansion ratio	4.45	5.07	4.65	4.43	4.58
Water uptake	255.00	301.50	299.09	307.50	252.69
Kernel length after elongation (mm)	9.99	11.59	13.81	10.80	10.62
Elongation ratio	2.05	2.11	2.23	2.30	2.58
Alkali spreading value	5.75	6.90	6.73	6.75	6.85
Amylose content (%)	21.33	22.35	19.20	22.78	21.15
Gel consistency	57.13	48.30	48.55	51.88	47.62
	Red zomu, Zomu, Kalodhan, Tabrey, Thulo attey, Takmaru, Marsee, Chirakey (8)	Chinidhan, Kataka, Sijali, Jhapaka, Sanokhamti, Khimti, Khamti, Brihmphool, Charinangrey, Musuli (10)	Nepaldhan, Phouryal, Lalbacchi, Charimasini, Champey, Zornalli, Dhoodkalam, Kalsati, Anandhi, Dhutraj, Japani (11)	Tauli, Kaleybungey Bhangeri, Tulasi, Attey Dudheyjuari, Phoolpatta, Yeidehi (8)	Timburey, Ramzeera, Rambhog, Ramsaree, Baelbutty, Krishnabhog, Kalonunia, Sanoattey, Katti, Taichung, Pahelodalle, Takmaru (LL), Zokub (13)

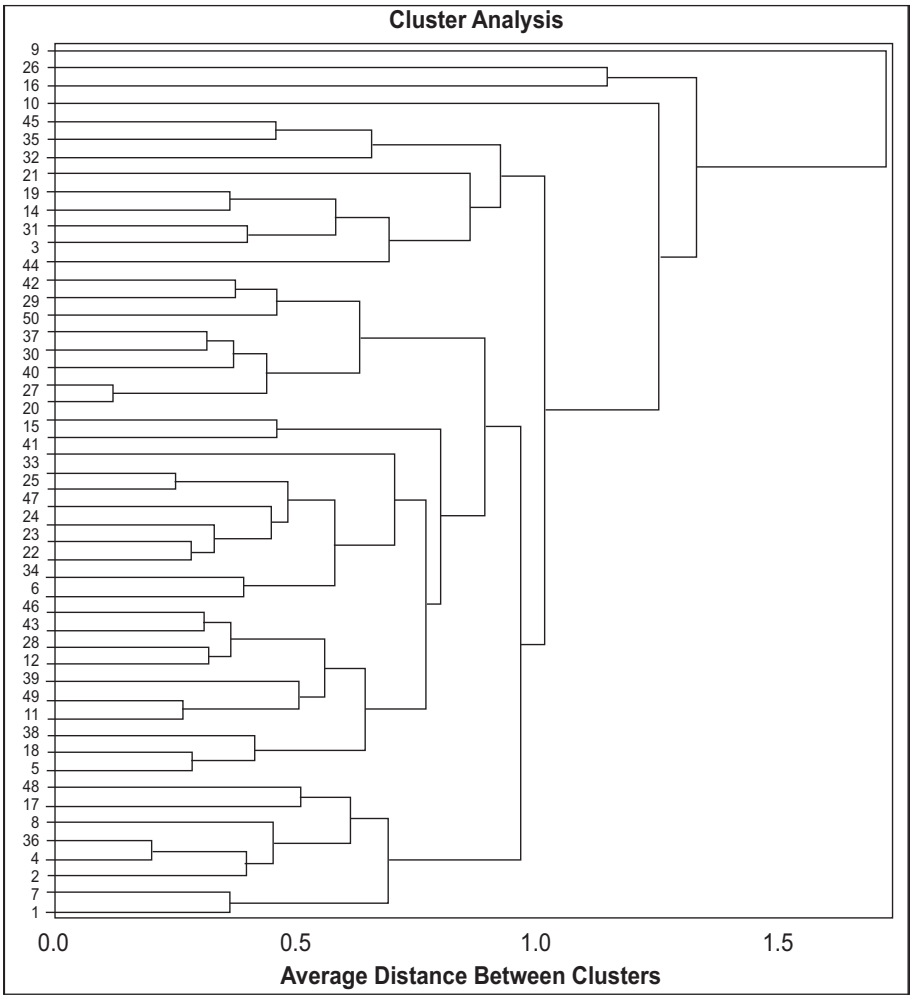

Fig. 1. UPGMA based clustering of rice germplasm based on five principal components

likewise entries in cluster III have better plant type for high yield but needs improvement in number of effective tillers and higher number of filled grains per panicle. Characters having maximum value for PC1 are days to maturity, days to heading and KLAC while kernel breadth contributed negatively towards first PC. Variables highly and positively correlated for PC2 were amylose content and plant height while seed weight and kernel length contributed negatively towards PC2. First component differentiated those accessions having long flowering and maturity duration alongwith minimum kernel breadth, hence PC1 identified mainly the phenological variables for differentiating the accessions while PC2 identified plant height and amylose content along with the grain parameters which contributed most negatively. Ahmed *et al*. (2016) reported first three PCs for 72.24% of the total variation among 10 agro-morphological traits and obtained seven cluster groups using UPGMA. They also found that first five PCs with vector value >1 for 82.90% of total variation and grouped 31 rice germplasm in to four clusters. These above findings are in line with Caldo *et al.* (1996) who reported maturity, heading and plant height among characters contributed to the parental lines of Philippines rice cultivars while Sharma *et al.* (2012) and Guei *et al.* (2005) reported negative contributions of phenological like days to flowering and maturity characters for PC1.

Maintaining landraces *ex-situ* is one of the best conservation strategies for genetic resources as marginal farmers prioritize reaping maximum production instead of maintaining genetic diversity (Lipton and Longhurst, 1989). The information generated during the present study is worthy of attention and could be utilized for conservation, utilization and improvement of local rice cultivars.

Conclusions

Sikkim is one of the smallest states in India and the occurrence of tremendous variability in rice landrace germplasm within the small geographical area is indeed noteworthy with many of the landraces collected having not been reported in the past. The agro- morphological characterization unraveled important traits of immense value to the breeders having ample scope for improvement of popular landraces like *Attey*, *Kalonunia*, *Bhrimphool* and *Krishnabhog* for the economic benefit of local farmers. Due to their small population size, these landraces are prone to genetic erosion and oblivion without any benefit to local farmers. Therefore, registration of important landraces with Protection of Plant Varieties and Farmers Rights Authority under Farmers' Variety category by the biodiversity management committees may help the farming communities not only in protecting their varieties but also in accruing some royalties through access and benefit sharing mechanism.

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RESEARCH ARTICLE

Assessment of Genetic Variability and Pollen Viability in Rice (*Oryza sativa* L.) Germplasm for Low Temperature Tolerance at Reproductive Stage

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A panel of rice genotypes consisting of the mini core, lowland and upland genotypes were evaluated for their ability to withstand cold stress to assess their genetic variability and pollen viability. The analysis of variance revealed highly significant differences among the genotypes for all the traits studied in ENV 1. Days to 50% flowering, plant height and spikelet fertility showed highest genotypic and phenotypic variance in all the three environments. Overall correlation analysis showed that spikelet fertility and biological yield were highly correlated with grain yield. Pollen viability test under cold stress condition relative to timely sown were studied and genotypes performing well were BAM 5889, BAM 5179, and BAM 4408. Among the mini core rice accessions studied, genotypes performing well under both timely and late sown environment were BAM 4115, UR 100, BAM 5179, BAM 3808, LR 11, BAM 4483 and UR 3.

Key Words: Cold Stress, Genetic variability, Rice, Reproductive Stage.

Introduction

Rice occupies a pivotal place in Indian agriculture as it provides 43% calorie requirement for more than 70% of population. In India, it is cultivated in an area of 43.94 million hectares which is the largest among all rice growing countries with, annual production of about 104.8 million tonnes and productivity of 2.3 tonnes ha^{-1} in 2014-15 (GoI, 2016). To feed the increasing population, it is projected that yields of rice, must increase by at least 70% before 2050 (Furbank and Tester, 2011). Cold stress causes various injuries to rice in low-temperature and high-altitude areas affecting rice production stage pollen sterility can be induced by cold, decreasing the final number of grains which will ultimately leads to drastic reduction in grain yield. Low temperature stress is manifested at different growth stages such as germination, seedling, vegetative, reproductive, and grain maturity (Andaya and Mackill, 2003). Cold temperature during the reproductive phase leads to seed sterility, which reduces yield and grain quality. Low temperature at the booting stage has also been reported to cause degeneration of young microspores, hypertrophy, dissolution of tapetal cells and interrupting or decreasing the supply of nutrients from the anther walls to the pollens (Satake, 1989). Hence, from above fact it is well understood that cold stress is one of the threats to rice production. Hence, there is a need to breed cold tolerant rice varieties, for which exploitation of variability followed by selection is an important criteria. A concept of core and mini-core collection where the germplasm is representative of diverse genotype will be an excellent material to screen for cold tolerance. To improve the yield it is important to understand correlation between yield and its component traits. Keeping this in view, the present investigation is taken up to assess genetic variability, heritability and correlation among traits under low temperature stress.

Materials and Methods

Plant Material

The present study was conducted at CPGS (College of post graduate studies), Umiam, Meghalaya, situated at latitude of 25º41´ and longitude of 91º54´ and an elevation of 950 m above mean sea level in *Kharib* 2014-15. A panel of rice genotypes consisting of 85 mini core (Tiwari *et al*., 2015), 21 lowland rice genotypes and 11 upland and three checks were screened for their ability to withstand cold stress at reproductive stage. These genotypes were staggered planted at three different dates – timely sown (ENV 1), medium sown (ENV 2) and late sown (ENV 3) in an augmented field design with three

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blocks for each environment maintaining a spacing of 18 cm and 22 cm between plants and rows, respectively. Check varieties were randomly allotted in each block in each environment. Observations were recorded on ten randomly selected plants of each genotype per replication for ten traits *viz*. days to 50% flowering, flag leaf area, panicle exertion, panicle length, plant height, panicle number, spikelet fertility, test weight, spikelet per panicle, biological yield and grain yield per plant.

Statistical Analysis

Analysis of variance was carried out for the above mentioned characters separately for three environments, by using online augmented analysis software (Rathore *et al.*, 2004) available at http://www.iasri.res.in/SpadWeb/. The ANOVA table generated from augmented design was used to estimate genotypic and phenotypic components of variance and heritability. The genotypic and phenotypic components of variation were calculated by the formula given by (Burton and Devane 1953) and heritability by (Allard, 1960). Genotypic and phenotypic correlation coefficients for all possible comparisons were made as per the formulae suggested by (Miller *et al.,* 1958). To test the significant of correlation coefficients, the estimated values were compared with the table value at n-2 degrees of freedom at 5% and 1% level of significance. The partitioning of genotypic correlation coefficient of the character in study into direct and indirect effects was carried out by using the procedure of (Dewey and Lu, 1959).

Principal Component Analysis (PCA)

Data reduction method of PCA was used to distribute the genotypes along two major principal components that were derived on the basis of correlation coefficient of six characters that showed highest correlation with grain yield under ENV 3 and relative values of these characters with respect to ENV 1, expressed as the ratio of values in ENV 3 and values in ENV 1. Scatter plot was made using MS-EXCEL on the basis of principle component scores obtained by using SPAR 2.0 software developed by ICAR Indian Agricultural Statistics Research Institute, New Delhi. Outliers having high principal component values were identified as superior performers.

Pollen Viability Test

Spikelet and pollen fertility is an appropriate index to evaluate cold tolerance at the booting stage and it was estimated under controlled environmental conditions in contrasting genotypes shortlisted from the field experiment. Pollen with round shape and dark blue colour were considered to be fertile; otherwise they were recorded as sterile. Panicles, which had exserted more than two-thirds out of flag leaf sheath were chosen early in the morning just before anthesis around 07:00 – 09:000 AM and the spikelets were selected from the second or third branch from top on the primary rachis. Third or fourth spikelets on the branch were used to estimate pollen viability. From each genotype, florets from three plants were collected and again from each plant, three spikelets were used for pollen viability test. Soon after collection, anthers from each spikelet were excised with a needle on the glass slide. The six anthers from each spikelet were crushed and stained with 10 µl of 1% (v/v) of I_2 (iodine) in 3% (v/v) KI (potassium iodide) solution. And from this, 1 µl was sampled and observed under light microscope at 10x magnification and digital images were taken and evaluated number of viable pollen. Afterwards, the numbers of pollen grains stained with I_2-KI as well as unstained pollen grains were counted. Pollen viability was estimated as the ratio of no. of stained pollen to the total no. of pollen grains and expressed as percentage i.e. Pollen viability = (No. of stained pollen/ Total no. of pollen grains) × 100.

Results and Discussion

Analysis of Variance

The analysis of variance revealed highly significant differences within the genotypes for all the traits studied in ENV 1 (Table 1). This indicated that the genotypes were possessing inherent genetic variation among themselves with respect to the traits studied. These findings are in accordance with the findings of (Rashmi *et al.,* 2017; Singh *et al.,* 2015; Singh *et al*., 2007). In ENV 3, panicle exsertion and spikelet per panicle were highly significant. Proportion of total variance due to genotypes was studied and variance components were further used to compute heritability estimates for each trait. Days to 50% flowering, Plant height and Spikelet fertility showed highest genotypic and phenotypic variance in all the three environments. Heritability in broad sense plays a predictive role in selection procedures and gives an idea of the total variation ascribable to genotypic effects, which are exploitable portion of variation (Sanghera *et al.,* 2013). Among all the traits, heritability of plant height (1.00, 0.98) in ENV 1 and ENV 2 was found to be highest whereas in ENV 3, spikelet per panicle (0.86) shown highest heritability. This finding was in close agreement with (Abebe *et al.,* 2017).

Table 1. Analysis of variance for characters under study

ANOVA		DTF	FLA	PE	PL	PH	PN	SF	TW	SPP	BY	GY
	ENV 1	76.04**	28.94	2.73*	5.82**	134.83**	1.25	0.97	0.02**	207.15	25.42*	8.53
Block(adj)	ENV 2	14.78	14.81	0.99	1.34	26.94*	1.29	5.08	0.02	428.40	196.76**	53.64*
	ENV 3	4.00	0.25	0.81	0.49	45.27	2.32	164.89	0.01	16.33	33.59*	7.70
	ENV 1	209.92**	135.86**	5.56**	7.72**	318.42**	11.33*	346.90**	0.37**	2013.2*	528.38**	215.59**
Treatment	ENV 2	166.30**	82.36**	4.35**	9.89	293.86**	11.29*	499.70*	0.26*	730.65*	162.84**	42.88*
	ENV 3	132.86*	14.92	5.64**	5.68	172.91*	3.33	492.10*	0.27	302.03**	15.64*	2.98
	ENV 1	1.41	8.57	0.33	5.82	0.12	1.33	3.02	0.00	292.82	2.09	13.59
Error	ENV 2	10.28	4.40	0.24	6.33	2.31	1.94	65.52	0.03	110.17	1.59	4.22
	ENV 3	12.83	5.07	0.44	0.49	27.52	2.15	58.51	0.23	15.69	2.15	1.72
	ENV 1	86.11	31.41	3.91	22.03	97.28	8.44	61.73	2.44	94.05	42.01	22.99
Mean	ENV 2	83.32	22.36	3.26	19.71	86.58	8.73	60.84	2.35	53.69	23.37	8.52
	ENV 3	82.42	17.29	0.07	16.35	72.51	4.91	40.31	2.30	43.52	8.93	2.41
	ENV 1	1.38	9.32	14.76	0.33	0.36	13.68	2.81	1.34	18.19	3.44	16.04
CV	ENV 2	3.85	9.38	14.87	12.76	1.76	15.97	13.30	7.56	19.55	5.40	24.11
	ENV 3	4.35	13.02	1015.44	8.71	7.23	29.92	18.97	20.95	9.10	16.44	54.38
	ENV 1	69.50	42.43	1.74	0.63	106.10	3.33	114.63	0.12	573.49	175.43	67.33
V_g	ENV 2	52.01	25.99	1.37	1.19	97.18	3.11	144.73	0.08	206.83	53.75	12.89
	ENV 3	40.01	3.29	1.73	1.73	48.47	0.39	144.53	0.01	95.45	4.50	0.42
	ENV 1	70.91	51.00	2.08	6.45	106.22	4.67	117.64	0.12	866.31	177.52	80.92
V_p	ENV 2	62.28	30.38	1.61	7.51	99.49	5.06	210.25	0.11	317.00	55.34	17.11
	ENV 3	52.84	8.35	2.17	2.22	75.98	2.54	203.04	0.25	111.14	6.65	2.14
	ENV 1	0.98	0.83	0.84	0.10	1.00	0.71	0.97	0.99	0.66	0.99	0.83
H	ENV 2	0.83	0.86	0.85	0.16	0.98	0.62	0.69	0.71	0.65	0.97	0.75
	ENV3	0.76	0.39	0.80	0.78	0.64	0.15	0.71	0.05	0.86	0.68	0.20

DTF – Days to Flowering, FLA – Flag Leaf Area, PE- Panicle Exsertion, PL – Panicle Length, PH – Plant Height, PN – Panicle Number, SF – Spikelet Fertility, TW – Test Weight, SPP – Spikelet Per Panicle, BY – Biological Yield and GY – Grain Yield, CV – Coefficient of varience, Vg – Genotypic varience, Vp – Phenotypic varience, H – Heritability, ENV – Environment.

Identifications of Important Traits

Correlation Coefficient Analysis

Traits such as yield, which are of interest to breeders are complex and are the result of the interaction among number of components. Knowing the relationship between grain yield and its components is of paramount importance for making the best use of these relationships in selection (Sarawagi *et al*., 1997). The correlation coefficient study on grain yield based on yield contributing traits recorded for ten characters was done for three different environments (Table 2). For timely sown environment (ENV 1), overall correlation analysis showed that days to 50% flowering (r = 0.368), flag leaf area (r = 0.421), panicle length (r = 0.436), spikelet fertility (r = 0.588), spikelet per panicle (r = 0.643), biological yield (r = 0.493) were highly significant and correlated with grain yield. The study of correlation coefficient from medium sowing (ENV 2) revealed spikelet fertility (r = 0.491), spikelet per panicle (r = 0.520) and biological yield (r = 0.448) were significantly correlated to grain yield. In case of late sown environment (ENV 3), panicle exsertion (r = 0.253), panicle number (r = 0.490), spikelet fertility (r = 0.389), biological yield (r = 0.651) were positively correlated to grain yield. Overall correlation analysis showed that spikelet fertility and biological yield were highly and correlated with grain yield in all the three environments.

Path Coefficient Analysis

Path coefficient analysis developed by Sewall Wright (1918) determines the significance of correlations between yield components and assigns relative importance to yield relations. The correlation coefficients generated by path analysis measure the direct and the indirect influence of a variable upon another (Nakawuka and Adipala, 1999). The direct effect of days to 50% flowering, panicle length and biological yield was observed negligible but correlation coefficient was positive in ENV 1 (Table 3). Interestingly, correlation coefficient between the traits and yield of spikelet

Table 2. Path coefficient analysis for grain yields under three environments

		DTF	FLA(cm)	PE(cm)	PL(cm)	PH(cm)	PN	SF%	TW(g)	SPP	BY(g)	GY(g)
DTF	ENV 1	1	0.371**	-0.237*	0.366**	0.301**	0.409**	-0.019	-0.158	0.423**	0.605**	0.368**
	ENV 2	1	0.397**	-0.231*	0.056	0.285*	0.316**	-0.228*	-0.227*	0.291*	0.599**	0.146
	ENV 3	1	0.269*	-0.290*	0.139	-0.032	0.051	-0.486**	-0.191	-0.119	0.171	-0.118
FLA	ENV 1	0.371**	1	-0.096	0.522**	0.390**	0.066	-0.02	-0.153	0.495**	0.593**	0.421**
	ENV 2	0.397**	1	-0.029	0.315**	0.377**	0.024	-0.148	0.149	0.384**	0.423**	0.148
	ENV 3	0.269*	1	-0.309**	0.539**	0.351**	0.029	-0.344**	-0.089	0.459**	0.318**	0.049
PE	ENV 1	-0.237*	-0.096	1	0.16	-0.174	-0.436**	0.136	0.199	-0.1	-0.236*	-0.026
	ENV 2	-0.231*	-0.029	1	0.374**	0.023	-0.104	-0.112	0.202*	-0.074	-0.024	0.005
	ENV 3	-0.290*	-0.309**	1	0.019	0.134	0.214*	0.187	0.225*	-0.043	0.083	0.253*
PL	ENV 1	0.366**	0.522**	0.16	1	0.270*	-0.127	0.268*	-0.102	0.549**	0.345**	0.436**
	ENV 2	0.056	0.315**	0.374**	1	0.278*	-0.022	-0.017	0.059	0.316*	0.257*	0.228*
	ENV 3	0.139	0.539**	0.019	1	0.629**	-0.029	-0.092	-0.011	0.422**	0.300**	0.029
PH	ENV 1	0.301**	0.390**	-0.174	0.270*	1	0.217*	-0.105	-0.149	0.391**	0.650**	0.277*
	ENV 2	0.285*	0.377**	0.023	0.278*	1	0.08	-0.121	-0.009	0.228*	0.441**	0.033
	ENV 3	-0.032	0.351**	0.134	0.629**	1	-0.024	0.054	0.108	0.375**	0.351**	0.147
PN	ENV 1	0.409**	0.066	-0.436**	-0.127	0.217*	1	-0.251*	-0.236*	-0.01	0.521**	0.208*
	ENV 2	0.316*	0.024	-0.104	-0.022	0.08	1	-0.1	-0.142	-0.210*	0.593**	0.141
	ENV 3	0.051	0.029	0.214*	-0.029	-0.024	1	-0.063	-0.128	-0.364**	0.698**	0.490**
SF	ENV 1	-0.019	-0.02	0.136	0.268*	-0.105	-0.251*	1	0.279*	0.161	-0.049	0.588**
	ENV 2	-0.228*	-0.148	-0.112	-0.017	-0.121	-0.1	1	0.192	0.204	-0.123	0.491**
	ENV 3	-0.486**	-0.344**	0.187	-0.092	0.054	-0.063	1	0.339**	-0.166	0.077	0.389**
TW	ENV 1	-0.158	-0.153	0.199	-0.102	-0.149	-0.236*	0.279*	1	-0.244*	-0.096	0.069
	ENV 2	-0.227*	0.149	0.202*	0.059	-0.009	-0.142	0.192	1	-0.084	-0.006	0.267*
	ENV 3	-0.191	-0.089	0.225*	-0.011	0.108	-0.128	0.339**	1	0.048	-0.105	0.187
SPP	ENV 1	0.423**	0.495**	-0.1	0.549**	0.391**	-0.01	0.161	-0.244*	1	0.459**	0.643**
	ENV 2	0.291*	0.384**	-0.074	0.316*	0.228*	-0.210*	0.204*	-0.084	1	0.369**	0.520**
	ENV 3	-0.119	0.459**	-0.043	0.422**	0.375**	-0.364**	-0.166	0.048	1	0.017	-0.07
BY	ENV 1	0.605**	0.593**	-0.236*	0.345**	0.650**	0.521**	-0.049	-0.096	0.459**	1	0.493**
	ENV 2	0.599**	0.423**	-0.024	0.257*	0.441**	0.593**	-0.123	-0.006	0.369**	1	0.448**
	ENV 3	0.171	0.318**	0.083	0.300**	0.351**	0.698**	0.077	-0.105	0.017	1	0.651**
GY	ENV 1	0.368**	0.421**	-0.026	0.436**	0.277*	0.208*	0.588**	0.069	0.643**	0.493**	1
	ENV 2	0.146	0.148	0.005	0.228*	0.033	0.141	0.491**	0.267*	0.520**	0.448**	1
	ENV 3	-0.118	0.049	0.253*	0.029	0.147	0.490**	0.389**	0.187	-0.07	0.651**	1

(*) = significant at 5% level of significant; (**) = significant at 1% level of significant

fertility was almost equal to its direct effect. So, direct selection through this trait will be effective. Among the component traits, spikelet fertility had highest direct effect (0.579) to grain yield along with positive indirect effects through days to 50% flowering, panicle exsertion, test weight, spikelet per panicle, biological yield and negative indirect effect through flag leaf area, panicle length, plant height, panicle number. Biological yield, indirect effect of panicle number (0.233) and spikelet per panicle (0.251) had more correlation towards grain yield. It was observed that plant height (0.031) had no direct effect to grain yield but it was supported by positive indirect effect of high magnitude via spikelet per panicle (0.213) which is highly correlated to grain yield. In ENV 2, following partitioning of individual correlation values of the traits into direct and indirect effects, spikelet fertility (0.394), spikelet per panicle (0.374), biological yield (0.436) and test weight exhibit direct effect of correlation to grain yield. Among these, biological yield (0.436) had high magnitude of direct effect. Days to flowering had recorded a negligible contribution via direct effect to grain yield but high correlation via indirect effect on biological yield (0.261). Panicle number (0.055) was not directly correlated to grain yield but indirectly correlated via biological yield (0.259). Among the component traits, spikelet fertility (0.279) and biological yield (0.649) contributed direct effects on yield but biological yield had more directly correlated on yield with high magnitude in ENV 3. Flag leaf area (0.100) and panicle number (0.044) had negligible correlation via direct effect to grain yield but it was indirectly correlated with biological yield. Similarly,

direct effect of plant height (-0.080) was not correlated to grain yield but indirectly correlated through biological yield (0.227). The direct effect of spikelet fertility was highest and also highly correlated to grain yield in the three environments. These results are similar to the finding of (Pallavi *et al*., 2017; Shoumik *et al*., 2017).

Principal Component Analysis (PCA)

Based on the six characters and their relative values (value in ENV 3/ value in ENV 1), principal component analysis for data reduction was performed and the genotypes were plotted across the first two principal components (Fig. 1). The first two components explained 33% of total variability, PC 1 contributing 18.7% and PC 2 contributing 14.93%. Similar findings were obtained by (Gana *et al*., 2013; Sanni *et al*., 2010). The genotypes were distributed with respect to the first two components and genotypes showing high value for first two principal components UR 100, BAM 4115, BAM 759, BAM 4483 and LR 11 were selected as potential reproductive stage cold tolerant genotypes.

Pollen Viability Test

Pollen viability test of different mini core rice genotypes under cold stress condition relative to timely sown condition were studied and tolerant and susceptible genotypes were identified according to their viability of pollen at reproductive stage separately for timely and late sown environment (Fig. 2). The ratio of pollen viability under ENV 3 and pollen viability under ENV 1 led to the identification of superior genotypes with respect to the trait. Genotypes performing well in cold stress were BAM 5889, BAM 5179, and BAM 4408 whereas genotypes with inferior performances were BAM 56, BAM 811 and BAM 4138. Pollen viability under cold stress has been reported to be an important indicator for cold tolerance (Zhou *et al.,* 2010).

Table 3. Path Coefficient studies

			Indirect Effect Through										
		Direct Effect	DTF	FLA	PE	PL	PH	PN	SF	TW	SPP	BY	GY (g)
DTF	ENV 1	-0.028		0.082	-0.034	-0.028	0.009	0.183	-0.011	-0.022	0.231	-0.014	0.368**
	ENV 2	-0.001		-0.045	-0.005	0.004	-0.05	0.017	-0.09	-0.054	0.109	0.261	0.146
	ENV 3	-0.033		0.027	-0.041	-0.021	0.003	0.002	-0.136	-0.027	-0.003	0.111	-0.118
FLA	ENV 1	0.221	-0.011		-0.014	-0.04	0.012	0.029	-0.012	-0.022	0.27	-0.014	0.421**
	ENV 2	-0.113	0		-0.001	0.022	-0.066	0.001	-0.058	0.036	0.143	0.185	0.148
	ENV 3	0.1	-0.009		-0.044	-0.08	-0.028	0.001	-0.096	-0.013	0.011	0.206	0.049
PE	ENV 1	0.144	0.007	-0.021		-0.012	-0.005	-0.195	0.079	0.028	-0.055	0.005	-0.026
	ENV 2	0.02	0	0.003		0.026	-0.004	-0.006	-0.044	0.048	-0.028	-0.011	0.005
	ENV 3	0.141	0.01	-0.031		-0.003	-0.011	0.009	0.052	0.032	-0.001	0.054	0.253*
PL	ENV 1	-0.077	-0.01	0.115	0.023		0.008	-0.057	0.156	-0.015	0.3	-0.008	0.436**
	ENV 2	0.068	0	-0.036	0.008		-0.049	-0.001	-0.007	0.014	0.118	0.112	0.228*
	ENV 3	-0.149	-0.005	0.054	0.003		-0.05	-0.001	-0.026	-0.002	0.01	0.195	0.029
PH	ENV 1	0.031	-0.009	0.086	-0.025	-0.021		0.097	-0.061	-0.005	0.213	-0.015	0.277*
	ENV 2	-0.176	0	-0.043	0	0.019		0.004	-0.047	0.002	0.085	0.192	0.033
	ENV 3	-0.08	0.001	0.035	0.019	-0.093		-0.001	0.015	-0.009	0.009	0.227	0.147
PN	ENV 1	0.447	-0.012	-0.021	-0.063	0.01	0.097		-0.146	-0.033	-0.005	-0.012	0.208*
	ENV 2	0.055	0	0.003	-0.002	-0.001	0.004		-0.039	-0.034	-0.079	0.259	0.141
	ENV 3	0.044	-0.002	-0.031	0.03	0.004	-0.001		-0.018	-0.018	-0.009	0.453	0.490**
SF	ENV 1	0.579	0.001	-0.004	0.02	-0.021	-0.003	-0.112		0.04	0.088	0.001	0.588**
	ENV 2	0.394	0	0.017	-0.002	-0.001	0.021	-0.005		0.046	0.076	-0.054	0.491**
	ENV 3	0.279	0.016	-0.034	0.026	0.014	-0.004	-0.003		0.049	-0.004	0.05	0.389**
TW	ENV 1	0.142	0.004	-0.034	0.029	0.008	-0.005	-0.106	0.162		-0.134	0.002	0.069
	ENV 2	0.24	0	-0.017	0.004	0.004	0.002	-0.008	0.075		-0.031	-0.003	0.267*
	ENV 3	0.143	0.006	-0.009	0.032	0.002	-0.009	-0.006	0.095		0.001	-0.068	0.187
SPP	ENV 1	0.546	-0.012	0.109	-0.014	-0.042	0.012	-0.004	0.093	-0.035		-0.01	0.643**
	ENV 2	0.374	0	-0.043	-0.001	0.022	-0.04	-0.012	0.08	-0.02		0.161	0.520**
	ENV 3	0.024	0.004	0.046	-0.006	-0.063	-0.03	-0.016	-0.046	0.007		0.011	-0.07
BY	ENV 1	-0.023	-0.017	0.131	-0.034	-0.027	0.02	0.233	-0.028	-0.014	0.251		0.493**
	ENV 2	0.436	-0.001	-0.048	0	0.018	-0.078	0.033	-0.048	-0.001	0.138		0.448**
	ENV 3	0.436	-0.001	-0.048	0	0.018	-0.078	0.033	-0.048	-0.001	0.138		0.651**

(*) = significant at 5% level of significant; (**) = significant at 1% level of significant

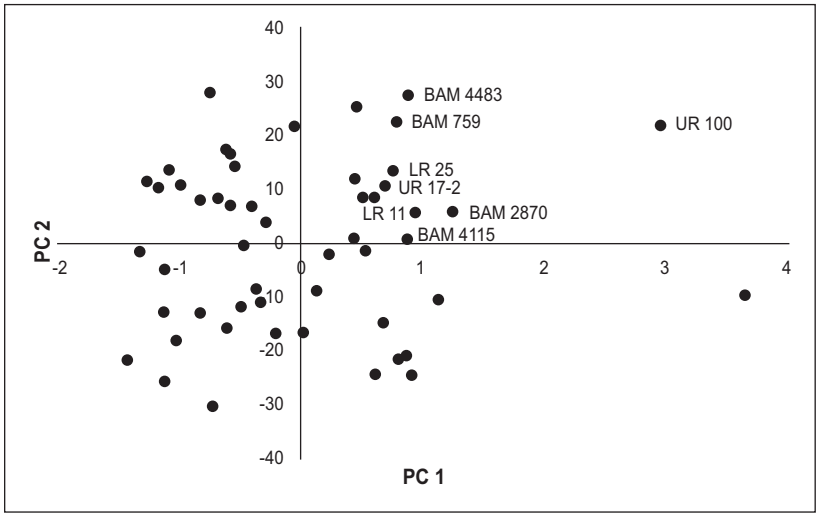

Fig.1. Principal Component Analysis based on rice characters found to be important under late sown condition and their relative value as compared to timely sown condition

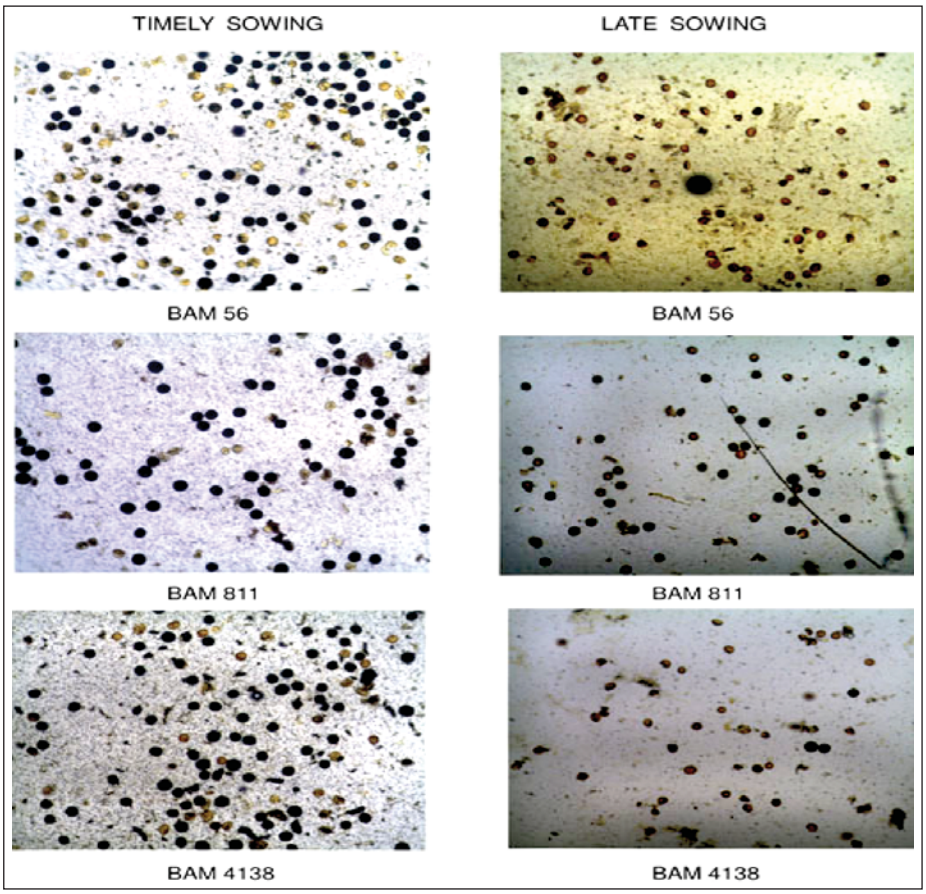

Fig. 2. Identification of tolerant and susceptible genotypes with respect to pollen viability

Identification of Important Genotypes under Cold Stress at Reproductive Stage

Important characters having significant correlation and path coefficient values with respect to grain yield under low temperature at flowering stage (late sown condition) were identified. The characters which were identified were biological yield, spikelet fertility, panicle exsertion, test weight, grain yield and spikelet per panicle. Among the

mini core rice accessions studied, genotypes performing well under both timely (ENV 1) and late sown (ENV 2/ ENV 3) environment identified from the selected six traits were BAM 4115, UR 100, BAM 5179, BAM 3808, LR 11, BAM 4483 and UR 3.

Conclusion

The genotypes identified in the study, especially the local landraces, can be used as potential donors for breeding cold tolerant varieties for mid- and high altitude areas of the North Eastern Hill Region. The important characters identified should help designing effective selection indices in such breeding programmes. Selected tolerant and susceptible genotypes could be further utilized and studied in detail to elucidate the genetic, molecular and physiological basis of cold tolerance at both, seedling and reproductive stage.

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RESEARCH ARTICLE

Exploration, Germplasm Collection and Variability Study on an Underutilized Root Tuber Crop "*Soh-phlong*" (*Flemingia procumbens* Roxb.) from Meghalaya, India

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This article deals with the study on morphological variability of underutilized root crop 'Soh-*phlong*'(*Flemingia procumbens* Roxb.) collected through exploration in Meghalaya, India. A total of 27 accessions studied for tuber characters, viz., size, shape, skin colour, taste and texture of epidermis, revealed significant good variation. Thrust areas for future aspects of plant genetic resource (PGR) value and its management have been highlighted.

Key Words: Collection, Conservation, Exploration, *Soh-phlong*, Underutilized tuber crop.

Introduction

Flemingia procumbens Roxb. [syn. *Moghania vestita* (Baker) Kuntze] locally called as *'Soh-phlong'* belongs to the family Fabaceae (Arora and Pandey,1996; Pandey and Arora, 2004, Sawian, 2007; Rahnam, 2017). It is an underutilized minor tuber crop domesticated in Indian sub-continent, but has remained confined to its cultivation in Khasi and Jaintia hills of Meghalaya, a Northeastern hill state of India (Jyrwa *et al*., 2017). *Soh-phlong* ("*Soh*" means fruit and "*Phlong*" means grass in Khasi language) term is used for tubers valued as fresh fruit (as they are juicy and refreshing) which are dug from field totally covered with its trailing stems and branches. It is distributed in the western Himalaya extending eastwards upto Khasi Hills, where it is locally used for its medicinal importance. Unlike many other tuber crops, this has gained very little attention though it has having multiple uses (Edison *et al*., 2006; Vidhan, 2010; Divya and Pushpa Bharati, 2017).

Soh-phlong is a little known edible root tuber crop from Meghalaya, North-East India. Meagre research information pertaining to different aspects of genetic resources (Singh and Arora, 1973), agronomic practices (Gangwar and Ramakrishnan, 1989) and pharmaceutical value (Jyrwa *et al*., 2017). It's ethnobotanical use by the tribals as wormicide has drawn on attention of the researchers more towards identification of active principles and its purification and pharmaceutical evaluation (Roy *et al*., 1996; Das *et al*., 2004; Das *et al*., 2006; Tandon and Das, 2007). Despite of several reported use, there is neither any systematic collection and conservation effort nor variability studies conducted in this important crop originated from the region.

Keeping this knowledge gap in view, the ICAR-National Bureau of Plant Genetic Resources has recently conducted an exploration trip for germplasm collection in *Soh-phlong* during November 2017 from Meghalaya, India which resulted in augmentation of good tuber variability (Nivedhitha *et al*., 2017). The variability of the mature root tubers collected from selected areas of five districts of Meghalaya was studied to find the pattern within the collected germplasm.

Materials and Methods

A crop-specific exploration was undertaken within the area of North-Eastern state of Meghalaya (25º.76′ to 25º.38′ N latitude; 92º.18′ to 91º.48′E longitude; altitude ranging from 1399-1837m) covering five districts i.e East Khasi Hills, West Khasi Hills, South-west Khasi Hills, West Jaintia Hills and Ri-Bhoi of Meghalaya, India during November 2017. A total of 27 accessions of *Soh-phlong* germplasm were collected, using standard random sampling method (Arora and Paroda, 1991). Ten samples/per accession of physiologically matured root tubers were collected from farmers' field, cleaned

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from soil particles and stored in muslin bag. Only a few samples with morphologically distinct type were collected from Ri-Bhoi district.

Morphological characters include visual observations and scoring of size, shape, skin colour, texture of epidermis and ease of peeling. Since the root tuber characters were allied to cassava (*Manihot esculenta* Crantz) the characterisation methodology was followed based on the scoring of cassava (Fukuda *et al*., 2010). For recording of quality data (taste, bitterness of tubers *etc*.) authors undertook sensory evaluation/organoleptic test with the help of 25 local people. Statistical analysis of collected diversity was done using DARwin6 (version 6.0.015), a software package developed for diversity analysis. Information on crop cultivation areas and ethnobotanical use of the species was recorded based on semi-formal structured interview with local tribal farmers and sellers of village markets and validated with published literature.

A total of 50 herbarium specimens of cultivated and wild type *Soh-phlong* located at Botanical Survey of India, Eastern Regional Centre, Shillong and Northern Regional Centre, Dehra Dun and Forest Research Institute, Dehradun, were studied for morphological characters especially leaves, root tubers, etc. A set of the collected germplasm was deposited at ICAR-NBPGR, Regional station, Shillong for establishment, regeneration and evaluation studies, while the other set was used for variability study and biochemical analysis at the ICAR-NBPGR, New Delhi. Vouchers of herbarium specimens (HS23047a, 23047b) were deposited in the National Herbarium of Cultivated Plants (NHCP), New Delhi.

Result and Discussion

Collection Sites

A total of 27 accessions of *Soh-phlong* collection showed diverse morphological traits. The crop was reported under cultivation in selected districts of Meghalaya and this was not however preferred in the neighbouring states (Fig. 1). Emphasis was given on tuber characters i.e. shape, taste, skin colour, and ease of peeling during collection and those characters were less variable as compared with tuber size (Table 1).

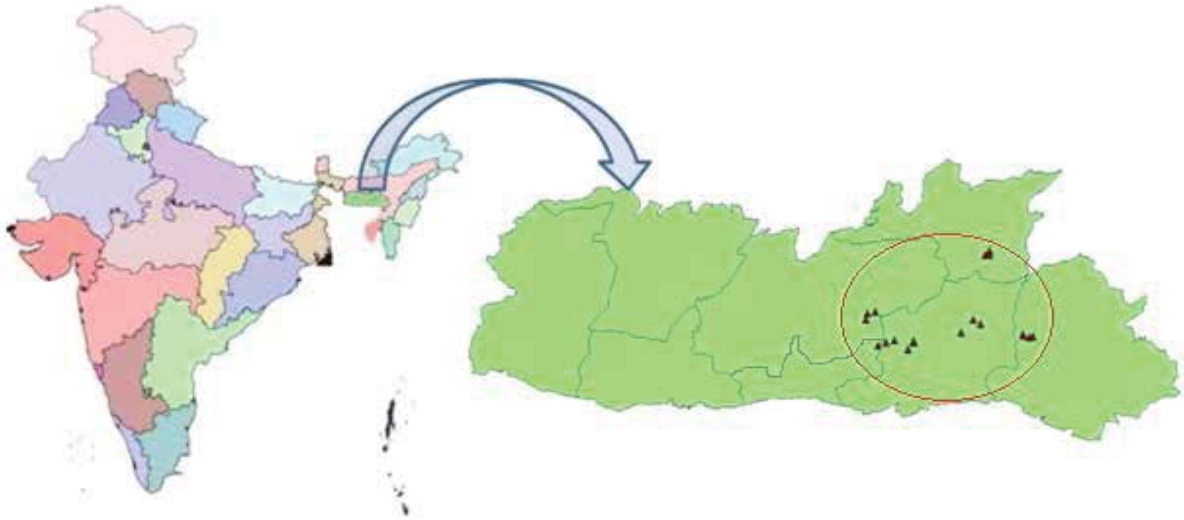

Fig. 1. Sites of germplasm collections, areas of cultivation and markets for *Soh-phlong* (shown by triangles)

Root Tuber Variability Study

Cluster analysis using DARwin6 (version 6.0.015) grouped 27 accessions into two major clusters namely cluster I and II, based on qualitative traits (Table 1) and average length and circumference of the tubers. Cluster I was divided into two sub-clusters, sub-cluster I and II. Sub-cluster I again formed two groups with accessions NST 22, 4, 25, 28, 21, 13, 6, 15, 24 and 2 in IA sub-cluster and accessions NST 12, 9, 10, 1, 11, 17, 20, 14 and 3 in IB sub-cluster. In sub-cluster II accessions NST 8, 7, 27, 23 and 16 placed in II A sub-cluster and accessions NST 29 and 18 placed in IIB sub-cluster.

NST 26 was placed separately and formed cluster II. The detailed cluster characteristics with their represented traits are described below:

Cluster I: Sub-cluster I was a major group clustering 19 accessions which represented all the five collection sites. This cluster showed grouping again into ten accessions in IA cluster and nine accessions in IIB cluster. All accessions in this group mostly formed a homogenous group of representatives. The tubers were characteristically fusiform shape, with dark-pale yellow skin, easily peelable epidermis, with intermediate-nutty (sweet) taste and average length of 5.54 cm and circumference of 7.08 cm.

Sub-cluster II had seven accessions represented three collection sites such as East Khasi Hills, West Jaintia Hills and Ri-Bhoi districts where, five accessions placed in II A cluster and remaining two accessions placed in II B cluster without much variability in characters. Mostly root tubers had fusiform-cylindrical shape, pale-dark yellow skin and ease of peeling. This group had heterogenous types with mostly nutty tubers with average length of 8.18 cm and circumference 7.40 cm.

Cluster II: Accession NST 26 collected from Ri-Bhoi district was classified into entirely a separate cluster with the average length of 14 cm (highest among the collected accessions) with the tuber circumference of 3 cm which forms the lowest among rest of the accessions. The tuber was cylindrical in shape, yellow, and easy-to-peel indermediate epidermis with nutty taste.

The authors were informed that for marketing purpose, mostly big sized tubers were preferred but for propagation purpose, farmers usually prefer to store small-medium sized tubers.

Table 1. Morphological characterization of qualitative traits* of root tubers of *Soh-phlong*

S.No.	Collector No. with IC no.	Area of collection	Shape	Skin colour	Texture of epidermis	Taste	Ease of peeling
1	NST 1 (NA)	West Khasi Hills	Fusiform	Dark yellow	Rough	**Intermediate	Easy
2	NST 2 IC-0627400	West Khasi Hills	Fusiform	Dark yellow	Rough	**Intermediate	Easy
3	NST 3 IC-0627401	West Khasi Hills	Fusiform	Dark yellow	Rough	**Intermediate	Difficult
4	NST 4 IC-0627402	West Khasi Hills	Conical	Pale yellow	Rough	**Intermediate	Difficult
5	NST 6 IC-0627403	East Khasi Hills	Fusiform	Pale yellow	Rough	**Intermediate	Easy
6	NST 7 IC-0627404	East Khasi Hills	Fusiform	Pale yellow	Intermediate	Nutty	Difficult
7	NST 8 IC-0627405	East Khasi Hills	Fusiform	Pale yellow	Intermediate	Nutty	Difficult
8	NST 9 IC-0627406	East Khasi Hills	Oval	Dark yellow	Rough	**Intermediate	Easy
9	NST 10 IC-0627407	East Khasi Hills	Oval	Dark yellow	Rough	**Intermediate	Easy
10	NST 11 IC-0627408	East Khasi Hills	Fusiform	Yellow	Rough	Nutty	Easy
11	NST 12 IC-0627409	East Khasi Hills	Conical	Dark yellow	Rough	**Intermediate	Easy
12	NST 13 IC-0627410	South-west Khasi Hills	Fusiform	Pale yellow	Rough	Nutty	Easy
13	NST 14 IC-0627411	South-west Khasi Hills	Cylindrical	Dark yellow	Rough	Nutty	Difficult
14	NST 15 IC-0627412	South-west Khasi Hills	Fusiform	Pale yellow	Rough	Bitter	Easy
15	NST 16 IC-0627413	East Khasi Hills	Fusiform	Dark yellow	Intermediate	Bitter	Difficult
16	NST 17 IC-0627414	West Jaintia Hills	Cylindrical	Pale yellow	Rough	Bitter	Easy
17	NST 18 IC-0627415	West Jaintia Hills	Fusiform	Dark yellow	Intermediate	Nutty	Easy
18	NST 20 IC-0627416	West Jaintia Hills	Fusiform	Dark yellow	Rough	Nutty	Difficult
19	NST 21 IC-0627417	West Jaintia Hills	Conical	Pale yellow	Rough	Nutty	Easy
20	NST 22 IC-0627418	West Jaintia Hills	Conical	Pale yellow	Rough	Nutty	Difficult
21	NST 23 IC-0627419	West Jaintia Hills	Fusiform	Pale yellow	Intermediate	Nutty	Difficult
22	NST 24 IC-0627420	West Jaintia Hills	Fusiform	Dark yellow	Rough	Nutty	Difficult
23	NST 25 IC-0627421	East Khasi Hills	Fusiform	Pale yellow	Rough	**Intermediate	Difficult
24	NST 26 IC-0627422	Ri-Bhoi	Cylindrical	Yellow	Intermediate	Nutty	Easy
25	NST 27 IC-0627423	Ri-Bhoi	Fusiform	Pale yellow	Intermediate	**Intermediate	Difficult
26	NST 28 IC-0627424	Ri-Bhoi	Fusiform	Pale yellow	Rough	Bitter	Difficult
27	NST 29 IC-0627425	Ri-Bhoi	Cylindrical	Dark yellow	Intermediate	Nutty	Easy

*: based on Fukuda *et al.* (2010); ** bitter and itchy; nutty (sweet), NA- Not allotted

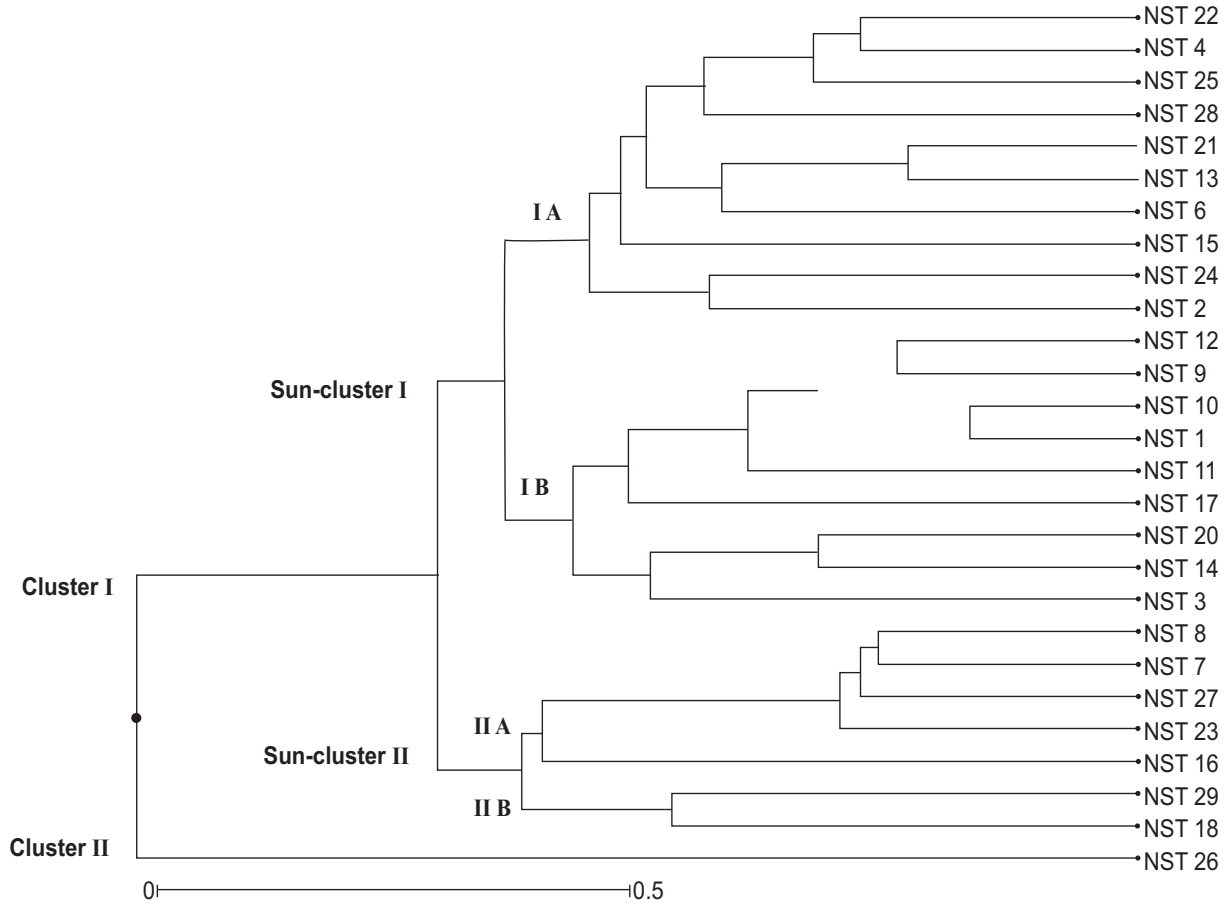

Fig. 2. Cluster diagram-DARwin6 (version 6.0.015) showing classification of 27 accessions into two major discrete clusters

Cultivation Practices, Harvest, Storage and Market

Agronomic practices followed for cultivation in the Hills of North-Eastern region especially Meghalaya has been worked out for diverse crops growing in this area (Awasthi *et al*., 1981; Awasthi and Borthakur, 1986; Jeeva *et al*., 2006). Rainfall and soil conditions of the area of cultivation are very specific (Pugh, 1951).

Soh-phlong is generally propagated vegetatively through small tubers during March-April. It is planted in a virgin soil for one year. After that the land is left fallow or utilized for plantation of other crops. Farmers used to grow the crop after the gap of minimum five years in rotation. Mixed cropping also practiced. Mostly, it grown along with turmeric, ginger, taro and potato. *Soh-phlong* is planted in jhum land, on bund at 30-45 cm plant to plant distance and 35-40 cm between the rows and covered with soil. Earthing-up and weeding are done as soon as weeds emerge, but preferably when plants attain a height of about 8-10 cm during June-July. The flowering starts from August onwards and the crop takes seven months to reach maturity. Harvesting starts in mid November. The tubers are manually harvested by digging up with a spade and stored in a pit covered with soil and they are taken out as per market demand (Awasthi *et al*., 1981).

Freshly harvested tubers are graded on the basis of large, medium and small sizes. Large-medium sized tubers fetch better price in market. However, an average yield of 3,000 kg/ha has been reported in Meghalaya (Singh and Arora, 1973). *Soh-phlong* is grown as a cash crop and sold as peeled raw tubers at Rs. 100-300/ kg in the markets of Meghalaya. Tubers are available in the local/village markets and Shillong markets by October till May.

After grading, freshly harvested tubers of small size are kept for self consumption and next season growing

Fig. 3. 1. Khasi farmer digging out the root tubers 2.Tribal women showing freshly harvested tuber 3. Storing seed tubers for next year 4. Processing tubers for market sale 5.Tubers sold in village market 6. Peeled tubers sold in market with chutney made with (*Perilla frutescens*) 7. Root tuber variability collected and studied 8. Variability in peeled tubers 9. Harvested tubers with dried aerial parts.

as propagating material. For propagation purpose tubers are stored for the next planting season (5-6 months) inside a pit (size approximately 40-50 × 30cm or depending on the quantity of 'propagules/tubers' to be stored) dug near the field and undisturbed till planting season (Fig. 3.3). For market sale, tubers are stored underground intermittently and delivered periodically based on market demand. For, market use, generally medium-large sized, round to fusiform shaped tubers with good taste are preferred.

Botany of *Soh-phlong* and its Use

Flemingia procumbens Roxb. Fl. Ind., ed. 3:338. 1832. *Flemingia vestita* Baker; *Maughania procumbens* (Roxburgh) Mukerjee; *M. vestita* (Baker) Kuntze.

A perennial herb, 60-80cm, stem densely pubescent prostrate, highly branched. The roots are tuberous (6 cm or longer), fusiform, round and cylindrical, skin colour–dark to pale yellow, Leaves digitately 3-foliolate obovate-cuneate, pubescent; stipules ovate, 4-8 mm, persistent; petiole 1-2 cm, wingless, pubescent; petiolules

short, densely hairy; leaflets papery; terminal leaflet oblong or nearly obovate, 1-5 x 0.5-2.5 cm, lateral leaflets obliquely elliptic, smaller, both surfaces sparsely pubescent or glabrous, abaxial surface with dense dark red glands, basal veins 3, lateral veins 3-5 pairs, apex and base rounded. Raceme axillary or terminal, 2 to 10 cm, densely pubescent; bracts lanceolate, ca. 5 mm, striate. Flowers ca. 6 mm, clustered; pedicel ca. 1 mm. Calyx 5-lobed; lobes linear-lanceolate, lower one is longer than the tube. Corolla slightly longer than calyx; standard elliptic, base with claw and auricles at 2 sides; wings narrowly oblong, base with claw and auricle at one side; keels falcate, clawed. Ovary elliptic, sparsely hairy; style linear, glabrous flowers bright-red; Fruits are hairy sub-cylindrical pods and elliptic, ca. 5×4 mm, shortly villous, black and glandular; seed globose, brown or black in colour; Flowering- in August-September.

Uses

This crop is reported for anthelmintic and vermifungal properties (Yadav *et al.,* 1992; Tandon *et al*., 1997; Tandon and Saha, 2004; Das *et al.,* 2004; Sunita, 2014). Research on phytochemistry has led to identification of active principles (Bidyadha *et al.,* 2008). During the inter-action, the authors has got feed-back on associated indigenous knowledge on use, propagation and conservation aspects that was cross-checked with local people.

Soh-phlong crop is known to improve soil quality being a leguminous crop by fixing atmospheric nitrogen (Gangwar and Ramakrishnan, 1989). Edible tuber are consumed by the Khasi and Jaintia hill tribals after peeling off the outer yellowish skin along with grinded Perilla seeds (locally known as 'Neileih'). The tubers have vermifugal property and are administered to children for deworming and stomach upset and cooling (Singh and Arora, 1973; Das *et al.,* 2004; Sunita, 2014).

Future Thrust

Under climate change, with serious concerns about sustainability of agricultural production and food security worldwide, interest in the under-utilized tuberous vegetable such as *Soh-phlong* which has been domesticated long back in India needs renewed attention.

Despite all the above mentioned uses of this crop, it has remained hidden and unknown to rest of the geographic region. The conservation of biodiversity of tuber crops is most desirable in the context of food security, climate change and crop diversification. In the context, the under-utilized tuber crop like *Soh-phlong*, efforts to conserve it in field genebank, establish propagation methods, and *in vitro* studies are warranted. The following aspects are needed to be prioritized in respect to plant genetic resource management.

Collection: fine grid survey, exploration and collection of selected desired types

Evaluation: morphological and biochemical evaluation of tubers (plant-type, easy-to-peel types, etc.) for market

Conservation: traditional and *in vitro* methods

Storage: traditional *vis-a-vis* new methods

Use: edible tuber and other value added products

Developing the descriptor for this crop, conservation of elite types through tissue culture and on-farm conservation need to be attended. More systematic collection of variability in plant types and tubers and the medicinal properties of this underutilized crop needs to be given major thrust for strengthening genetic resources programme.

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RESEARCH ARTICLE

Phenotypic Characterization of Pigeonpea (*Cajanus cajan*) Germplasm Collected from Jharkhand, India

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The present investigation was carried out with an aim to study genetic diversity, trait correlation and path analysis among one hundred pigeonpea accessions collected from geographically diverse locations of Jharkhand state of India. These accessions were evaluated in an augmented block design for two years for nine yield attributing traits and analysis of variance, path analysis, correlation and hierarchical cluster analysis was performed on the recorded data to estimate the extent of variability among genotypes and to measure the direct and indirect contribution of different yield attributing traits on yield. Significant genetic variability was observed among the accessions for all the studied traits with high estimates of heritability and genetic advance. Highly significant and positive correlation was found between different traits and yield per plant. The hierarchical cluster analysis grouped the genotypes into 11 distinct non-overlapping clusters. It was concluded that the genotypes possess significant variability enabling them as candidate genotypes to be utilized as parents for breeding of pigeonpea varieties with desired attributes.

Key Words: Cluster analysis, Genetic divergence, Germplasm, Phenotypic characterization, Pigeonpea.

Introduction

Pigeonpea (*Cajanus cajan* L. Millspaugh) is a highly adaptable, stress-tolerant and high in protein grain legume, which possesses valuable traits for enhancing the sustainability of dry sub-tropical and tropical agricultural systems (Khoury *et al.,* 2015; Kaoneka *et al.,* 2016). It stands next only to chickpea in terms of importance as a mainstream pulse crop in India. However, the yield potential of pigeonpea remains unrealized due to various biotic and abiotic stresses (Khalekar *et al.,* 2013). India is the principal pigeonpea growing country and contributes nearly 90% of world's acreage and production, followed by Myanmar, Kenya, and Malawi (FAOSTAT, 2014). The total area under pigeonpea cultivation is reported to be 3.88 mha in India with a total production of 3.17 mt and an average productivity of 849 kg per ha (Indiastat, 2015). India has now been conclusively recognized as the centre of origin as proposed by Vavilov (1951) and primary centre of diversity for pigeonpea (Saxena *et al.,* 2014; Kaoneka *et al.,* 2016). In India, the North-Eastern part of the Deccan Plateau along with the adjoining Chhotanagpur plateau, forming the parts of the present day Odisha, Chhattisgarh and Jharkhand states, boast tremendous genetic diversity in pigeonpea germplasm. Therefore, there is a high probability of finding elite germplasm in the form of locally adapted land races with desirable agro-morphological traits which may culminate into breeding of pigeonpea varieties with higher productivity. The role of genetic diversity in conducting successful plant breeding programmes involving productivity, quality parameters and stress tolerance is very important (Walunjkar *et al*., 2015). The market demand of pigeonpea is bound to increase in demographically expanding India, where *per capita* pulse availability has declined from 69 grams in 1961 to 32 grams in 2005 (Swaminathan and Bhavani, 2013). The importance of pigeonpea in India is manifold as protein malnutrition is a major problem due to widespread poverty and vegetarian food habit of a significant proportion of the population. Further, as the Eastern India is being viewed as a potential ground for second green revolution in order to realize the national dream of permanent and complete food self-sufficiency, the genetic erosion of precious germplasm is highly likely as was the case with the first green revolution in the Northern India. Moreover, the introduction of

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pigeonpea hybrids in the area targeted for exploration and germplasm collection is also a potent threat to the locally adapted precious land races. Therefore, collection and characterization of the pigeonpea germplasm from this part of the country assumes great significance. The characterization data provides valuable information about genetic diversity in the germplasm collections and this information is helpful in understanding the pattern of genetic variation in a crop species (Rao and Hodgkin 2002) and its further utilization. The pattern of variation enables the breeders to select effective and efficient parent for a crossing programme. In view of these facts the present study was conducted with the objectives to phenotypically characterize, classify and to study divergence and relationship among the collected pigeonpea germplasm accessions in order to estimate the genetic worth of the material for subsequent inclusion in the breeding programmes.

Material and Methods

The experimental material consisted of 100 accessions of pigeonpea germplasm collected from northern Jharkhand during the year 2012 as per the standard germplasm collection guidelines of the National Bureau of Plant Genetic Resources, New Delhi. These germplasm accessions were characterized in an augmented block design (ABD) along with two checks viz. Bahar (local check) and NDA-1 (National check) for two years under sub-tropical, sub-humid climate of Ranchi. The observations were recorded on nine quantitative traits viz. days to 50% flowering, days to maturity, primary branches per plant, 100 seed weight (g), plant height (cm), number of pods per cluster, pod length (cm), number of grains per pod and seed yield per plant (g) on the 100 accessions collected from Jharkhand. The mean values of the recorded observations were used for statistical analysis to assess the genetic diversity among listed accessions. The data was analysed by using the software Windostat Version 9.2 from Indostat services, Hyderabad. The genetic parameters i.e. genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability and genetic advance at 5% selection intensity and correlation coefficients were estimated as per Singh and Chaudhary (1979). The correlation coefficients were partitioned into direct and indirect effects using the path coefficient analysis according to Dewey and Lu (1959). Euclidean distances can theoretically estimate the genetic distances between parents to maximize the transgressive segregation upon crossing and cluster analysis is an appropriate method for determining family relationships. Among various methods used for genetic diversity analysis the Tocher's method is one of the most popular ones. Mahalanobis (1936) generalized distance (D^2) was used to determine the degree of divergence and cluster analysis was performed following Tocher's method (Rao, 1952).

Results and Discussion

The descriptive statistics of the studied traits revealed that the trait seed yield per plant (CV=35.06%) exhibited maximum variation while minimum variation was observed for days to 80% maturity (CV=5.65%) (Table 1). Among all the traits investigated, seed yield per plant had highest standard error, standard deviation as well as highest coefficient of variation. It indicated that the characterized accessions differ considerably on the basis of yield per plant. The descriptive statistics clearly indicated the presence of significant genetic variability among genotypes for other traits also. The traits seed yield per plant and primary branches per plant exhibited maximum variability among all the traits. Both of these two traits are important yield component traits and thus they are determinative of the genetic worth of the germplasm. Similar findings with significant

Table 1. Descriptive statistics of nine traits in pigeon pea

Trait	Mean	Standard Error (±)	Minimum	Maximum	Standard Deviation	CV (%)
Days to 50% flowering	175.90	1.36	139.65	197.65	13.75	7.82
Days to 80% maturity	227.88	1.28	194.80	252.30	12.88	5.65
Primary branches per plant	22.75	0.52	12.22	36.36	5.29	23.25
100 Seed weight (gm)	11.16	0.15	7.80	14.21	1.52	13.63
Plant height (cm)	270.54	2.90	197.05	347.55	29.27	10.82
Pods per cluster	3.58	0.06	2.42	5.49	0.59	16.51
Pod length (cm)	4.94	0.07	2.98	7.00	0.67	13.48
Seeds per pod	4.57	0.04	3.85	5.83	0.44	9.70
Seed yield per plant (g)	98.40	3.42	46.69	190.17	34.50	35.06

variation in seed yield and primary branches per plant were reported by Saroj *et al.* (2013) and Kumara *et al.* (2014). The analysis of variance showed that genotype mean squares for all studied traits were highly significant except for primary branches per plant and hundred seed weight. Among test entries versus control all the traits except primary branches per plant, plant height and seeds per pod showed significant differences for the genotypes under study. It was inferred that the diversity in pigeonpea genotypes is mainly due to the variability in yield per plant, plant height, days to 50% flowering, days to maturity and primary branches per plant which is in agreement with the earlier reports of De *et al.* (1992); Katiyar *et al.*, 2004 and Nag *et al.* (2012). The environmental variance was lower than the corresponding genotypic variance for all the studied traits. The lower environmental variance implied that the studied traits were under genetic control and selection should be effective in their improvement. Similar results were reported by Vange and Egbe (2009), Mahiboobsa *et al.* (2012) and Saroj *et al.* (2013).

The GCV was highest for yield per plant followed by the traits primary branches per plant and pods per cluster (Table 2). Linge *et al.* (2010) Saroj *et al.* (2013) reported similar results regarding GCV in pigeonpea. High GCV and minimum difference with the corresponding PCV indicates a lesser role of environment in the expression of these traits and their amenability for improvement through selection. High broad sense heritability estimates were found for yield per plant (0.97), seeds per pod (0.94), pod length (0.93), pods per cluster (0.88), plant height (0.88), days to 80% maturity (0.88) and days to 50% flowering (0.87). The heritability estimates were moderate for primary branches per plant (0.52) and low for 100 seed weight (0.14). The estimates of heritability indicated the possibility of trait improvement through selection and generally, similar pattern of heritability estimates for different traits were reported by Dahiya and Brar (1977) and Manyasa *et al.* (2008). However, Saroj *et al.* (2013) have reported low broad sense heritability for number of seeds per pod and high heritability for 100 seed. The traits with high heritability can be used as descriptor for classification of studied germplasm (Abu-Alrub, 2004). Genetic advance is an indicator of the genetic progress in a trait under selection. In the present study high genetic advance was estimated for the traits yield per plant (66.97), pods per cluster (27.40), pod length (24.55) and primary branches per plant (21.28). When both genetic advance and heritability are high for a trait, it indicates the predominance of additive gene effects and as per Panse, (1957) selection should be effective in the improvement of these traits. To assist and accelerate the process of selection, positive and significant phenotypic association between yield and its component traits is highly desirable. In the present investigation, positive correlation with yield per plant was exhibited by all the traits under study (Table 3). Pods per cluster exhibited significant positive correlation with yield per plant and highly significant positive correlation was exhibited by the traits days to 50% flowering and days to 80% maturity, days to 50% flowering and plant height, days to 50% flowering and pods per cluster, days to 80% maturity and plant height and pods per cluster, primary branches per plant and plant height and pod clusters per plant, 100 seed weight with pod length and seeds per pod, plant height with pods per cluster, pod length with seeds per pod. The traits 100 seed weight and pods per cluster were found to be negatively correlated and the correlation was significant. The positive correlation among traits indicated that selection based on these traits

Table 2. Genetic parameters of the studied genotypes

Trait	Mean	Variance			Heritability			
		Environmental	Genetic	Phenotypic		GCV	PCV	GA
Days to 50% flowering	175.76	23.17	152.03	175.20	0.87	7.02	7.53	13.46
Days to 80% maturity	228.03	19.08	138.43	157.51	0.88	5.16	5.50	9.96
Primary branches per plant	22.80	9.90	10.68	20.59	0.52	14.34	19.90	21.28
100 seed weight	11.13	1.38	0.22	1.60	0.14	4.22	11.36	3.22
Plant height	270.43	98.14	740.03	838.17	0.88	10.06	10.71	19.47
Pods per cluster	3.57	0.03	0.26	0.29	0.88	14.15	15.05	27.40
Pod length	4.92	0.03	0.37	0.39	0.93	12.33	12.76	24.55
Seeds per pod	4.57	0.01	0.16	0.17	0.94	2.11	8.66	17.32
Yield per plant	97.78	35.56	1044.74	1080.30	0.97	33.06	33.61	66.97

Table 3. Phenotypic correlation coefficients

Trait	Number of days to 50% flowering	Number of days to 80% maturity	Number of primary branches per plant	100 Seed weight (gm)	Plant height (cm)	Number of pods per cluster	Pods length (cm)	Number of grains per pod	Seed yield per plant (g)
Days to 50% flowering	**1.000**	0.773***	-0.090	-0.048	0.428***	0.216*	-0.108	-0.179	0.101
Days to 80% maturity		**1.000**	0.039	0.015	0.334***	0.264**	-0.058	-0.035	0.127
Primary branches per plant			**1.000**	0.144	0.196*	0.201*	0.028	0.184	0.154
100 Seed weight (gm)				**1.000**	0.004	-0.006*	0.211*	0.375***	0.154
Plant height (cm)					**1.000**	0.413***	-0.075	0.050	0.158
Pods per cluster						**1.000**	0.006	0.030	0.263**
Pods length (cm)							**1.000**	0.607***	0.129
Seeds per pod								**1.000**	0.150
Seed yield per plant (g)									1.000

* Significant at 5% ** Significant at 1% *** Significant at 0.1%

should positively contribute towards improvement of the seed yield. These results are supported by the earlier findings of Salunke *et al*. (1995) and Sodavadiya *et al*. (2009). Padi (2003) have reported significantly positive correlation between pods per plant and yield per plant. However, Vange and Moses (2009) reported negative correlation between days to 50% flowering and days to maturity which was not observed in the present study. The path coefficient analysis for seed yield per plant through estimated genotypic correlation coefficients between yield per plant and its component traits is presented in the Fig. 1. It revealed that number of pods per cluster gave the maximum direct effect (0.217) on the yield followed by 100 seed weight (0.111). The maximum direct effect of pods per cluster followed by 100 seed weight, primary branches per plant, days to 50% flowering, seeds per pod, plant height and number of days to 80% maturity as revealed by path analysis are in general agreement with the results reported by Brar *et al.* (1991) and Nag and Sharma (2012). However, Udensi and Ikpeme (2012) reported that 100 seed weight was having highest direct effect on yield per plant. The trait, pods per plant, manifested through number of pods per cluster was having the highest direct effect on seed yield and was found to be the main yield component trait in the present study. Similar findings have been reported by Padi (2003). The collected genotypes were subjected to genetic diversity and relationship analysis

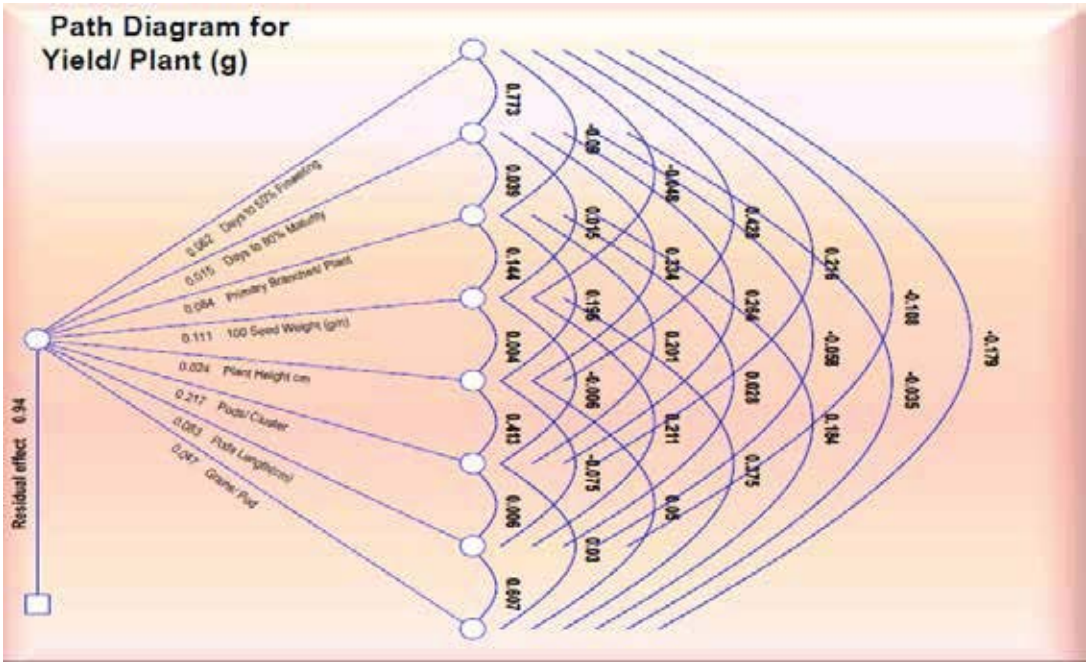

Fig. 1. Path diagram for yield per plant (R^2= 0.1168 Residual effect = 0.94)

by estimating Euclidean inter-cluster and intra-cluster standard distances. The dendrogram based on Ward's minimum variances (Ward, 1963) was constructed which grouped all accessions under study into 11 distinct non-overlapping clusters. The number of clusters and the inter-cluster distances indicated presence of significantly high genetic diversity in the collected germplasm accessions. Cluster I comprised of 14 genotypes which were maximum in any cluster followed by cluster V and X with 13 genotypes each, cluster VIII and IX with 10 genotypes each, cluster III, IV and XI with 9 genotypes each and cluster II, VI and VII with 6, 5 and 4 genotypes respectively. The cluster means for the studied nine quantitative traits were estimated and based on it the desirable accessions were selected from cluster II which had accessions with lowest mean for days to maturity (211.38) and cluster VII which had accessions with maximum mean yield per plant (153.08). The inter-cluster distance was maximum between cluster II and VI (38.13) and minimum between clusters VIII and IX (11.65). The intra-cluster distance was minimum in cluster XI (6.61) and maximum in cluster VI (11.30). Both Bahar and NDA-1, used as checks were grouped in the same cluster in the present study. Different genotypes of the same geographic origin were found to be scattered across different clusters and thus the geographic site of collection and genetic divergence were found to lack a definite relationship in the present study. The main factor behind the presence of high genetic diversity in the collected accessions appeared to be due to genetic drift and selection in differing environments rather than the geographic origin of the germplasm. The free movement of the germplasm across different environments represented by the collection sites could also be a reason of the lack of a definite relationship between the geographic site of collection and genetic identity of the accession. The experimental findings of cluster analysis are in general agreement with the findings of Sharma and Roy (1994), Mahamad *et al.* (2006), and Pandey *et al.* (2015). Kumari *et al.* (2014) have reported similar results in an SSR primer based genetic diversity study in pigeonpea. The inter-cluster distances were greater than the intra-cluster distances revealing that considerable amount of genetic diversity existed among the accessions. It is assumed that maximum amount of heterosis would be manifested in cross combinations involving the parents belonging to the most divergent clusters. The percent contribution of different traits for genetic divergence showed that the contribution of complex trait yield per plant was maximum (47%) towards genetic divergence followed by that of plant height (40%). The traits days to 50% flowering and days to 80% maturity contributed 6% each while primary branches per plant contributed only 1% to the total genetic divergence. These findings are in agreement with those of Kumara *et al.* (2014) who reported that yield is the biggest contributing trait towards genetic divergence in pigeonpea. In the present study, the parents having traits contributing maximum towards genetic divergence, for example, yield per plant and plant height were successfully identified for further use in the pigeonpea improvement programmes.

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RESEARCH ARTICLE

Assessment of Genetic Diversity in Cultivated and Wild Species Germplasm of Barley based on Morpho-agronomical and Root Architecture Traits

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Genetic variation in a selected set of 213 accessions of wild and cultivated barley germplasm was evaluated for 23 agro-morphological and root traits for two years. Wide range of phenotypic expression was observed for traits such as days to spike emergence (53.50-113.50), plant height (PH) (39.53-141.87 cm), spike length (1.83-13.57 cm), spikelet triplet groups (STG) 10-35.67, 100-grain weight (HGW) (0.12-6.56 g), total root length (RL) (6.96-97.25 cm) and root dry weight (RDW) (0.20-12.84 mg). Wild germplasm grouped under cluster I and cultivated under cluster II with different sub-groups for hull-less, two-rowed and six-rowed germplasm, irrespective of the geographical diversity. Principal component analysis indicated that first five components accounted for 77.23% of the total multivariate variation and it was mainly contributed by grain yield (GY), grain area (GA), STG, RL, total root surface area (RSA), total root volume (RV) and RDW. Correlation coefficients of root traits were positively significant with PH, SL, HGW, GY and grain size, indicating root architecture can play a pivotal role for future yield enhancement in rain-fed crop like barley. Donors for various traits were also identified such as short duration (IC445542, IC542197, IC470019), spike length (IC113052), dwarf plant habit (IC113045), root biomass (EC578716, EC492340).

Key Words: Barley germplasm, Genetic diversity, Root morphology, Wild species.

Introduction

Barley (*Hordeum vulgare* L; 2n=14) is one of the oldest cultivated cereal and has been grown in India since ancient times. It was domesticated in southwest Asia from two-rowed wild progenitor *H. vulgare* ssp. *spontaneum* and Himalayas is considered as it's center of diversification (Badr *et al.,* 2000). Presently, it is the fourth most important cereal crop worldwide after maize, rice and wheat in terms of production (FAO, 2018). Barley provides essential raw material for malting and brewing industries besides being an important feed and fodder crop. It also constitutes traditional staple food and beverage crop in many high altitude regions of world including India. Although, it is an ancient food and feed crop grown in India, its cultivation is presently facing stiff challenge from wheat crop, in terms of area and also fetches less remunerative prices, since green revolution. Further, about 44% area under barley cultivation in India is rain-fed. Therefore, owing to its cultivation as a rain-fed crop in marginal areas or on residual moisture, the increase in the yield of this crop has not been considerable (Ceccarelli *et al.*, 1999; Kumar *et al*., 2013). The rapid spread of improved varieties all over the world has led to narrow genetic base due to replacement of genetic resources and loss of specific alleles. New sources of genetic diversity through evaluation of germplasm collections must be incorporated into plant breeding to diversify the parental material for sustained and continued increase in yield and quality (Dawson *et al.*, 2015; Kaur *et al.*, 2018a, Kaur *et al.*, 2018b). Assessment of genetic diversity in germplasm collections helps breeders to select the accessions for crossing program and broadening the genetic base. Although genetic diversity assessment in barley germplasm assembly from different regions of world *viz*. North Africa (Ben Naceur *et al.,* 2012); Ethiopia (Abebe *et al.*, 2010); Spain (Lasa *et al.,* 2001); North America (Mikel and Kolb, 2008) and India (Manjunatha *et al*., 2007; Sarkar *et al*., 2010, 2014; Kaur *et al*., 2018) have been carried out using above ground agro-morphological traits, there is limited knowledge of genetic variation present in root system architecture and its association with above-ground plant morphology. The root architecture of a crop can influence the efficiency of

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water capture and hence yield and thus are particularly important to study in a rain-fed crop like barley. An advantage of having superior root traits such as root number, branching, root diameter, total root length/ surface area, root length density, and root volume under water scarce conditions have been proved recently in rice and wheat (Henry *et al.*, 2012; Christopher *et al.,* 2013). The present study was therefore, conducted to assess the nature and magnitude of variation in a diverse set of barley germplasm based on agro-morphological and root traits and to identify superior accessions which may have implications in future barley breeding program.

Materials and Methods

Plant Materials and Field Experiment

The plant material used in present study comprised of a diverse set of 213 germplasm accessions of spring barley, of which 126 accessions were indigenous collections (IC) and 87 were exotic collections (EC) including seven accessions of wild barley belonging to *Hordeum marinum*, *H. murinum* and *H. vulgare* ssp. *spontaneum*. The germplasm accessions under study were selected from field characterisation of a large collection of 5000 barley accessions conserved at National Gene Bank of ICAR-National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India. The agro-morphological and phenological data pertaining to this huge set were subjected to diversity analysis using PowerCore (v.1.0) software to select the set of accessions used in present study. Field experiments were carried out during the *Rabi* season consecutively for two years (2016–2017 and 2017–2018) at Issapur experimental farm of ICAR-National Bureau of Plant Genetic Resources located between 28°57′ N, 76°84′ E, altitude 218 m above sea level in south-west of Delhi, India. Issapur has semi-arid sub-tropical climate with around 400 mm average annual precipitation, sandy loam to loamy sand with pH 8.

Phenotyping of Morpho-agronomic Traits and Root System Architecture

Observations were recorded on 23 traits of which field phenotyping was done on 17 agro-morphological traits and six root morphological traits were recorded at seedling stage in laboratory experiment. For field evaluation 11 traits *viz.* days to 75% spike emergence (DSE), days to 80% maturity (DPM), plant height (PH, cm), spike length (SL, cm), spikelet triplet groups (STG), number of grains per spike (NGS), 100-grain weight (HGW, g), and grain yield per meter row (GY, g), grain length (GL, mm), grain width (GW, mm), grain area (GA, mm^2) were recorded quantitatively, while six traits *viz.* growth habit (GH), spike row (SR), awn type (AT); spike density (SD); grain type (GT); grain pericarp color (GPC) were scored qualitatively. Data were recorded on five randomly chosen plants per plot as per minimal descriptors for barley (Mahajan *et al.,* 2000). The grain morphometry (GL, GW and GA) was done using scanner based Grain Analysis software (version 1.3).

For root phenotyping, the surface sterilized seeds were germinated on damp germination paper segment placed in aseptic petri dishes of 90 mm diameter and 15 mm height providing enough moisture for germination. After sprouting for 3 days, uniformly germinated seeds were kept in half strength Hoagland solution (Hoagland and Arnon, 1950) for a period of 10 days in a growth chamber, under a long-day photoperiod (16 h light/ 8 h dark) at 22°C and a relative humidity of 65-75% using germination paper roll method. On 10^{th} day, the roots were washed, cut from root-shoot junction, and were spread out in a root positioning tray (30 × 40 cm) with 1 cm of water. Root samples were then scanned in greyscale at 600 dpi using a desktop scanner (Epson flatbed scanner) and the images obtained in tiff format were analysed using WinRHIZO Pro software (v2009, Regent Instruments, Montreal, QC, Canada), which is an image analysis system specially designed for root measurements. The WinRhizo software provided phenotypic data on total root length (RL; cm), total root surface area (RSA; cm^2), root diameter (RD; µm), root volume (RV; mm^3) and seminal root number (SRN). In addition, root dry weight (RDW; mg) was recorded after drying the root samples in a hot air oven at 70°C for 72 hrs. Ten seedling of each accession were used for recording observations.

Statistical Analysis

The germplasm accessions were evaluated in Augmented Block Design (Federer, 1956) with five standard check varieties *viz.* BH902, RD2552, BH959, BHS352 and DWRB101 in 6 blocks. Each block had 36 accessions and five check varieties which were randomized within blocks. Each accession was sown in two rows of 2 m row length with 30 × 10 cm spacing. The quantitative traits were analysed for various statistical parameters *viz.* mean, range, variances, principal component analysis (PCA)

and Ward's agglomerative hierarchical clustering (Ward, 1963) using SAS (version 9.3, 2009). Before undertaking statistical analysis on the basis of adjusted pooled mean values, homogeneity of variances was tested as per Levene (1960). Phenotypic and genotypic coefficients of variation (PCV and GCV) for each trait were computed as PCV = $\sqrt{V_P}$/mean × 100, GCV = $\sqrt{V_G}$/mean × 100 as per Burton (1952) and the range was categorized as per Sivasubramanian and Madhavamenon (1978). Broad sense heritability was estimated as h^2 (bs) = V_G/V_P × 100 as per Lush (1940) and further classified into low, medium and high (Robinson, 1966). Expected genetic advance was calculated as EGA = k × V_G/V_P × $\sqrt{V_P}$ as per Johnson *et al.* (1955). Here the standard value of k is 2.06 assumed at 5% selection intensity; V_G is genotypic variance; and V_P is phenotypic variance. Genetic advance was expressed as % of mean as GA (%) = EGA/mean × 100. In addition, Pearson correlation coefficients were computed at $P_{0.05}$ for all morpho-agronomic variables including root traits.

Results and Discussion

Amongst qualitative traits, six-row type accessions represented 83% of the total collection under study. Approximately 90% of the accessions were awned. Single accession was recorded as awnless while nine accessions had very short awns and six were with hooded plant type. Spike density was recorded intermediate in 51% of the accessions followed by dense in 44% and lax in 5% accessions only. Seventy seven per cent accessions were hulled types while 23% were naked or hull-less. Grain pericarp color varied widely, *viz*., white, blue, black, red and purple in some accessions, however white/light yellow pericarp was dominant (90%). Wild barley *H. vulgare* ssp. s*pontaneum*, *H. marinum* and *H. murinum* were characterized by awned, two-row spike with brittle rachis form. The spikes were very long and awned in *H. vulgare* ssp. *spontaneum*, while other *Hordeum* sp. were having awnleted short spikes. Our results are in agreement with earlier diversity assessment studies in barley germplasm by Sarkar *et al.* (2010) and Manjunatha *et al.* (2007) who also reported almost similar frequency distribution for major qualitative traits such as row-type, grain type and grain color. Thus in the present study, the most common type of barley germplasm accessions were characterized by semi spreading nature of GH, six-row type spike with intermediate spike density, awned spikes and hulled grains.

Statistical analysis on qualitative traits showed wide range of phenotypic expression for important agro-morphological traits such as PH (39.53-141.87 cm), SL (1.83-13.37 cm), NGS (11.00-83.83), HSW (0.12-6.99 g) and GY (1.11-191.25 g) (Table 1). Mean DSE was 89, but it ranged from 53.50 for accession EC578711

Table 1. Statistical parameters of genetic variability in barley germplasm for various morpho-agronomic and root traits

Traits (n=213)	Range	Mean ± SE	Variance (P)	Variance (G)	PCV (%)	GCV (%)	Heritability	Genetic advance as % of mean
DSE	53.50-113.50	89.03±0.88	159.82	153.86	14.20	13.93	96.27	28.16
DPM	103.00-137.50	123.6±0.41	32.43	20.98	4.61	3.70	64.69	6.14
PH	39.53-141.87	95.94±0.95	172.69	141.70	13.70	12.41	82.05	23.15
SL	1.83-13.57	8.15±0.11	2.52	2.09	19.48	17.74	82.94	33.28
STG	10.00-35.67	23.09±0.33	21.22	15.36	19.95	16.97	72.38	29.75
NGS	11.00-83.83	46.12±1.15	280.72	251.06	36.33	34.36	89.43	66.93
GY	1.11-191.25	83.93±2.70	2226.00	1543.00	47.69	39.71	70.32	68.10
HGW	0.12-6.56	3.98±0.07	1.02	0.99	25.38	25.00	97.06	50.74
GL	6.29-12.43	9.98±0.10	2.04	1.74	14.31	13.22	85.29	25.15
GW	1.76-4.11	2.97±0.05	0.48	0.37	14.23	12.49	77.08	22.59
GA	9.73-34.62	23.80±0.52	56.24	41.88	22.19	19.15	74.47	34.04
RL	6.96-97.25	50.41±0.98	183.33	168.93	26.86	25.78	92.15	50.98
RSA	0.42-15.19	8.64±0.19	6.66	5.97	29.87	28.28	89.64	55.16
RV	2.60-287.60	119.08±3.10	1769.69	1232.03	35.82	29.89	70.62	51.38
RD	265.08-762.35	538.00±4.60	4028.57	2865.24	11.82	9.97	71.12	17.32
SRN	1.50-9.00	5.48±0.07	1.05	0.61	18.70	14.25	58.10	22.38
RDW	0.20-12.84	5.46±0.14	3.86	3.66	35.98	35.04	94.82	70.28

DSE, days to spike emergence; DPM, days to physiological maturity; PH, plant height (cm); SL, spike length (cm); STG, spikelet triplet groups; NGS, number of grains per spike; GY, grain yield per meter row (g); HGW, hundred grain weight (g); GL, grain length (mm); GW, grain width (mm); GA, grain area (mm^2); RL, total root length (cm); RSA, total root surface area (cm^2); RV, total root volume (mm^3); RD, root diameter (μm); SRN, seminal root number; RDW, root dry weight (mg).

to 113.50 for accession EC667569. The early maturing genotypes reached physiological maturity in 103 days and late maturing genotype took 138 days to mature. Similar range for DSE and DPM was also reported by Ebrahim *et al.* (2015) while evaluating genetic diversity in barley. HGW, an important yield character, varied extensively from 0.12 g in accession EC123148 (*H. marinum*) to 6.56 g in DWRB-91 with an average of 3.98 g. The two-rowed accessions showed higher HSW and grain size due to better grain filling than six-row types as also reported by Sarkar *et al.* (2010). Spike emergence and maturity were late in wild accessions. Smallest spike (1.77 cm) and lowest HSW (0.10 g) was recorded in wild EC578252. For root morphological traits, mean RL was 50.41 cm, while the range was 6.95 cm in *H. marinum* accessions to 97.25 cm in EC578523. Accessions EC578821, IC533320 had maximum RSA. Mean SRN was 5.48 while mean RDW was 5.46 g. The wide range of variation observed in this study also corroborated earlier diversity assessment studies in barley by Sarkar *et al.*(2010) for DSE, PH and HSW, Ebrahim *et al.* (2015) for DPM, Sarkar *et al.* (2014) for HSW, SL, NGS and Sun *et al.* (1999) for PH.

The extent of the genetic variability can be better assessed by estimation of GCV in relation to PCV. Small difference between the two indicates underlying genetic factors while larger difference indicates influence of the environment. PCV and GCV were high for GY, NGS, HGW, RL, RSA, RV and RDW and low for rest of the traits. In studied barley germplasm, DSE, SL, NGS, GY, GL, RL, RSA and RDW showed high heritability in broad sense coupled with high genetic advance and are predominantly under additive type of gene action. Accessions IC113052, IC118656, EC578517, IC82573, EC339572, EC578523, EC578821, EC578716 and IC533320 showed high value of these traits and can be exploited further for grain yield through simple phenotypic performance based selection methods using these traits. These traits showed >80% heritability and may be useful for exploiting additive gene action to produce widely adopted genotypes in barley breeding. On the other hand, parameters such as DPM, STG, SRN, GA, SRD, which have low variance, heritability and genetic advance showed the presence of non-additive gene interaction and needed to be improved. Accessions EC328972, IC533203, EC578370, IC445342, IC334463 and EC578653 showed low heritability and genetic advance for above-mentioned traits.

To understand the nature of correlations between traits, data were analysed to determine Pearson simple correlation coefficients among these traits (Table 2). Correlation coefficient between PH, grain yield and yield attributes such as SL, STG, HGW and NGS were significant and positive. Plant height is an important trait directly linked with productive potential of the plant *i.e.* yield. In addition, it is desirable trait for using barley as

Table 2. Correlation coefficients of different quantitative variables for barley germplasm accessions

Traits	GA	GW	DPM	DSE	GL	PH	SL	STG	GY	HGW	NGS	SRN	RSA	RV	RL	RDW
GW	0.88*															
DPM	-0.04	-0.18*														
DSE	-0.21*	-0.38*	0.61*													
GL	0.83*	0.50*	0.08	-0.02												
PH	0.23*	0.33*	-0.01	-0.03	0.05											
SL	0.45*	0.48*	-0.08	-0.10	0.27*	0.42*										
STG	0.24*	0.29*	0.10	0.18*	0.60	0.40*	0.57*									
GY	0.40*	0.44*	-0.12	-0.22*	0.30*	0.28*	0.18*	0.14*								
HGW	0.66*	0.76*	-0.18*	-0.36*	0.33*	0.33*	0.47*	0.24*	0.45*							
NGS	0.11	0.12	-0.05	-0.02	0.10	0.39*	0.12	0.24*	0.21*	-0.10						
SRN	0.47*	0.59*	-0.28*	-0.35*	0.18*	0.40*	0.53*	0.35*	0.37*	0.57*	0.20*					
RSA	0.58*	0.59*	-0.07	-0.23*	0.40*	0.44*	0.38*	0.31*	0.51*	0.59*	0.10	0.56*				
RV	0.49*	0.48*	-0.03	-0.18*	0.33*	0.40*	0.29*	0.24*	0.41*	0.48*	0.40	0.42*	0.93*			
RL	0.56*	0.57*	-0.06	-0.23*	0.38*	0.47*	0.41*	0.36*	0.48*	0.58*	0.15*	0.57*	0.92*	0.75*		
RDW	0.54*	0.57*	-0.13	-0.27*	0.35*	0.33*	0.35*	0.27*	0.46*	0.55*	0.07	0.56*	0.84*	0.80*	0.76*	
RD	0.37*	0.41*	-0.10	0.17*	0.16*	0.27*	0.24*	0.12	0.19*	0.36*	0.08	0.33*	0.57*	0.75*	0.29*	0.56*

*Correlation is significant at $P_{0.05}$

GA, grain area; GW, grain width; DPM, days to physiological maturity; DSE, days to spike emergence; GL, grain length; PH, plant height; SL, spike length; STG, spikelet triplet groups; GY, grain yield per meter row; HGW, hundred grain weight; NGS, number of grains per spike; SRN, seminal root number; RSA, total root surface area; RV, total root volume; RL, total root length; RDW, root dry weight; RD, root diameter.

fodder crop. Ruzdik *et al.* (2015) also observed significant and positive correlations between grain yield and plant height in barley. DSE was negatively correlated with GA, HGW and GY, which means early spike emergence may lead to more filled bold grains and thus yield in barley. Al-Tabbal and Al-Fraihat (2012), and Sarkar *et al.* (2014) also reported negative correlation of HGW, GY with DSE. DSE and DPM were positively correlated ($r=0.615$, $p_{0.05}$) with each other suggesting accessions showing early spike emergence were also early maturing. All the root traits *viz.* SRN, RSA, RV, RL, RDW and RD were correlated with each other and with GA, GW, GL, PH, SL, GY, HGW. Selection for one trait will cause change in its mean through additive gene effect and simultaneous indirect effect in mean of correlated trait. Therefore, in the present study, positive correlation between root traits and yield components suggests root structure architecture should be paid attention for further yield enhancement in a rain-fed crop like barley.

The clustering pattern based on multivariate analysis of quantitative variables revealed that all the accessions grouped into two main clusters- C1 and CII, constituted by wild and cultivated germplasm, respectively (Fig. 1). Cluster I grouped wild accessions of *H. marinum* and *H. murinum* except *H. vulgare ssp. spontaneum,* which were characterized by late spike emergence and maturity, small PH, SL and small sized grains. *H. vulgare* ssp. s*pontaneum* along with some other two-rowed accessions formed a sub-group under cluster II owing to its more plant height and spike length. It forms the primary gene pool and is fully inter-fertile with cultivated barley and has been known to harbor beneficial sources of genes utilized in breeding for important agricultural traits (Von Korff *et al.,* 2004, Vanhala and Stam, 2006). However, the extent of utilization of other *Hordeum* sp. such as *H. marinum* and *H. murinum* is low because they belong to tertiary gene pool. To depict the genetic relationship among cultivated germplasm, a dendrogram was generated by Ward's agglomerative hierarchical clustering method based on squared Euclidean distances which revealed that CII could be further divided in four sub-groups with 93 accessions in cluster II-A, followed by 21 accessions in cluster II-B, 57 accessions in cluster II-C and 39 accessions in cluster II-D (Table 3). The mean values of accessions grouped under each sub-cluster showed that cluster II-B grouped majority of the two-rowed barley accessions which showed more SL, STG, GY, HSW, RL, RSA, RV, RD, SRN and RDW and were bold seeded. Most of the hull-less barley accessions grouped under cluster II-C which showed late DSE, DPM, lesser yield and small grain size. Cluster II-A and II-D were constituted by hulled six-row barley accessions with a sub-group of two-rowed accessions under both. Manjunatha *et al.* (2007) had also reported separate clustering for hulled barley from hull-less types. Thus, resulting four sub-clusters distinguished hull-less from hulled and two-rowed from six-rowed barley accessions, but failed to separate from different geographical areas in the region. Our results are in congruence with Shekhawat *et al.* (2001) and Mittal *et al.* (2010) who carried out genetic divergence

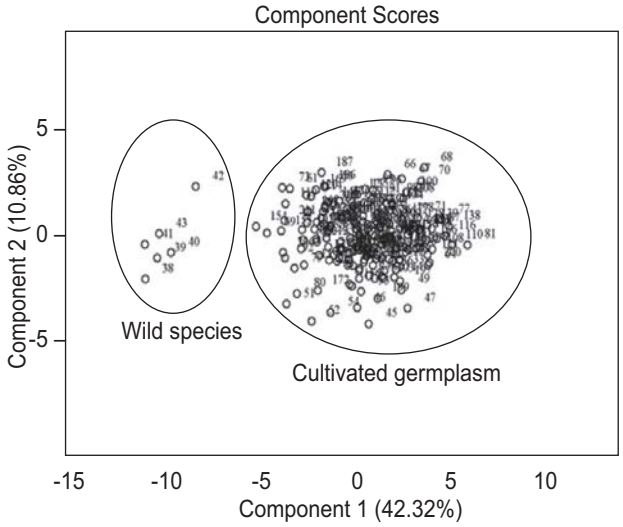

Fig. 1. Principal component biplot showing distribution of 213 barley accessions

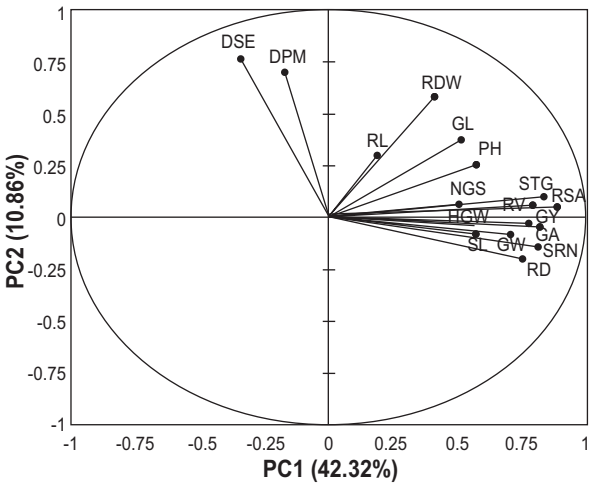

Fig. 2 Scatter plot of different morpho-agronomic and root architecture variables loaded on PC1 and PC2

Table 3. Means of quantitative variables in different clusters of barley germplasm accessions

Cluster variables	C I	C II-A	C II-B	C II-C	C II-D
DSE	111.66	89.63	86.85	93.10	78.31
DPM	129.6	124.99	123.32	125.46	117.71
PH	48.78	100.43	96.13	97.52	90.35
SL	3.11	8.32	9.66	7.78	8.27
STG	13.46	23.19	27.62	23.19	21.80
NGS	16.34	52.17	29.37	45.60	45.85
GY	2.22	97.99	116.68	52.55	82.57
HGW	0.21	4.18	5.00	3.48	4.26
GL	9.50	10.77	10.77	8.45	10.16
GW	2.10	5.11	5.57	4.45	5.05
GA	13.31	37.50	41.25	26.96	35.45
RL	8.72	55.97	65.64	42.98	45.71
RSA	1.18	9.94	11.86	6.83	7.45
RV	7.01	144.21	176.44	88.17	88.59
RD	368.55	565.85	579.07	512.68	510.88
SRN	1.75	5.46	6.49	5.20	5.86
RDW	0.86	6.23	8.11	4.12	4.78
No. of accessions	6	93	21	57	39

DSE, days to spike emergence; DPM, days to physiological maturity; PH, plant height (cm); SL, spike length (cm); STG, spikelet triplet groups; NGS, number of grains per spike; GY, grain yield per meter row (g); HGW, hundred grain weight (g); GL, grain length (mm); GW, grain width (mm); GA, grain area (mm^2); RL, total root length (cm); RSA, total root surface area (cm^2); RV, total root volume (mm^3); RD, root diameter (μm); SRN, seminal root number; RDW, root dry weight (mg).

studies in barley and reported that geographic diversity not necessarily reflect genetic diversity. However, our clustering pattern does not corroborate the studies by Shafaeddin (2002) and Abebe *et al.* (2010) who stated fair relationship between the genetic and geographical classifications among origins of samples, which is not found in our study. Germplasm lines from same geographical regions may fall apart in clusters which may happen due to genetic similarity, selection pressure and environmental influence or due to free exchange of material across different locations. Germplasm belonging to the same cluster are considered similar with respect to aggregate effect of studied traits. Hence, divergent parents can be selected from these clusters as genetic distance among them is due to different genetic constitutions.

The PCA based on correlation was used to identify determinants of quantitative trait variability. First five PCs having Eigen value >1.0 contributed 77.23% of the total multivariate variation present in the germplasm, 42.32% of which was accounted in the first axis, and 10.86% in the second (Fig. 2). According to Johnson and Wichern (2002), based on Eigen values and vectors, it is possible to identify traits which are mainly responsible to explain the variation. Therefore, a genotype-trait biplot was constructed based on first two principal components revealed that GA, GY, STG, SRN, RSA and RV contributed more positively to PC1, while DSE, DPM and RDW contributed to PC2 which suggests that germplasm accessions could be distinguished by characters associated with PCs having more relative contribution in total variation. PC3, PC4 and PC5 explained 9.11, 8.53 and 6.41% variation, respectively, loaded partially on PH, NGS, GL, GA, SL and HGW. Our results are in congruence with Ebrahim *et al.* (2015) and Manjunatha *et al.* (2007) who reported high contribution of above-mentioned agro-morphological traits while studying diversity assessment in barley.

The superior accessions identified in the first year were planted at two locations during second year evaluation for morpho-agronomic traits. Based on the pooled performance for location and year combination, few trait specific promising germplasm accessions were identified such as hulled barley IC445542 and EC578370 showed early maturity in 103 and 105 days, respectively, compared to checks BH902 (130 days) and BH959 (128 days). Hull-less IC542197 and IC470019 showed short duration/earliness in 107 days compared to check BHS352 (125 days). Early types are essential in most barley producing areas in India as rain-fed barley production needs early maturing varieties to escape drought and terminal heat in late March and early April. Early maturity is the main breeding target in India owing

to rain-fed sowing of barley under low input conditions in chief producer states of UP, Rajasthan, Bihar, hilly areas of Uttrakhand and JK and under limited moisture in Punjab and Haryana. Average plant height was 95.94 cm while one accession IC113045 showed dwarf plant habit with 42.51 cm height compared to variety BH959 (91.32 cm) used as check, while EC578707 was recorded to be the tallest attaining the height upto 142 cm. In addition, these accessions also showed early maturity. HGW was more than 5.5 g in accessions EC492340 and IC542206 compared to DWRB101 (4.84 g) used as check for two-row types. For SL, two-rowed accession IC113052 (13.57 cm) and six-rowed IC118656 (12.7 cm) were found promising compared to check DWRB101 (8.15 cm) and BH959 (8.93 cm), respectively. Seed morphometry revealed that accession IC578281 and IC533165 had maximum GL (12.43 mm, 11.28 mm), GW (3.93 mm, 4.11 mm) and GA (34.62 mm^2, 32.05 mm^2), and thus was bold seeded compared to best check BH959. For RDW, accessions EC578716 (12.84 mg) and EC492340 (11.01 mg) were found superior in comparison to best check BH902 (5.8 mg).

In conclusion, present study identified STG, GA, GY, RSA, SRN and RDW as the major traits contributing towards phenotypic diversity based on comprehensive information derived from PCA and cluster analysis. This indicates that yield traits and root morphology have contributed highest genetic variability in barley germplasm under study. The positive significant inter-relationship between the root traits and yield and its attributes suggested that improvement in root architecture could play a pivotal role for future yield enhancement in barley, predominantly sown on residual moisture in India. Moreover, the phenotyping of root traits reported in this study is high throughput, rapid, easy and cost effective technique to screen large number of germplasm accessions for better root structure architecture, which can be further evaluated in field conditions. The trait specific promising germplasm accessions such as EC578711 (early heading), IC445542, IC542197 (short duration), IC533320 (root length) and EC578716, EC492340 (root biomass) identified are likely to be of specific advantage in dry conditions as they represent the best compromise to escape water scarcity, which is main production constraint for rain-fed barley cultivation in India.

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RESEARCH ARTICLE

Characterization of Rice Landraces from Dibrugarh District of Assam

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Twenty five different traditional rice landraces from Dibrugarh district of Assam were characterized. In case of morphological characters, high heritability coupled with high genetic advance was recorded for number of grains per plant and number of panicle per plant. For biochemical parameters, high heritability coupled with high genetic advance was recorded for amylose and fat contents. Correlation coefficient analysis revealed that number of grains per plant, plant height, stem length and panicle length exhibited positive correlation with grain yield per plant. Thus, stem length and numbers of grains per plant can be considered in breeding programme. The landraces were with low to intermediate gelatinization temperature range (55-74 ºC) indicating good cooking quality. The study identified Kolajoha, Miatong, Bor Aijung, Ronga Bordhan, Bor Salpona, Dolkochi, and Athabora as promising landraces that could be utilized in the development of high yielding landraces.

Key Words: Biochemical, Genetic variability, Morphological, Physicochemical, Rice.

Introduction

Rice (*Oryza sativa* L.) is the second most widely grown cereal crop in the world after wheat. North East India including Assam is considered as one of the primary centre of origin of rice representing a rich source of genetic diversity and reservoir of valuable rice gene pool. Due to favourable agro-climatic conditions, people in this region cultivate both local and high yielding varieties of rice including non-glutinous, glutinous as well as scented ones. There are various communities of Assam, who prefer and are mainly dependent on traditional local rice cultivars/ landraces. All the districts of Assam have their own traditional local rice cultivars. One of the districts of Assam is Dibrugarh, located at upper Brahmaputra Valley Zone extending from 27º5'38" N to 27º42'30" N latitude and 94º33'46"E to 95º29'8"E longitude inhabiting different communities. The rice cultivars grown in this area are of indigenous type and are not generally grown in other parts of Assam. The rice varieties grown are mostly scented and glutinous. However, there is lack of compilation depicting the key diagnostic characters of these cultivars that are essential to carry out scientific seed production and certification. India is the signatory of World Trade Organization (WTO) and as an obligation, the Protection of Plant Varieties and Farmers' Rights Act (2001) has been implemented in India from January 2005 (Yadav, 2004). Under this Act, Plant Varieties and Farmers' Rights Protection Authority has been established (Anonymous, 2005). Enforcement of these international agreements is possible only if the identity and ownership of the plant genotypes are established. Therefore, characterization of the cultivars is very essential from the view point of Plant Variety Protection of the farmers' varieties.

Genetics plays a decisive role in determining the chemical or physical characteristics of a plant and its products. Morphological traits, SDS-PAGE and rapid chemical tests have been used extensively for germplasm characterization and classification in rice and other crops (Chauhan and Nanda, 1984; Devi and Hazarika, 2000; and Patra, 2000). The conduct of DUS tests for distinctness, uniformity and stability of new varieties of plants is essential for the purpose of granting the breeder's right. In this light, the present investigation aimed for proper characterization of traditional rice landraces of Dibrugarh district, Assam based on DUS criteria coupled with biochemical evaluation.

Materials and Methods

In the present investigation, twenty five rice cultivars, traditionally cultivated in Dibrugarh district of Assam were collected and three rice varieties, one each from

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non-glutinous (Ranjit), glutinous (Aghoni Bora) and scented type (Keteki Joha) were taken as standards (Table 1). The field experiment was laid out in Randomized Complete Block Design (RCBD) with three replications. Observations were recorded for the morphological characters as per the International Union for the Protection of New Varieties of Plants guidelines (IUPOV), 1985 and National guidelines for DUS test. Observations were recorded for leaf characters viz., coleoptiles colour, basal leaf sheath colour, leaf anthocyanin colour, presence or absence of auricle, anthocyanin colour of auricle, presence or absence of ligule, anthocyanin colour of the ligule, leaf collar, flag leaf attitude, length and width of the leaf blade; panicle characters *viz.,* panicle attitude of branching, panicle exertion, colour tip of lemma, number of panicle per plants and panicle length; grain character like grain length, grain width, decorticated grain length, decorticated grain width, decorticated grain shape, decorticated grain colour, grain length/grain width ratio, decorticated grain length/ decorticated grain width ratio, 1000 grain weight, number of grains per panicle, number of grains per plant; plant characters *viz.,* stem length, stem thickness, time of heading, time of maturity and yield per plant. The cultivars were also tested for biochemical and physiochemical parameters, such as starch content (Chopra and Konwar, 1976), amylose content (Juliano, 1971), fat content (AOAC, 1970), crude protein content (Scales and Harrison, 1920), phenol test (Walls, 1965), aroma test (Sood and Siddique, 1978) and gelatinization temperature (Little *et al*., 1958).

Trial data were analyzed using ANOVA and the genetic parameters such as phenotypic and genotypic coefficient of variation (PCV and GCV) were worked out as suggested by Johnson *et al.* (1955). Correlation coefficients were calculated according to Al-Jibouri *et al.* (1958). Path coefficient analysis was calculated according to Wright (1921).

Results and Discussion

The present investigation aimed to identify the diagnostic characteristics both morphological and biochemical, of twenty five indigenous rice cultivars traditionally grown by the farmers of Dibrugarh district of Assam with three notified cultivars (Keteki Joha, Aghoni Bora and Ranjit) as control for determining varietal identity and purity and to provide assurance to the farmers about seed quality of the cultivars.

The analysis of variance revealed that all the landraces under investigation exhibited significant variation (Table 2). Morphological traits are being used as potential markers for identification of genotypes. Forty-six morphological traits were studied, out of which twenty eight traits were qualitative and eighteen traits were quantitative. Variations were observed in several qualitative characters like basal leaf sheath colour (Fig. 1), leaf anthocyanin colouration, pubescence of blade surface, leaf anthocyanin colouration of collar (Fig. 2), panicle exertion, panicle attitude of branching, colour tip of lemma (Fig. 3), density of pubescence of lemma, lemma anthocyanin colouration of apex, presence or absence of awn, awn colour, distribution of awn, decorticated grain shape and gelatinization temperature (Table 3). Of the twenty eight rice germplasm under study, twenty one was found to have green basal leaf sheath colour. Parikh *et al.* (2012) investigated 71 rice germplasms and found that majority of genotypes (84.5%) had green basal leaf sheath colour. Such variations in qualitative traits in rice landraces were also reported by Moukoumbi *et al.* (2011) and Sarawgi *et al*. (2013).

Mean performance of different quantitative traits are shown in Table 4. Leaf blade length and width of the rice germplasm were found to vary between 40.07–56.50 cm and 1.12–1.87 cm respectively. Wide range of variations regarding leaf length (38.0-76.3 cm) and leaf width (0.76-1.72 cm) were also reported by

Table 1. Rice genotypes used in the study

S. No.	Genotype Name	S. No.	Genotype Name	S. No.	Genotype Name	S. No.	Genotype Name
1	Johadhan	8	Cheni Lahi	15	Mainajan	22	Dolkochi
2	Kolajoha	9	Bor Malbhog	16	Malbhog	23	Dharia
3	Bor Aijung	10	Nalsati	17	Boga Bordhan	24	Athabora
4	Ranga Bordhan	11	Lakhman Dhan	18	Bora Dhan	25	Miatong
5	Sadiya Lahi	12	Ranga Bas	19	Bor Salpona	26	Aghoni Bora
6	Suwag Moni	13	Aibing Sali	20	Joybangla	27	Ranjit
7	Haldhar sali	14	Saru Salpona	21	Sagar Sali	28	Keteki Joha

Table 2. Analysis of variance for quantitative traits of rice land races of Dibrugarh district of Assam

Source	Degrees of freedom	LLB	LWB	PH	PL	SL	ST	PNP	TH	TM	NG Plant	LG	WG	LG/WG	LDG	WDG	LDG/WDG	Seed weight	Yield/plant	Starch	Amylose	Fat	Crude Protein
Replication	2	1.4680	0.0001	0.4672	0.0830	3.1691	0.0011	4.2512	0.1570	0.5830	55654.4201	0.0007	0.0007	0.0014	0.0006	0.0020	0.0013	0.2624	47.8321	0.79	37.03	0.005	0.005
Genotype	27	62.2751*	0.1016*	575.8220*	9.1771*	480.4513*	0.0025*	55.6691*	154.3863*	117.4401*	1034191.1501*	1.9240*	0.4941*	0.5921*	0.8197*	0.3673*	0.5414*	38.3712*	571.6511*	13.98*	137.33*	0.91*	0.55*
Error	54	0.2240	0.0004	2.5031	0.0709	1.0780	0.00002	0.2481	0.0111	0.2871	3832.7220	0.0007	0.0004	0.0004	0.0009	0.0006	0.0004	0.0170	2.4702	0.50	0.06	0.004	0.002
CV		1.0319	1.4091	0.4880	0.9710	0.8497	1.0185	2.9678	0.0892	0.3271	2.99	0.31	0.63	0.69	0.47	0.95	0.81	0.52	3.03	3.81	33.37	10.07	10.86

* P≤0.5 and ** P≤0.01

LLB: Length of leaf blade, LWB: Width of leaf blade, PH: Plant height, PL: Panicle length, SL: Stem length, ST: Stem thickness, PNP: Panicle no. per plant, TH: Time of heading, TM: Time of maturity, NGPlant: No. of grains per Plant, LG: Grain length, WG: Grain width, LDG: Length of decorticated grain, WDG: Width of decorticated grain

Fig. 1. Representative variations in basal leaf sheath colour of some rice cultivars.

Fig. 2. Representative variations in anthocyanin colouration of collar of some rice cultivars.

Fig. 3. Representative colour tip of lemma of some rice cultivars.

Table 3. Performance of different qualitative characters

Landraces & varieties	BLSC	LAC	PBS	LACC	PE	PAB	CTL	DPL	ACA	AWN	AC	DOA	DGS	GT
Johadhan	Green	Absent	Absent	Absent	Well exserted	Erect to semi erect	Black	Weak	Strong	No	Absent		Long Slender	High
Kolajoha	Green	Absent	Absent	Absent	Well exserted	Erect to semi erect	Black	Weak	Strong	No	Absent		Long Slender	High
Bor Aijung	Green	Absent	Medium	Absent	Well exserted	Semi erect	Yellowish	Absent	Absent	No	Absent		Long Bold	High
Ranga Bordhan	Green	Absent	Absent	Absent	Well exserted	Semi erect	Black	Weak	Medium	Yes	Reddish Brown	Upper half only	Long Bold	High
Sadiya Lahi	Green	Absent	Weak	Absent	Well exserted	Semi erect	Yellowish	Weak	Absent	No	Absent		Short Bold	High
Suwag Moni	Green	Absent	Medium	Absent	Well exserted	Erect to semi erect	Yellowish	Weak	Medium	Yes	Yellowish White	Upper half only	Long Bold	High
Haldhar Sali	Green	Absent	Weak	Absent	Well exserted	Erect to semi erect	Black	Weak	Absent	Yes	Yellowish Brown	Tip only	Long Bold	High
Cheni Lahi	Green	Absent	Medium	Absent	Well exserted	Erect to semi erect	Yellowish	Weak	Absent	Yes	Brown	Tip only	Long Slender	High
Bor Malbhog	Light Purple	Absent	Medium	Absent	Well exserted	Erect to semi erect	Yellowish	Weak	Absent	No	Absent		Long Bold	Low
Nalsati	Light Purple	Absent	Medium	Absent	Well exserted	Semi erect	Purple	Weak	Strong	Yes	Black	Upper half only	Long Bold	High intermediate
Lakhman Dhan	Green	Absent	Weak	Absent	Well exserted	Erect to semi erect	Yellowish	Weak	Absent	Yes	Yellowish White	Tip only	Long Bold	High
Ranga Bas	Green	Absent	Medium	Absent	Well exserted	Erect	Brown	Medium	Absent	No	Absent		Medium Slender	High
Aibing Sali	Light Purple	Absent	Weak	Absent	Well exserted	Erect to semi erect	Yellowish	Weak	Absent	Yes	Yellowish White	Tip only	Long Bold	High
Saru Salpona	Light Purple	Absent	Weak	Present	Well exserted	Semi erect	Purple	Weak	Strong	No	Absent		Long Slender	High
Mainajan	Green	Absent	Weak	Absent	Mostly exserted	Erect to semi erect	Brown	Medium	Absent	No	Absent		Short Bold	High
Malbhog	Light Purple	Absent	Medium	Absent	Well exserted	Semi erect to spreading	Yellowish	Weak	Absent	No	Absent		Long Bold	Inter-mediate
Boga Bordhan	Green	Absent	Weak	Absent	Well exserted	Erect to semi erect	Yellowish	Weak	Absent	No	Absent		Long Bold	High inter-mediate
Bora Dhan	Green	Absent	Weak	Absent	Well exserted	Semi erect to spreading	Brown	Weak	Absent	No	Absent		Long Bold	Inter-mediate
Bor Salpona	Light Purple	Absent	Weak	Present	Well exserted	Semi erect	Purple	Weak	Strong	No	Absent		Long Bold	High
Joybangla	Green	Absent	Medium	Absent	Well exserted	Erect to semi erect	Yellowish	Weak	Absent	No	Absent		Long Bold	High
Sagar Sali	Green	Absent	Absent	Absent	Mostly exserted	Erect to semi erect	Purple	Weak	Absent	No	Absent		Short Bold	High inter-mediate

Landraces & varieties	BLSC	LAC	PBS	LACC	PE	PAB	CTL	DPL	ACA	AWN	AC	DOA	DGS	GT
Dolkochi	Green	Absent	Medium	Absent	Well exserted	Semi erect to spreading	Brown	Weak	Absent	No	Absent		Long Bold	High
Dharia	Green	Absent	Medium	Absent	Well exserted	Semi erect to spreading	Yellowish	Weak	Medium	No	Absent		Short Bold	High
Athabora	Uniform Purple	Present	Medium	Present	Well exserted	Semi erect to spreading	Brown	Weak	Absent	No	Absent		Long Bold	Inter-mediate
Miatong	Green	Absent	Medium	Absent	Well exserted	Semi erect to spreading	Brown	Medium	Absent	Yes	Yellowish White	Upper half only	Long Bold	Inter-mediate
Aghoni Bora	Green	Absent	Weak	Absent	Mostly exserted	Erect to semi erect	Yellowish	Weak	Absent	Yes	Yellowish White	Upper half only	Long Slender	Low
Ranjit	Green	Absent	Weak	Absent	Well exserted	Semi erect to spreading	Brown	Weak	Absent	No	Absent		Medium Slender	High
Keteki Joha	Green	Absent	Weak	Absent	Well exserted	Erect to semi erect	Brown	Weak	Absent	Yes	Yellowish White	Whole length	Long Slender	High

Table 4. Mean performance of different quantitative traits

	LLB (cm)	PH (cm)	PL (cm)	PNP	TH (days)	TM (days)	NGP	LG/WG	LDG/ WDG	1000 GW (g)	Yield/ Plant (g)	Starch	Amylose	Crude fat	Crude protein
Johadhan	42.63	144.33	27.70	29.03	120.53	169.33	3284.87	3.33	3.04	21.47	70.51	78.61	27.86	3.26	7.55
Kolajoha	42.17	146.47	28.00	25.27	120.37	170.00	2803.27	3.33	3.06	21.70	60.83	78.64	26.43	3.22	7.61
Bor Aijung	46.00	155.87	29.67	13.57	117.93	158.33	2902.93	2.69	2.32	25.54	74.14	72.26	21.25	3.09	7.45
Ranga Bordhan	45.67	160.87	30.70	13.80	124.73	172.33	2208.13	2.35	2.18	30.08	66.42	71.25	23.06	3.77	7.83
Sadiya Lahi	40.37	154.57	26.80	17.10	125.43	160.33	2597.30	2.34	1.97	26.42	68.62	74.40	15.29	3.16	7.41
Suwag Moni	52.60	156.17	28.20	17.40	119.63	161.33	1648.23	2.54	2.41	28.24	46.55	76.09	15.94	2.93	7.65
Haldhar sali	42.80	153.97	30.30	16.97	123.07	175.67	1951.77	2.25	2.09	27.59	53.85	77.00	14.12	2.85	7.51
Cheni Lahi	45.83	176.10	28.53	18.50	125.87	162.67	2664.90	3.25	3.40	23.52	62.69	72.18	27.47	3.51	7.78
Bor Malbhog	43.30	143.27	24.97	17.77	123.97	163.67	1334.57	2.96	2.78	30.11	40.19	75.31	14.51	3.49	7.88
Nalsati	45.80	155.33	26.77	11.23	127.97	166.67	1383.03	3.43	2.91	26.50	36.65	76.45	15.68	2.93	8.08
Lakhman Dhan	49.57	157.53	29.13	13.80	116.07	160.33	1207.07	2.95	2.63	24.43	29.49	75.81	15.29	2.82	7.89
Ranga Bas	45.00	140.13	25.23	23.37	98.10	150.33	2678.60	3.13	2.50	20.27	54.30	78.32	7.52	3.09	7.57
Aibing Sali	45.43	155.33	28.63	20.53	126.90	171.33	2213.43	2.78	2.56	28.47	63.02	74.19	17.88	2.86	7.75
Saru Salpona	44.20	148.03	29.43	20.60	125.17	172.33	1959.93	3.47	3.18	23.33	45.73	75.60	15.94	3.10	7.22
Mainajan	45.50	142.47	26.13	16.57	103.27	155.33	2374.90	2.12	2.08	22.86	54.28	75.75	12.70	2.85	7.60
Malbhog	45.57	144.20	25.23	18.80	122.83	161.33	1472.27	2.76	2.20	32.59	47.99	74.34	13.99	3.66	7.82
Boga Bordhan	44.27	152.10	28.17	13.43	125.00	172.33	1670.63	2.76	2.45	27.67	46.24	75.47	7.52	4.13	7.83
Bora Dhan	52.40	152.37	27.73	10.27	118.00	168.33	2168.87	2.31	2.02	29.67	64.35	76.60	6.22	4.27	7.08
Bor Salpona	40.07	175.03	28.20	14.63	123.17	169.67	3065.90	3.14	2.62	26.24	80.45	73.56	16.58	3.05	7.26
Joybangla	43.23	151.37	25.90	19.50	118.20	166.33	2212.27	2.60	2.21	26.44	58.48	76.20	7.65	3.06	7.26
Sagar Sali	43.60	141.53	29.40	15.33	121.93	160.67	2129.40	1.79	1.72	21.44	45.66	78.57	7.65	2.85	7.74
Dolkochi	44.50	157.90	26.00	14.57	126.97	162.33	1772.57	3.07	2.64	26.64	47.21	74.48	19.18	3.08	7.16
Dharia	43.63	117.47	26.73	15.10	122.70	158.33	2499.73	2.93	2.47	20.23	50.58	78.73	10.88	2.92	7.10

	LLB (cm)	PH (cm)	PL (cm)	PNP	TH (days)	TM (days)	NGP	LG/WG	LDG/ WDG	1000 GW (g)	Yield/ Plant (g)	Starch	Amylose	Crude fat	Crude protein
Athabora	43.70	149.03	27.77	14.90	117.63	155.67	1560.80	2.61	2.44	27.60	43.08	77.80	8.27	4.15	7.23
Miatong	59.93	167.70	27.77	14.60	114.13	157.00	1674.67	3.06	2.62	26.26	43.98	78.65	15.29	2.91	7.11
Aghoni Bora	48.87	116.97	25.17	12.07	130.07	163.67	1311.27	3.29	3.23	25.31	33.19	75.48	1.87	4.39	7.18
Ranjit	56.50	134.50	26.17	11.73	125.93	159.00	1234.67	3.08	2.95	20.33	25.10	76.79	24.36	4.83	9.22
Keteki Joha	43.67	129.60	23.83	19.60	131.03	161.67	2077.10	3.46	3.01	18.04	37.48	72.47	20.21	3.07	7.70
Mean	45.96	149.29	27.44	16.79	121.31	163.80	2073.68	2.85	2.56	25.32	51.82	75.75	15.38	3.71	7.58
S.E.	0.27	0.42	0.15	0.29	0.06	0.31	35.74	0.01	0.01	0.08	0.91	0.58	0.20	2.19	0.04
C.D. 5%	0.78	1.19	0.44	0.82	0.18	0.88	101.34	0.03	0.03	0.22	2.57	1.64	0.57	0.15	0.11
C.D. 1%	1.03	1.59	0.58	1.09	0.24	1.17	134.96	0.04	0.05	0.29	3.43	2.10	0.76	0.20	0.14

Patra (2000). Panicle length in Assam rice was found comparatively in a narrower range (23.83-30.70 cm) than that of rice landraces of Odisha (19.0-34.3 cm) as reported by Patra (2000). Grain length/grain width ratio ranged from 1.79 to 3.47 which were at par with the findings of Siddique *et al*. (2007). Bor Salpona was the highest yielder possessing shortest length of leaf blade (40.07 cm), medium width of leaf blade (1.52 cm), long stem length (144.67 cm), thicker stem (0.43 cm), highest grains per panicle (209.5), decorticated grain width (2.58 mm) and high 1000-grain weight. The cultivar Bor Aijung was the second highest yielder followed by landraces Kola Joha, Sadiya Lahi, Ranga Bordhan, Johadhan, Boradhan, Aibing Sali, Cheni Lahi, Joybangla, Mainajan and Ranjit.

Significant variations were also observed in biochemical parameters studied. Highest amount of starch (78.73%) was found in the cultivar Dharia. Amylose content in rice under study ranged from 1.87 to 27.86 %. Higher amylose contents (above 20%) were observed in five (Johadhan, Kolajoha, Bor Aijung, Ranga Bordhan and Cheni Lahi) of the rice landraces while six rice landraces, viz. Ranga Bas, Boga Bordhan, Bor Dhan, Joybangla, Sagar Sali and Athabora were found to have lower amylose content (6.22–8.27%). The glutinous variety Aghoni Bora had only 1.87% of amylose. The popular rice variety Ranjit had the highest crude fat and crude protein content amongst all the rice genotypes used in the study (Table 4). The observed differences in qualitative and quantitative traits of rice genotypes might be due to the differences in their genetic make-up.

Twenty two landraces showed positive result for the phenol test except for Keteki Joha, Bor Aijung, Ranga Bordhan, Saru Salpona, Mainajan and Bor Salpona. In view of high heritability and stability of phenol colour reaction, it could be used as primary diagnostic character for distinguishing the rice genotypes (Anitalakshmi *et al.,* 2014). Phenol test, in the present study, helped in grouping the rice landraces into two groups. Aroma is an important economic trait of quality rice mostly preferred by the consumers and only three landraces, *viz.*, Keteki Joha, Johadhan and Kola Joha had aroma. The quality and quantity of starch and gelatinization temperature strongly influence the cooking quality of rice. Rice with high gelatinization temperature requires more water and time to cook than with low or intermediate gelatinization temperature. Landraces with intermediate gelatinization temperature are preferred by consumers (Sharma *et al.,* 2008). Intermediate gelatinization temperature was found in five landraces *viz*., Nalsati, Malbhog, Bora Dhan, Athabora and Miatong.

In case of morphological characters, number of grains per plant and number of panicles per plant showed the higher value of GCV, heritability and genetic advance. Time of maturity, time of heading, stem thickness showed high heritability and low genetic advance. Progeny testing might help in the selection process for these traits. In case of biochemical characters, amylose content showed highest GCV with highest heritability and genetic advance. This trait might be helpful in selection for grain quality. The difference between the GCV and PCV were very low for all the characters indicating very low influence of the environment (Table 5).

Correlation coefficient analysis revealed that morphological characters such as grains per plant, plant height, panicle length, width of decorticated grain and stem length exhibited positive correlation with grain yield per plant (Table 6a & 6b). But length of leaf blade and stem thickness was negatively correlated with yield per plant. Of the biochemical character, starch content

Table 5. Variability of quantitative characters of rice land races of Dibrugarh district of Assam

Characters	Genotypic variance	Phenotypic variance	GCV (%)	PCV (%)	Heritability (broad sense) (%)	Genetic advance as % mean	
						5%	1%
Length of leaf blade (LLB)	20.68	9.89	9.89	9.94	98.92	20.27	25.98
Width of leaf blade (LWB)	0.03	0.03	12.46	12.54	98.73	25.51	32.70
Plant height (PH)	191.76	192.29	9.27	9.28	99.75	19.08	24.45
Panicle length (PL)	3.03	3.10	6.34	6.42	97.73	12.93	16.57
Stem length (SL)	159.79	160.86	10.34	10.37	99.36	21.23	27.21
Stem thickness (ST)	8.00 x10^{-4}	9.00 x10^{-4}	6.76	6.84	97.72	13.77	17.65
Panicle no. per plant (PNP)	18.47	18.72	25.6	25.7	98.68	52.39	67.14
Time of heading (TH)	51.45	51.46	5.913	5.914	99.91	12.18	15.60
Time of maturity (TM)	39.05	39.33	3.81	3.82	99.23	7.83	10.03
No. of grains per Plant	343452.80	347285.50	28.26	28.41	98.91	57.89	74.19
Grain length (LG)	0.64	0.64	9.45	9.46	99.87	19.46	24.94
Grain width (WG)	0.16	0.16	13.42	13.43	99.73	27.61	35.39
LG/ WG	0.19	0.19	15.55	15.57	99.82	32.01	41.02
Length of decorticated grain (LDG)	0.27	0.27	8.20	8.22	99.61	16.87	21.63
Width of decorticated grain (WDG)	0.12	0.123	13.80	13.83	99.53	28.36	36.34
LDG/WDG	0.18	0.18	16.58	16.60	99.72	34.12	43.73
Seed weight	12.78	11.80	14.11	14.13	99.83	29.07	37.25
Yield/plant	189.72	192.2	26.57	26.57	98.74	54.4	69.71
Starch	4.49	4.99	2.79	2.95	89.91	5.50	6.99
Amylose	45.75	45.82	43.98	44.01	99.86	86.99	97.17
Amylopectin	45.75	45.82	43.98	44.01	99.86	16.45	21.08
Fat	0.30	0.31	16.55	16.67	98.55	33.86	43.73
Crude Protein	0.18	0.18	5.63	5.66	98.75	11.53	14.77

Table 6a. Estimation of correlation coefficient of morphological characters of rice land races of Dibrugarh district of Assam

	LLB	LWB	PH	PL	SL	ST	PAN/ PLAN	TH	TM	NG/ RAINS	LG	WG	LG/ WG	LDG	WDG	LDG/ WDG	1000GW	Y/ P
LLB	1.000	-0.046	0.041	-0.003	0.041	0.361*	-0.414	-0.142	-0.286	-0.483**	0.197	0.079	0.033	0.056	-0.091	0.091	0.062	-0.441**
LWB	-0.044	1.000	0.109	0.316	0.071	0.193	-0.565**	0.215	0.315	-0.229	0.150	0.538**	-0.393*	0.021	0.470**	-0.390*	0.555**	0.066
PH	0.041	0.108	1.000	0.533**	0.993**	-0.039	-0.065	-0.049	0.257	0.191	0.267	0.273	-0.091	0.377*	0.327*	-0.085	0.431*	0.459**
PL	-0.003	0.311	0.528**	1.000	0.433*	-0.061	-0.098	0.031	0.462**	0.218	-0.183	0.277	-0.341*	0.010	0.281	-0.219	0.195	0.373*
SL	0.042	0.072	0.988**	0.426*	1.000	-0.044	-0.049	-0.051	0.211	0.163	0.312	0.262	-0.055	0.405*	0.317*	-0.062	0.434*	0.427*
ST	0.355*	0.186	-0.039	-0.065	-0.046	1.000	-0.406*	0.339*	0.191	-0.515**	0.491**	0.079	0.199	0.495**	-0.072	0.281	0.303	-0.371*
PAN/PLANT	-0.411*	-0.561**	-0.065	-0.094	-0.049	-0.394*	1.000	-0.205	0.104	0.524**	-0.084	-0.359*	0.284	0.123	-0.217	0.260	-0.318	0.315
TH	-0.141	0.213	-0.049	0.030	-0.051	0.336*	-0.204	1.000	0.539**	-0.246	0.324*	-0.080	0.269	0.335*	-0.172	0.345*	0.154	-0.150
TM	-0.285	0.313	0.255	0.453**	0.208	0.191	0.103	0.537**	1.000	0.091	0.257	0.139	0.058	0.399*	0.124	0.117	0.328*	0.285
NGP	-0.478**	-0.229	0.189	0.216	0.160	-0.501**	0.527**	-0.245	0.091	1.000	-0.374*	-0.215	-0.012	-0.241	-0.048	-0.057	-0.340*	0.866**
LG	0.196	0.149	0.267	-0.180	0.311	0.485**	-0.083	0.323*	0.256	-0.371*	1.000	0.056	0.505**	0.899**	-0.040	0.451**	0.581**	-0.102
WG	0.079	0.534**	0.273	0.274	0.261	0.078	-0.357*	-0.080	0.137	-0.214	0.056	1.000	-0.829**	-0.042	0.903**	-0.762**	0.764**	0.196
LG/WG	0.033	-0.391*	-0.091	-0.337*	-0.055	0.196	0.282	0.269	0.059	-0.011	0.504**	-0.829**	1.000	0.536**	-0.800**	0.915**	-0.343*	-0.218
LDG	0.055	0.021	0.377*	0.009	0.404*	0.489**	0.122	0.334*	0.398*	-0.240	0.898**	-0.041	0.535**	1.000	-0.088	0.547**	0.490**	-0.016
WDG	-0.091	0.464**	0.326*	0.279	0.315	-0.074	-0.215	-0.171	0.123	-0.048	-0.040	0.900**	-0.798**	-0.088	1.000	-0.874**	0.694**	0.324*
LDG/WDG	0.090	-0.386*	-0.084	-0.218	-0.061	0.278	0.257	0.345*	0.117	-0.057	0.451**	-0.760**	0.913**	0.547**	-0.873**	1.000	-0.359*	-0.265
1000GW	0.061	0.551**	0.430*	0.192	0.432*	0.300	-0.316	0.154	0.327*	-0.337*	0.581**	0.762**	-0.342*	0.489**	0.692**	-0.358*	1.000	0.161
Y/P	-0.437*	0.062	0.454**	0.366*	0.421*	-0.360*	0.320*	-0.149	0.284	0.867**	-0.101	0.194	-0.216	-0.016	0.321*	-0.263	0.161	1.000

* P≤ 0.5 and ** P≤ 0.01

Upper matrix= Genotypic correlation coefficient, Lower matrix= Phenotypic correlation coefficient

Table 6b. Estimation of correlation coefficient of biochemical parameters of rice land races of Dibrugarh district of Assam

	Starch	Amylose	Amylopectin	Fat	Protein	Yield
Starch	1	-0.317*	0.317*	-0.068	-0.108	-0.273
Amylose	**-0.303**	1	-1.000**	-0.127	0.394*	0.264
Amylopectin	**0.303**	**-1.000****	1	0.127	-0.394*	-0.264
Fat	**-0.057**	**-0.126**	**0.126**	1	0.316	-0.243
Protein	**-0.100**	**0.391***	**-0.391***	**0.316**	1	-0.404*
Yield	**-0.258**	**0.262**	**-0.262**	**-0.240**	**-0.398***	1

* P≤ 0.5 and ** P≤ 0.01

Upper matrix = Genotypic correlation coefficient, Lower matrix= Phenotypic correlation coefficient

showed positive correlation with amylopectin and negative correlation with amylose at genotypic level. Amylose showed positive correlation with protein and negative correlation with amylopectin at both genotypic and phenotypic level. Amylopectin showed negative correlation with amylose and protein at both genotypic and phenotypic level. Path analysis revealed that stem length followed by number of grains per plant had maximum positive direct effect with yield per plant (Table 7) and both exhibited positive correlation with grain yield per plant at both genotypic and phenotypic level. Therefore, these two traits deserve consideration in breeding programme to bring about improvement in grain yield. On the other hand plant height followed by panicle per plant had maximum negative direct effect with yield per plant but correlation was positive. So plant height and panicle per plant contributed towards yield through positive indirect effects. The mean performance of the landraces for yield and yield attributing traits along with mean performance of the landraces for biochemical parameters revealed that Kolajoha, Miatong, Bor Aijung, Ronga Bordhan, Bor Salpona, Dolkochi, Athabora were few promising landraces that could be utilized in the development of high yielding landraces.

Conclusion

Identification and characterization of indigenous rice cultivars of Assam is crucial in harnessing their potential in carrying out scientific seed production and certification. A previous knowledge of identity of these varieties is helpful in order to utilize them in rice improvement programmes. Among various morphological characters basal leaf sheath colour, leaf anthocyanin colouration, pubescence of blade surface, leaf anthocyanin colouration of collar, attitude of flag leaf, panicle exertion, panicle attitude of branching, colour tip of lemma, density of pubescence of lemma, lemma anthocyanin colouration of apex, presence absence of awn, awn colour, distribution of awn, decorticated grain shape could very well distinguish rice cultivars. These characters may be used as an index for identification of these cultivars. The cultivars showed a considerable variation in different parameters analyzed. Amongst grain quality attributes, amylose content was found to possess maximum genetic variation apparently controlled by additive genes. Thus, this trait deserves most priority in selection for grain quality. However, it is necessary to study the detailed amino acid composition, mineral content, vitamin content, effect of parboiling on different nutritional components etc. for nutritional evaluation of the cultivars as a whole.

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Table 7. Direct and indirect effects of yield components on grain yield per plant of rice land races of Dibrugarh district of Assam

	PATH matrix of Y/P								
	LLB	PH	PL	SL	ST	PPP	NGP	WDG	1000GW
LLB	**0.0217**	0.0009	-0.0001	0.0009	0.0079	-0.009	-0.0105	-0.002	0.0013
PH	-0.0634	**-1.5313**	-0.8155	-1.5206	0.0592	0.0988	-0.2929	-0.5005	-0.6598
PL	-0.0006	0.111	**0.2084**	0.0903	-0.0128	-0.0203	0.0454	0.0586	0.0406
SL	0.0591	1.4493	0.6326	**1.4595**	-0.0645	-0.0717	0.2375	0.4632	0.6331
ST	0.0096	-0.001	-0.0016	-0.0012	**0.0264**	-0.0107	-0.0136	-0.0019	0.008
PPP	0.0436	0.0068	0.0103	0.0052	0.0427	**-0.1053**	-0.0552	0.0229	0.0334
NGP	-0.5402	0.2142	0.244	0.1822	-0.5762	0.5871	**1.1195**	-0.0533	-0.3801
WDG	-0.0002	0.0006	0.0005	0.0006	-0.0001	-0.0004	-0.0001	**0.0019**	0.0013
1000GW	0.0297	0.2082	0.0941	0.2097	0.1465	-0.1535	-0.1641	0.3353	0.4833
Corr with Y/P	-0.4407**	0.4586**	0.3727*	0.4265*	-0.371*	0.3149	0.866**	0.3243*	0.1613

* P≤0.5 and ** P≤0.01

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RESEARCH ARTICLE

Molecular Characterization and Genetic Divergence Analysis of Traditional and Improved Aromatic Rice Varieties Using Microsatellite Markers

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Microsatellite profiling was employed to analyze the nature and extent of differentiation and divergence among eighteen traditional and improved varieties of aromatic rice. Analysis of 18 primers dependent genetic polymorphism revealed 89 shared and 91 unique allelic variants generated in the form of amplified products amongst 18 varieties. Polymorphic information content of the primers ranged from 0.60 to 0.88 with an average of 0.80. Ample genetic differentiation and divergence amongst entries was deduced by a comparison of similarity coefficients. Broadly, the entries were distinctly divided into three multi-genotypic groups, each of which accommodated traditional and improved varieties. However, the first and second groups were dominated by traditional varieties, whereas the third group was dominated by improved varieties. With maximum similarity in respect of targeted genomic regions, Champaran Basmati and Sanwal Basmati (0.45) were accommodated into the first group along with Jeerabati and Rajendra Kasturi (0.45), in addition to Baharni, Birsamati and Kasturi. Spatial distribution pattern of two dimensional ordinations of microsatellite primers specific genetic profiles from traditional and improved varieties completely validated the hierarchical classification pattern exhibited by sequential agglomerative clustering based dendrogram. The varieties from different groups could be utilized in breeding programs for genetic enhancement of aromatic rice germplasm base and molecular profiles could be helpful in preserving the integrity of these valuable aromatic rice varieties.

Key Words: Allelic diversity, Aromatic rice, Genetic diversity, Genetic profile, Microsatellite.

Introduction

Rice is an important cereal crop grown under diverse eco-geographical conditions in various tropical and sub-tropical countries of the world and consumed as a staple food by about fifty per cent of the human population worldwide. Aromatic rice constitutes a small but special group of landraces and varieties of rice well known for its nut like flavour, specific aroma and superior grain quality (Priyadarshini *et al*., 2018). Presence of aroma in the grain is one of the most important attributes of high quality rice, especially for price determination in the domestic and international markets. Aroma is best developed when aromatic rice is grown in areas where temperature is cooler during maturity (Pachauri *et al*., 2010). Although majority of the aromatic rice varieties popular in the world market have long grains, many indigenous aromatic rice varieties have short and medium sized grains.

India is well known as a country having an immense wealth of aromatic rice. Unfortunately, many accessions of aromatic rice have been lost as an aftermath of the green revolution where emphasis was mainly placed on productivity rather than quality. Among the aromatic rice accessions, the highly valued rice accessions because of their superior grain, cooking and eating quality characteristics are collectively called as basmati rice (Amarawathi *et al*., 2008). As a result of properties like extra-long superfine slender grains with chalky endosperm, pleasant and exquisite aroma, sweet taste and soft texture when cooked, length-wise kernel elongation with least breadth-wise swelling on cooking and tenderness of cooked rice, basmati rice commands a premium price in the world market (Pachauri *et al*., 2010). However, many other indigenous varieties of scented rice excel equally as far as aroma and cooking qualities are concerned (Vhora *et al*., 2013). But, unfortunately, these have somehow not got the sincere attention of rice

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scientists and traders, including exporters (Anonymous, 2003; Pachauri *et al*., 2010; Roy *et al*., 2016). As a result, most of this valuable wealth has either already vanished or is on a decline.

Since recognizable variation in respect of the type and intensity of aroma exists in different groups of aromatic rice varieties (Widjaja *et al*., 1996; Singh *et al*., 2007; Pachauri *et al*., 2010), precise study of the nature and extent of genetic differentiation and divergence in basmati gene pool and other aromatic rice types is necessary for conservation of accessions, identification of duplicate accessions, proper purity maintenance and varietal identification. Therefore, increasing attention is being paid towards comprehensive characterization of elite basmati quality rice germplasm, supplementing the existing morphological descriptors with reliable and repeatable genomic sequences and markers based molecular profiles (Amrawathi *et al*., 2008; Rabbani *et al*., 2010; Shah *et al*., 2013). Genetic analysis at the molecular level is being considered as an important approach to assure the export quality of basmati rice in order to maintain the distinctiveness of basmati varieties and to differentiate between the various grades of basmati rice (Nagaraju *et al*., 2002; Pal *et al*., 2004). Genomic markers that are genetically linked to fragrance along with the advantage of being inexpensive, simple and reliable, requiring small amounts of tissue have been developed for their utilization in the breeding and selection of fragrant rice (Cordeiro *et al.,* 2002).

Morphological evaluation and characterization is an important methodology for analyzing the differentiation and diversity, but it is less efficient and cumbersome. Advantages offered by the genomic sequences based markers that they remain unaffected across different stages, seasons and locations, in addition to their abundance and genome wide coverage, project them as a powerful tool in the assessment of genetic variation and elucidation of the genetic relationships. Among genomic markers, microsatellites are known to be highly polymorphic, easily automated, more reproducible, co-dominant and distributed throughout the genome. Usefulness of microsatellite markers for germplasm characterization in rice is well established (Mia *et al*., 2010; Singh *et al*., 2011; Samal *et al*., 2014; Singh *et al.*, 2016, Priyadarshini *et al*., 2018; Kumari *et al*., 2018a). Keeping all above in view, the present study was conducted to evaluate the genetic polymorphism at the molecular level and to investigate the nature and pattern of genetic differentiation and divergence among some traditional and improved varieties of aromatic rice.

Materials and Methods

Eighteen rice accessions comprising, landraces, advanced breeding lines and improved varieties of aromatic rice, namely Champaran basmati, Sanwal basmati, Jeerabati, Birsamati, Rajendra Kasturi, Baharni, Kasturi, Jasmine, Marcha, Lalmati, Kalanamak, Rajendra Bhagwati, Ranbir Basmati, Sugandha, Basmati 370, Rajendra Suwasini, RAU 3055 and Pusa Basmati-1, were subjected to molecular characterization in the present study conducted in the Molecular Biology Laboratory of the Department of Agricultural Biotechnology & Molecular Biology, Dr. Rajendra Prasad Central Agricultural University, Pusa. Using modified cetyl-trimethyl-ammonium bromide (CTAB) method (Ferdous *et al*., 2012) with slight modifications (Shamim *et al*., 2016a), the genomic DNA was extracted from the leaves of fifteen days old seedlings of these entries. The amplification of targeted genomic regions in extracted DNA samples was carried out utilizing a panel of eighteen primer pairs specific to the unique flanking sequences of the microsatellites distributed among six chromosomes present in the genome of rice (Table 1). The amplification was performed in a total volume of 15µl containing 3.8 µl water (Protease and Nuclease free), 3.0 µl 5X PCR buffer, 0.3 µl 10 mM $MgCl_2$, 3.0 µl 200 µM dNTPs mixture, 1.2 µl (5 µM) Primer F, 1.2 µl (5 µM) Primer R, 1.0 µl Taq Polymerase (1 unit) and 1.5 µl DNA template. The reaction condition along with thermal profile cycling was optimized in the Master Cycler (Eppendorf) using initial denaturation at 94 ºC for 5 min; 35 cycles of denaturation at 94 ºC for 1 min, annealing at 53º-58 ºC for 1 min and extension at 72 ºC for 2 min followed by a final extension at 72 ºC for 7 min. The amplicons were loaded onto 2% agarose gel containing ethidium bromide (0.5µg/ml) in 0.5X TBE buffer and resolved at 100 volts and then visualized and documented under gel documentation system (Alpha Innotech, USA). The size of the most intensely amplified products was determined in relation to the size of markers in 50 bp DNA ladder with the help of gel reader (Alpha View Gel Reader).

Molecular level genetic polymorphism was recorded on the basis of presence or absence of bands in different entries under investigation. All the entries were scored for the presence and absence of bands and the data recorded in this respect were entered into binary matrix as discrete variables and then this data matrix was

Table 1. Microsatellite primers used for amplification of targeted genomic regions in traditional and improved aromatic rice varieties

Locus	Ch. No.	Primer Sequence (5'- 3')	Repeat Motif	An. Tp. (^{0}C)
RM 13	5	(F) TCCAACATGGCAAGAGAGAG (R) GGTGGCATTCGATTCCAG	$(GA)_6$-$(GA)_{16}$	53
RM 16	3	(F) CGCTAGGGCAGCATCTAAA (R) AACACAGCAGGTACGCGC	$(TCG)_5(GA)_{16}$	56
RM 42	8	(F) ATCCTACCGCTGACCATGAG (R) TTTGGTCTACGTGGCGTACA	$(AG)_6(AG)_2$ T$(GA)_5$	56
RM 44	8	(F) ACGGGCAATCCGAACAACC (R) TCGGGAAAACCTACCCTACC	$(GA)_{16}$	56
RM 72	8	(F) CCGGCGATAAAACAATGAG (R) GCATCGGTCCTAACTAAGGG	$(TAT)_5C(ATT)_{15}$	55
RM 80	8	(F) TTGAAGGCGCTGAAGGAG (R) CATCAACCTCGTCTTCACCG	$(TCT)_{25}$	58
RM 223	8	(F) GAGTGAGCTTGGGCTGAAAC (R) GAAGGCAAGTCTTGGCACTG	$(CT)_{25}$	54
RM 225	6	(F) TGCCCATATGGTCTGGATG (R) GAAAGTGGATCAGGAAGGC	$(CT)_{18}$	53
RM 252	4	(F) TTCGCTGACGTGATAGGTTG (R) ATGACTTGATCCCGAGAACG	$(CT)_{19}$	54
RM 256	8	(F) GACAGGGAGTGATTGAAGGC (R) GTTGATTTCGCCAAGGGC	$(CT)_{21}$	54
RM 284	8	(F)ATCTCTGATACTCCATCCATC (R) CCTGTACGTTGATCCGAAGC	$(GA)_8$	54
RM 330	8	(F) CAATGAAGTGGATCTCGGAG (R) CATCAATCAGCGAAGGTCC	$(CAT)_5$	56
RM 337	8	(F)GTAGGAAAGGAAGGGCAGAG (R)CGATAGATAGCTAGATGTGC	$(CTT)_{4-19}(CTT)_8$	54
RM 339	8	(F) GTAATCGATGCTGTGGGAAG (R)GAGTCATGTGATAGCCGATG	$(CTT)_8$ CCT $(CCT)_5$	53
RM 426	8	(F) ATGAGATGAGTTCAAGGCCC (R) AACTCTGTACCTCCATCGCC	$(CA)_{10}$	54
RM 444	9	(F) GCTCCACCTGCTTAAGCATC (R) TGAAGACCATGTTCTGCAGG	$(AT)_{12}$	54
RM 505	7	(F) AGAGTTATGAGCCGGGTGTG (R) GATTTGGCGATCTTAGCAGC	$(CT)_{12}$	54
RM 515	8	(F) TAGGACGACCAAAGGGTGAG (R) TGGCCTGCTCTCTCTCTCTC	$(GA)_{11}$	56

Ch. No.: Chromosome number; An. Tp.: Annealing temperature

subjected to further analysis. Suitability of the marker based polymorphism for molecular differentiation and characterization of the entries was assessed by computing the polymorphism per cent (Kumari *et al*., 2018b). Allelic diversity exhibited by the microsatellite marker based polymorphism for identification of polymorphic and informative markers to characterize and differentiate the aromatic rice entries was evaluated on the basis of comparison of polymorphism information content (Anderson *et al*., 1993) of the primer pairs. Efficacy of individual primers in differentiation of the genotypes was investigated by computing the discrimination coefficient (DC) and non-discrimination coefficient (NDC) of the primer pairs (Kumar *et al*., 2018). Similarity coefficient (Dice, 1945) was computed for pair-wise combinations of the entries on the basis of comparison of presence and absence of bands and proportion of shared bands produced by the primers as;

$$Similarity\ coefficient = 21/(2a + b + c),$$

where, a, b and c represent number of bands shared between J^{th} and K^{th} genotypes, number of bands present in J^{th} genotype but absent in K^{th} genotype and number of bands absent in J^{th} genotype but present in K^{th} genotype, respectively.

Using sequential agglomerative hierarchical non-overlapping (SAHN) clustering based on similarity coefficients as the method for tree building in the cluster analysis, the dendrogram was generated by un-weighted pair-group method using arithmetic mean (UPGMA). Numerical taxonomic approach based analysis of the similarity matrix was performed with the help of

Table 2. Analysis of polymorphism revealed by microsatellite primers used for the amplification of targeted genomic regions in traditional and improved aromatic rice varieties

Primer	Allele size range (bp)	Allele size difference (bp)	No. of alleles	No. of unique alleles	No. of shared alleles	PP	PIC	DC	NDC
RM 13	127 - 153	026	07	04	3	57.1	0.803	0.84	0.15
RM 16	166 - 185	019	05	02	3	40.0	0.717	0.76	0.23
RM 42	166 - 538	372	19	14	5	73.6	0.850	0.94	0.05
RM 44	104 - 417	313	13	04	9	30.7	0.686	0.81	0.18
RM 72	146 - 211	065	09	06	3	66.6	0.810	0.85	0.14
RM 80	141 - 187	046	10	06	4	60.0	0.885	0.93	0.06
RM 223	159 - 309	150	11	06	5	54.5	0.870	0.83	0.16
RM 225	128 - 179	051	09	02	7	22.2	0.857	0.93	0.06
RM 252	213 - 811	598	17	09	8	52.9	0.840	0.92	0.07
RM 256	109 - 162	053	06	03	3	50.0	0.754	0.81	0.18
RM 284	144 - 161	017	06	02	4	33.3	0.791	0.83	0.16
RM 330	170 - 340	170	09	05	4	55.5	0.600	0.66	0.33
RM 337	155 - 216	061	08	03	5	37.5	0.871	0.92	0.07
RM 339	157 - 207	050	08	03	5	37.5	0.847	0.89	0.10
RM 426	177 - 228	051	08	02	6	25.0	0.873	0.92	0.07
RM 444	178 - 350	172	11	09	2	81.8	0.872	0.93	0.06
RM 505	180 - 507	327	15	08	7	53.3	0.696	0.80	0.19
RM 515	240 - 294	054	09	03	6	33.3	0.871	0.92	0.07

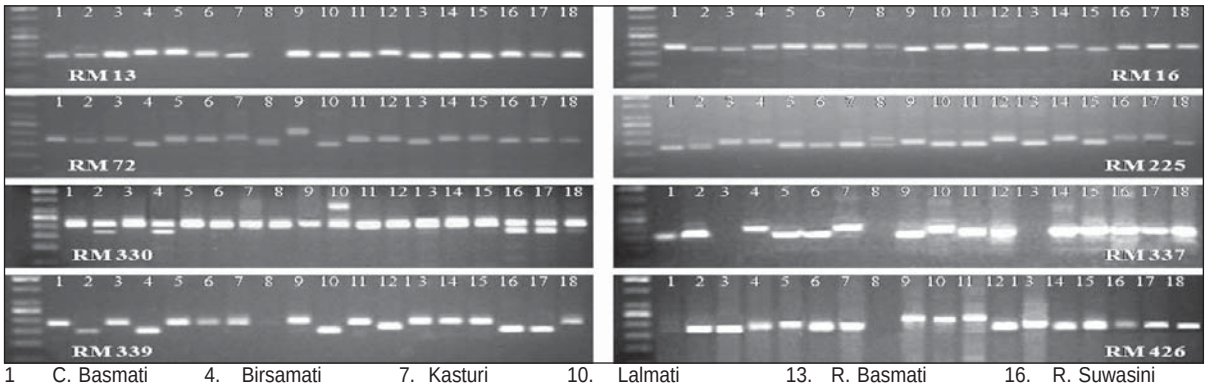
PP: Polymorphism per cent; PIC: Polymorphism information content; DC: Discrimination coefficient; NDC: Non Discrimination Coefficient

NTSYS-pc software (Rohlf, 1997). The information pertaining to the nature and magnitude of differentiation and divergence amongst the aromatic entries under evaluation was generated by identifying the clusters at appropriate phenon levels. Principal coordinate analysis of the microsatellite primers dependent genetic profiles of the entries was conducted and the two-dimensional spatial distribution pattern of the entries were compared with the results obtained from the cluster analysis and neighbour joining tree.

Results and Discussion

Differential banding patterns observed among the entries due to amplification of targeted genomic regions using eighteen microsatellite primers clearly indicated the existence of ample molecular level genetic polymorphism. The molecular level genetic polymorphism among the entries was recognized on the basis of presence or absence and size of bands, in addition to variation in respect of number and relative position of bands (Fig. 1). Strikingly different efficiency was exhibited by the primer pairs in terms of their ability to reveal variability amongst the entries. While some of the primer pairs generated several allelic variants as a result of variation in the length of microsatellite specific simple sequence repeats, others generated only a few allelic variants among the entries, corroborating the results of the several earlier researchers (Kumar *et al*., 2015; Shamim *et al*., 2016b; Kumari *et al*., 2018a; Priyadarshini *et al*., 2018). Further, all the primers utilized during molecular characterization generated unique allelic variants, but the number and proportion of unique alleles varied considerably among the primer pairs. Allele size range and allele size difference exposed the variability in the length of the simple sequence repeats at a microsatellite site, arising as a result of differences in the number of repeats existing in different entries and revealed by the primer pair involved in the amplification of targeted genomic region. Thus, microsatellite profiling clearly reflected the presence of plentiful genetic variability in terms of differences in the number of repeats at the primer specific microsatellite locus in evaluated aromatic rice entries.

Perusal of the data related to the analysis of polymorphism amongst traditional and improved aromatic rice varieties (Table 2) indicates that microsatellite primers used for the amplification of targeted genomic regions detected 180 allelic variants including 89 shared and 91 unique allelic variants. The number of alleles per primer ranged from six in the cases of RM 256 and RM 284 to nineteen in the case of RM 42 with an average of 10.0 alleles per primer. Experimental evidences using different panels of



1	C. Basmati	4.	Birsamati	7.	Kasturi	10.	Lalmati
2.	S. Basmati	5.	R. Kasturi	8.	Jasmine	11.	Kalanamak
3.	Jeerabati	6.	Baharni	9.	Marcha	12.	R. Bhagwati
13.	R. Basmati	16.	R. Suwasini				
14.	Sugandha	17.	RAU 3055				
15.	Basmati 370	18.	P. Basmati				

Fig. 1. Amplification patterns generated by microsatellite primers with eighteen traditional and improved varieities of aromatic rice

primer pairs and different set of genotypes of rice have documented considerably lower (<8.0) as well as higher (>12.0) number of allelic variants (Joshi and Behera, 2006; Brondani *et al*., 2006; Jayamani *et al*., 2007; Borba *et al*., 2009; Rabbani *et al*., 2010; Singh *et al*., 2011; Behera *et al*., 2012; Vhora *et al*., 2013; Kumar *et al*., 2015; Shamim *et al.,* 2016b; Priyadarshini *et al.*, 2018) than the allele number recorded in the present study. In general, the primer pairs yielding greater number of alleles per locus generated more number of unique alleles in accordance with the earlier reports (Joshi and Behera, 2006; Rabbani *et al*., 2010; Singh and Singh, 2012). Considerably greater percentage of unique alleles was generated by the primer pairs RM 444, RM 42, RM 72, RM 80, RM 223, RM 505, RM 330, RM 252, RM 13 and RM 256 in descending order of magnitude. Therefore, these ten primer pairs recorded remarkably greater polymorphism per cent.

Substantial differences were noticed in the polymorphism information content of the primer pairs, clearly reflecting ample variability in allelic diversity as well as allelic frequency among the entries under evaluation. The estimates, in general, reflected that the level of polymorphism revealed by the panel of primer pairs utilized during amplification was quite high but variable among the primers. Numerical value ranged from 0.600 in the case of RM 330 to 0.885 in the case of RM 80 with an average of 0.805 (Table 2). The values obtained in the present study are comparable to some of the earlier reports (Bansal *et al*., 2013; Priyadarshini *et al*., 2018; Kumari *et al*., 2018a; Kumar *et al*., 2018), but higher (Pal *et al*., 2004; Joshi and Behera, 2006, Brondani *et al*., 2006; Kibria *et al.*, 2009; Mia *et al.,* 2010; Das *et al*., 2012) or lower (Behera *et al.*, 2012) than the reports of other researchers in rice. Considering the number of alleles generated by different primer pairs in conjunction with the percentage of unique alleles and the level of polymorphism detected in the present study, the primers RM 42, 72, RM 80, RM 225, RM 252 and RM 444 appeared to be the more informative for the purpose of molecular characterization of aromatic rice entries under evaluation. Relatively greater efficiency of these primers was observed in discriminating the pair-wise combinations of entries evaluated.

Total repeat count of the di-nucleotide microsatellite loci was, in general, seemed to be more or less associated with the number of alleles detected per locus. Therefore, the results, in general, reflected that larger the repeat number involved in the microsatellite locus, the larger was the number of identified alleles. Further, the microsatellite loci with di-nucleotide repeat motifs, in general, tended to detect greater number of alleles than the repeat loci with tri-nucleotide repeat motifs in accordance with the earlier report (Kumar *et al*., 2018; Kumari *et al*., 2018a; Priyadarshini *et al*., 2018). Occurrence of null allele was inferred due to failure of amplification for a particular repeat locus specific to the unique flanking sequences of the microsatellite. The microsatellite locus associated with RM 13, RM 80, RM 337, RM 339, RM 426, RM 444 and RM 505 exhibited null allele ranging from one to three in the entries under evaluation. Similar results indicating the occurrence of null allele have also been reported earlier by (Pal *et al*., 2004; Shamim *et al*., 2016b; Priyadarshini *et al*., 2018; Kumari *et al*., 2018a). Appearance of more than one band in the same entry was noticed with the primer pairs RM 42, RM 44, RM

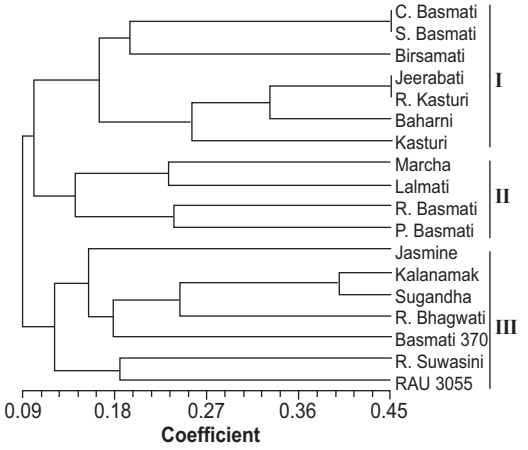


Fig. 2. Similarity indices based dendrogram showing interrelationship among traditional and improved aromatic rice varieties

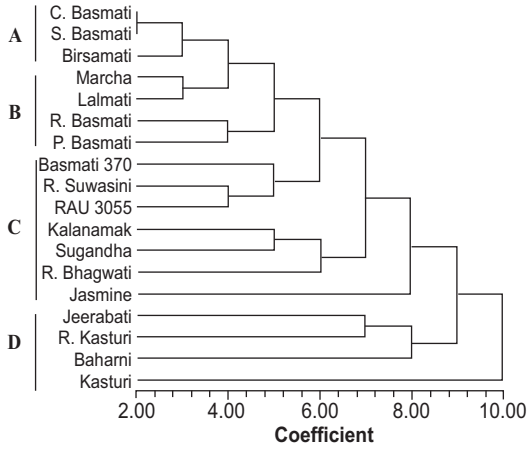


Fig. 3. Similarity indices based neighbour joining tree of traditional and improved aromatic rice varieties

223, RM 225, RM 252, RM 330 and RM 505, revealing most probably the existence of residual heterozygosity in genotypic background of some entries.

While assessing the genetic similarities and interrelationships, similarity coefficient between Sanwal Basmati and Champaran Basmati was found to be the maximum in magnitude (0.450) amongst pair-wise combinations of entries. Therefore, the molecular profiles of Sanwal Basmati and Champaran Basmati generated by using eighteen microsatellite specific primer pairs were relatively more similar with respect to the nucleotide sequence composition at primer binding sites and the molecular size of the genomic regions covered by the primer pairs. This was followed by remarkably higher magnitude of similarity coefficient between Jeerabati and Sanwal Basmati, Jasmine and Baharni, Kasturi and Baharni, Sugandha and Rajendra Bhagwati, Rajendra Kasturi and Sanwal Basmati, Lalmati and Kasturi, Kalanamak and Jasmine and Kasturi and Rajendra Kasturi. However, the magnitude of similarity coefficient between Jasmine and Champaran Basmati, Jasmine and Jeerabati, Jasmine and Birsamati, Marcha and Baharni, Lalmati and Rajendra Kasturi, Rajendra Bhagwati and Champaran Basmati, Rajendra Bhagwati and Sanwal Basmati, Ranbir Basmati and Kasturi, Basmati 370 and Kasturi, RAU 3055 and Champaran Basmati, RAU 3055 and Sanwal Basmati, RAU 3055 and Birsamati, Pusa Basmati 1 and Kasturi, Rajendra Bhagwati and Marcha, Ranbir Basmati and Jasmine, Basmati 370 and Lalmati, RAU 3055 and Marcha was equal to zero. Therefore, experimental results revealed enormous molecular level diversity amongst the eighteen aromatic entries evaluated in the present study.

Hierarchical classification pattern based cluster analysis of the traditional and improved rice varieties, as it is evident from a perusal of dendrogram (Fig. 2), basically yielded three multi-genotypic groups in which the entries were conveniently accommodated. All the multi-genotypic groups had a combination of traditional and improved varieties. However, the first and second multi-genotypic groups were dominated by traditional aromatic varieties, whereas, the third group predominantly consisted of improved aromatic varieties. The multi-genotypic groups were further divided into clusters, sub-clusters and sub-sub clusters at higher phenon levels, accommodating only the entries with increasingly similar pattern of markers together.

Neighbour joining tree also exhibited more or less similar type of overall clustering pattern but slightly different type of interrelationship amongst the entries (Fig. 3). As it is well established, this method is based on the idea of parsimony and hence considered as a method for estimating phylogenetic trees. Since it attempts to find a tree that is usually close to the true phylogenetic tree, it does yield relatively short estimated evolutionary trees rather than attempting to obtain the shortest possible tree for a set of data. Linking together the two objects that are the closest mutual neighbours, trees are constructed. Therefore, slight deviation in interrelationship and clustering pattern may be reflected as exhibited in the present study, but the inferences

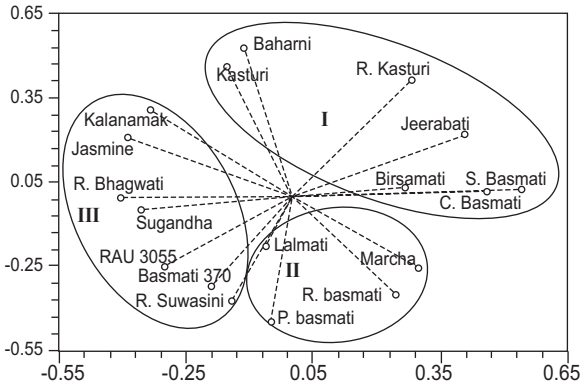


Fig. 4. Spatial distribution of the genetic profiles of traditional and improved aromatic rice varieties along principal coordinate axes

derived from neighbour joining tree were more or less in agreement with the results obtained from dendrogram.

Spatial distribution pattern of the traditional and improved aromatic varieties along the two principal axes in two dimensional plots of eighteen microsatellite primers dependent genetic profiles (Fig. 4), completely validated the numerical taxonomic approach based hierarchical classification pattern of the entries. Apparently it is recognizable that only some of the entries were placed around the centroid, while remaining entries were placed far away from the centroid, but the entries were distinctly divided into three major genotypic groups. The composition of these multi-genotypic groups is similar to that deduced from hierarchical classification approach based dendrogram of the entries.

Microsatellite scanning made it possible to discern adequate genetic variation at the molecular level amongst the aromatic rice entries and assisted in unique genotyping. Remarkably greater discrimination ability of microsatellite markers basically due to their polymorphic nature and allelic diversity, as reported earlier by several research workers (Rabbani *et al*., 2010; Behera *et al.*, 2012; Bansal *et al*., 2013; Shamim *et al*., 2016b; Singh *et al.*, 2016; Palanga *et al.*, 2016; Krupa *et al.*, 2017; Kumar *et al*., 2018; Kumari *et al*., 2018a; Priyadarshini *et al*., 2018) and also observed in the present study, indicates their usefulness and robustness in discrimination and unambiguous classification of aromatic rice varieties. Unique or genotype specific allele revealed by microsatellite based analysis of genetic polymorphism could be useful as molecular fingerprints in the identification and preservation of aromatic rice genotypes to benefit farmers, breeders and consumers. The varieties from different groups could be utilized in breeding programs for enhancement of genetic base of aromatic rice germplasm.

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RESEARCH ARTICLE

Genetic Divergence Studies in Doubled Haploids of Ethiopian Mustard (*Brassica carinata* A. Braun)

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The doubled haploids of Ethiopian mustard (*Brassica carinata* A. Braun) were evaluated along with mustard under two environments during *rabi,* 2010-11. Analysis of variance for different traits such as days to flower initiation, days to 50 per cent flowering, days to 75 per cent maturity, plant height, number of primary branches per plant, number of secondary branches per plant, siliquae per plant, length of main shoot, siliquae on main shoot, siliqua length, seeds per siliqua, 1000-seed weight, seed yield per plant, biological yield per plant, harvest index and per cent oil content revealed the presence of significant genetic variability for all characters except siliqua length and per cent oil content in Environment I (Env. I). On the other hand in Environment II (Env. II), the presence of significant genetic variability for most of the traits was observed. Pooled analysis over environments revealed the presence of g × e interactions for all characters except days to flower initiation and per cent oil content. All the 33 genotypes could be grouped into three clusters. Maximum genotypes were placed in cluster I. The cluster I exhibited maximum intra-cluster distance (1.12), while maximum inter-cluster distance was observed between cluster II and III (2.34). Maximum contribution towards genetic divergence was due to days to 75 per cent maturity.

Key Words: Cluster analysis, Ethiopian mustard, Genetic divergence.

Introduction

Oilseed crops are the backbone of Indian agricultural economy and occupy an important position in daily diet, being a rich source of fats and vitamins. India is the second largest rapeseed-mustard growing country and accounts for 21.7% area in the world after China. Among oilseeds, rapeseed-mustard is the second most important oilseed crop of the country after groundnut and plays a significant role in Indian oil economy by contributing about 28.6% to the total oilseed production (Shekhawat *et al*., 2014).

Rapeseed-mustard is the third important oilseed crop in the world after soybean (*Glycine max*) and palm (*Elaeis guineensis* Jacq.). The crop occupies an area of 33.58 million ha with a total annual production of 67.76 million tonnes and productivity 2018 kg/ha. In production**,** India (11.6%) ranks third after China (22.9%) and Canada (19.7%). The global production of rapeseed-mustard oil is around 12-14 million tonnes. In India, the crop occupies an area of 6.50 million ha with a total production of 8.02 million tonnes and productivity of 1262 kg/ha (Priyamedha *et al*., 2017).

Rapeseed-mustard in general, has shown a declining trend both in acreage and production largely due to lack of suitable cultivars for different ecosystems, fluctuations in weather conditions, cultivation in marginal and sub marginal lands and prevalence of various abiotic and biotic stresses. The present day varieties are more susceptible to *Alternaria* blight and white rust. Hence, the most suitable alternate way to increase productivity is by adoption of high yielding, input responsive genotypes having resistance against various biotic and abiotic stresses. The success of any breeding programme depends upon the nature and magnitude of variability present in the germplasm stock. The chances of initiating an effective breeding programme are greater if more genetic variability is available with the plant breeder. Thus, studies on genetic diversity are useful as a general guide for the choice of parents for future hybridization programme in order to obtain high heterotic response and superior transgressants. Estimation of degree of

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divergence within biological population and computation of relevant contribution of different components to the total divergence is done through Mahalanobis's generalized distance (D^2-statistic) method. Therefore, an attempt was made in the present study to estimate the nature and extent of genetic divergence and identify suitable donors on Ethiopian mustard.

Materials and Methods

The materials for the present investigation comprised 33 genotypes including 28 doubled haploids (DH) obtained through anther culture technique, one advanced breeding line (P-138) and four (3 mustard and 1 karan rai) check varieties *viz.,* Nav Gold, RCC-4, Pusa Jaikisan and Jayanti. The doubled haploids were obtained from the cross Jayanti × RCC-6-1 developed in the Department of Agricultural Biotechnology, CSK HPKV, Palampur. All the genotypes were raised at the experimental farm of Department of Crop Improvement, CSK HPKV, Palampur in randomized complete block design with three replications in the plot size of 3.0 × 0.60 m^2 on two different sowing dates *viz.*, 12th October, 2010 (Env. I) and 29th October, 2010 (Env. II). The row to row and plant to plant spacings were kept at 30 cm and 15 cm, respectively. The recommended cultural practices were followed to raise the crop. Data were recorded on various traits *viz.,* days to flower initiation, days to 50 per cent flowering, days to 75 per cent maturity, plant height (cm), number of primary branches per plant, number of secondary branches per plant, siliquae per plant, length of main shoot (cm), siliquae on main shoot, siliqua length (cm), seeds per siliqua, 1000-seed weight, seed yield per plant, biological yield per plant, harvest index (%) and percent oil content. The data were analysed statistically as per the method of Panse and Sukhatme (1985). Genetic diversity analysis was done as per D^2-analysis (Mahalanobis, 1936). Clustering was done by using Tocher's method (Rao, 1952).

Results and Discussion

Analysis of variance indicated the presence of sufficient genetic variability for all characters except siliqua length and per cent oil content in Env. I (Table 1). In Env. II, the presence of sufficient genetic variability for days to flower initiation, days to 50 per cent flowering, days to 75 percent maturity, plant height, number of primary branches per plant, number of secondary branches per plant, siliquae per plant, 1000-seed weight, seed yield per plant and harvest index was observed. Pooled analysis over environments revealed the presence of g × e interactions for all characters except days to flower initiation and per cent oil content (Table 2). The presence of g × e interaction has greatly influenced the variation due to genotypes to the extent that genotypic differences recorded in individual environments have vanished for these characters. Zehra and Gukan (2009) observed significant differences for plant height, number of branches per plant, number of pods per plant, pods

Table 1. Analysis of variance for different characters of *Brassica carinata* in Env. I and Env. II

S. No.	Characters	Env. I		Env. II	
		Mean Squares			
		Genotypes	Error	Genotypes	Error
		32	64	32	64
1	Days to flower initiation	153.08**	10.87	203.82**	37.03
2	Days to 50% flowering	68.91**	8.33	198.81**	8.43
3	Days to 75% maturity	189.79**	8.94	119.96**	44.47
4	Plant height (cm)	2318.26**	207.41	226.03*	117.46
5	Number of primary branches /plant	2.60**	0.57	2.19**	1.08
6	Number of secondary branches /plant	7.44**	0.97	7.8**	3.92
7	Siliquae/ plant	3703.66**	666.21	8929.05**	2579.37
8	Length of main shoot (cm)	127.24**	23.73	48.36	37.67
9	Siliquae on main shoot	180.78**	35.26	45.55	41.95
10	Siliqua length (cm)	0.263	0.217	0.23	0.160
11	Seeds /siliqua	2.36*	0.87	2.43	2.08
12	1000-seed weight (g)	1.15**	0.10	0.20*	0.11
13	Seed yield /plant (g)	7.36**	1.42	3.54**	1.47
14	Biological yield /plant (g)	140.18**	34.49	68.41	64.92
15	Harvest index (%)	44.47**	19.81	71.77*	39.51
16	Oil content (%)	11.54	12.56	12.75	15.50

* and **indicate significance at P ≤ 0.05 and 0.001, respectively

Table 2. Analysis of variance for different characters of *Brassica carinata* in pooled over the environments

S. No.	Characters	Mean Squares			
		Genotypes	Environments	Genotype × Environment (g × e)	Pooled error
		32	1	32	128
1	Days to flower initiation	337.18**	255.68**	17.72	23.95
2	Days to 50% flowering	235.09**	147.68**	32.63**	8.38
3	Days to 75% maturity	264.37**	6.55	45.38**	10.21
4	Plant height (cm)	1322.76	15254.73**	1221.53**	162.43
5	Number of primary branches / plant	1.52	20.97**	3.27**	0.82
6	Number of secondary branches / plant	7.57	250.26**	7.67**	2.44
7	Siliquae /plant	7940.97	1426.19	4691.74**	1622.49
8	Length of main shoot (cm)	90.82	955.68**	84.79**	30.70
9	Siliquae on main shoot	93.19	3224.25**	133.14**	38.60
10	Siliqua length (cm)	0.23	0.44	0.323*	0.20
11	Seeds /siliqua	2.21	52.08**	2.58**	1.47
12	1000-seed weight (g)	0.86*	0.19	0.48**	0.11
13	Seed yield /plant (g)	3.64	0.05	7.25**	1.44
14	Biological yield /plant (g)	105.19	251.16	103.41**	46.70
15	Harvest index (%)	47.92	199.20	68.29**	29.70
16	Oil content (%)	18.31**	81.08**	6.10	13.62

* and ** indicate significance at $P \leq 0.05$ and 0.01, respectively

per main stem, pod length, 1000-seed weight, seed yield per plant and percent oil content in two environments. Yared *et al.* (2012) also observed highly significant differences for days to flower initiation and days to maturity in Ethiopian mustard.

The cluster analysis revealed that all the genotypes could be grouped into three clusters (Table 3 and Fig. 1). Different clustering pattern in rapeseed-mustard was also reported by earlier workers (Yadav *et al.,* 1985; Verma and Sachan, 2000; Thul *et al.,* 2004; Patel and Patel, 2006; Mukesh *et al.,* 2007; Singh *et al.,* 2007; Mahmuda *et al.,* 2008). Different clustering pattern in different environments has also been reported by Goswami and Behl (2006).

Among all clusters, cluster I was found to be largest one. Verma and Sachan (2000), Patel and Patel (2006), Mukesh *et al.* (2007) and Singh *et al.* (2007) also grouped genotypes into different clusters and reported that cluster I was the largest one. Cluster I contained 29 genotypes such as P-43, P-45, P-51, P-103, P-122, P-96, P-62, P-26, P-74, P-101, P-89, Jayanti, P-33, P-133, P-24, P-63, P-77, P- 56, P-137, P-39, P-34, P-75, P-138, P-64, P- 117, P-17, P-92, P-31 and P-23. Cluster II had three genotypes *viz.,* RCC-4, Nav Gold and Pusa Jaikisan while cluster III had only one genotype *i.e.* P-12. The clustering pattern indicated that all the mustard checks formed separate clusters while all doubled haploids appeared in separate clusters. This further supported that Indian mustard (AABB, 2n = 36) has been originated in china by hybridization between *Brassica campestris* (AA) and *B. nigra* (BB) in nature while Ethiopian mustard (BBCC, 2n = 34) has been originated in Ethiopia by hybridization between *B. oleracea* (CC) and *B. nigra* (BB), though, one genome is common in both species. These results therefore, emphasized that the parents should be selected on the basis of total divergence for the traits used for an overall improvement in the yield.

The intra-cluster distances were comparable for clusters I (1.12) and II (1.00) (Table 4). Since the intra-cluster distance was low, the chances of developing

Table 3. Cluster composition in *Brassica carinata* following multivariate analysis in pooled over the environments

Cluster number	Number of genotypes	Genotypes
I	29	P-43, P-45, P-51, P-103, P-122, P-96, P-62, P-26, P-74, P-101, P-89, Jayanti, P-33, P-133, P-24, P-63, P-77, P- 56, P-137, P-39, P-34, P-75, P-138, P-64, P- 117, P-17, P-92, P-31 and P-23
II	3	RCC-4, Nav Gold and Pusa Jaikisan
III	1	P-12

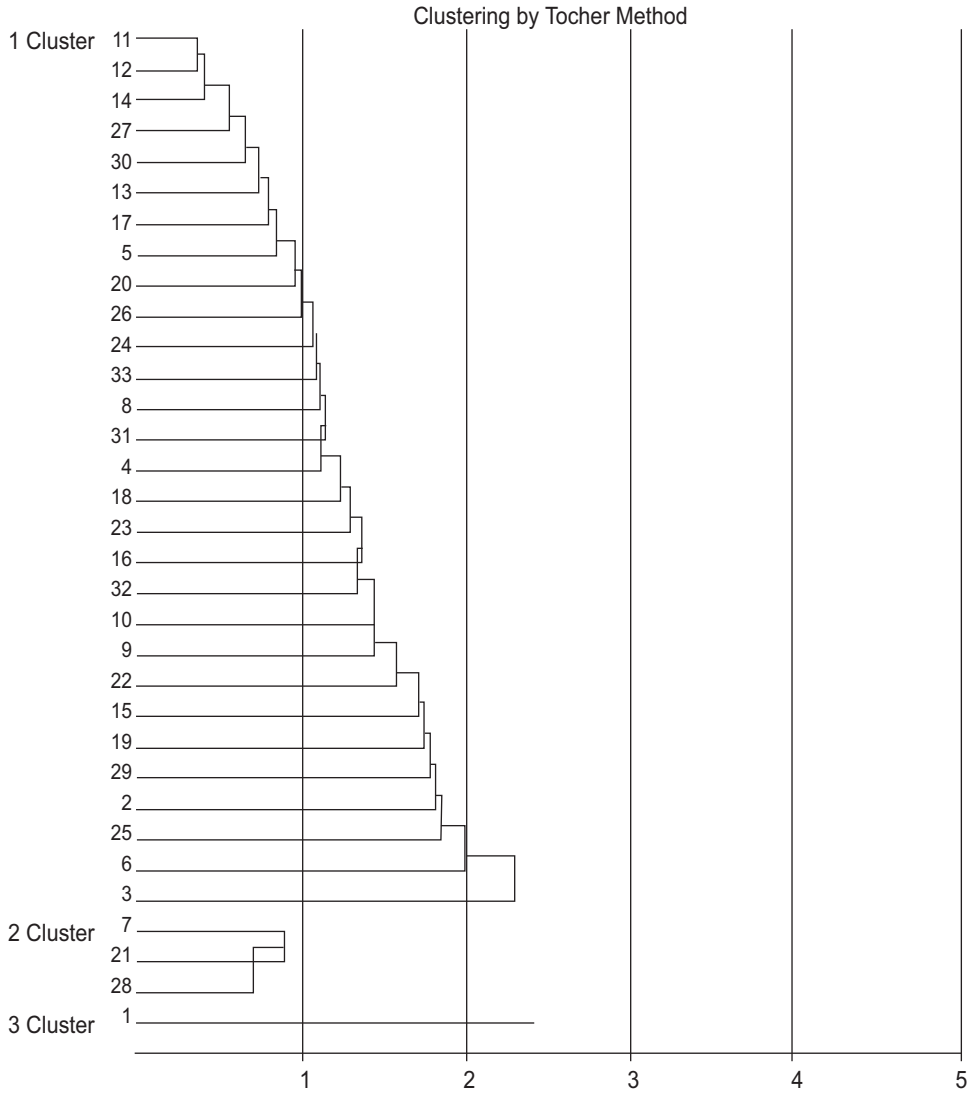

Fig.1. Dendrogram showing grouping of 33 *Brassica carinata* genotypes generated using D^2 multivariate analysis (Tocher's method) in pooled over the environment

good segregants by hybridization among parents within clusters would be low, therefore, it is logical to attempt crosses between the genotypes falling in different clusters based on inter-cluster distances. Patel and Patel (2006) reported highest intra-cluster distance in cluster II in Indian mustard.

The analysis of genetic divergence indicated the highest inter-cluster distance between clusters II and III (2.34) followed by distance between clusters I and II (2.21). The lowest inter-cluster distance was observed between clusters I and III (1.42). This clearly indicates that the genotypes included in these clusters are having sufficient genetic diversity and parents from diverse clusters could be used in hybridization programme for improving seed yield. Further, the clustering pattern suggested the parallelism between the genetic divergence and species-wise geographical distribution. However, Anand and Rawat (1984) and Gupta *et al.* (1991) suggested that geographical diversity of line does not necessarily reflect on index of its genetic diversity. No parallelism between geographical diversity and genetic diversity was reported by Verma and Sachan (2000) in Indian mustard. Geographical distribution of the cultivars did not significantly contribute to genetic divergence (Singh *et al.,* 2007).

Crosses involving parents belonging to most divergent clusters would be expected to manifest

Table 4. Average intra- and inter-cluster distance in pooled over the environments

Clusters	I	II	III
I	1.26 (1.12)	4.90 (2.21)	2.03 (1.42)
II		1.01 (1.00)	5.48 (2.34)
III			0.00 (0.00)

Values in bold letters are intra-cluster distances
Values in parenthesis are √ D2 = D values

maximum heterosis and release of desirable recombinants in segregating generations. Therefore, the parents should be selected from cluster combination between clusters II and III.

Based on the comparison of cluster means of different characters, it was observed that substantial differences existed among the cluster means for each character. Genotypes in cluster I had moderate values for all the characters studied (Table 5). Cluster II revealed maximum cluster means for siliqua length (4.06 cm), seeds per siliqua (10.79), 1000-seed weight (3.79 g) and biological yield per plant (37.89 g) and the genotypes recorded minimum days to flower initiation (65.94 days), days to 50 percent flowering (109.44 days), days to 75 percent maturity (148.50 days) and plant height (108.01 cm). Likewise, cluster III was characterized by maximum number of primary branches per plant (5.72), number of secondary branches per plant (11.70), siliquae per plant (329.37), length of main shoot (52.33 cm), siliquae on main shoot (33.00), seed yield per plant (6.91 g), harvest index (20.12%) and per cent oil content (40.00%). Based upon the inter-cluster distances and cluster means, the crosses among the genotypes belonging to clusters II and III, may give transgressants for higher seed yield, dwarf plant type, earliness in flowering and maturity, high biological yield and harvest index. Earlier workers have also attempted interspecific hybridization between *Brassica napus* × *B. carinata* (Sheikh *et al*., 2010b), *B. carinata* × *B. rapa* (Choudhary *et al.,* 2000), *B. juncea* × *B. carinata* (Sheikh *et al*., 2010a) and derived desirable transgressive segregants in the progeny by introgressing the desirable genes in Ethiopian mustard. Singh *et al.* (2010) also reported significant increase in shoot length, 1000-seed weight and significant decrease in maturity duration through interspecific hybridization in *B. carinata.*

The contribution of individual characters to divergence has been worked out in terms of number of times it appeared first (Table 6). Days to 75 per cent maturity contributed maximum (17.99%) towards total genetic divergence followed by plant height (17.42%) among 33 genotypes studied. Monalisa *et al*. (2005) reported that the number of siliquae per plant contributed maximum towards total genetic divergence followed by days to maturity and plant height.

The results from present study indicated that, selection of genotypes as superior and diverse parents for hybridization programme should be based on diverse clusters viz., II (Nav Gold, Pusa Jaikisan and RCC-4)

Table 5. Cluster means for different characters in pooled over the environments

S. No.	Characters	Clusters					
		I	II	III	Mean	Minimum	Maximum
1	Days to flower initiation	90.67	65.94	91.83	82.81	65.94	91.83
2	Days to 50% flowering	129.67	109.44	130.00	123.04	109.44	130.00
3	Days to 75% maturity	169.68	148.50	167.00	161.73	148.50	169.68
4	Plant height (cm)	113.73	108.01	118.80	113.51	108.01	118.80
5	No. of primary branches/ plant	4.91	4.00	5.72	4.88	4.00	5.72
6	No. of secondary branches/ plant	7.99	7.96	11.70	9.22	7.96	11.70
7	Siliquae / plant	172.09	156.41	329.37	219.29	156.41	329.37
8	Length of main shoot (cm)	42.43	40.33	52.33	45.03	40.33	52.33
9	Siliquae on main shoot	31.21	27.56	33.00	30.59	27.56	33.00
10	Siliqua length (cm)	3.64	4.06	3.83	3.84	3.64	4.06
11	Seeds/ siliqua	10.49	10.79	10.45	10.58	10.45	10.79
12	1000- seed weight (g)	2.56	3.79	2.57	2.97	2.56	3.79
13	Seed yield / plant	6.13	6.39	6.91	6.48	6.13	6.91
14	Biological yield / plant (g)	34.94	37.89	36.17	36.33	34.94	37.89
15	Harvest index (%)	18.45	12.28	20.12	16.95	12.28	20.12
16	Oil content (%)	36.67	37.36	40.00	38.01	36.67	40.00

Table 6. Contribution of individual characters to the divergence among 33 genotypes of *Brassica carinata*

S. No.	Characters	Times ranked Ist	Contribution (%)
1	Days to flower initiation	9	1.70*
2	Days to 50% flowering	67	12.69
3	Days to 75% maturity	95	17.99**
4	Plant height	92	17.42
5	Number of primary branches / plant	11	2.08
6	Number of secondary branches/ plant	24	4.55
7	Siliquae / plant	38	7.20
8	Length of main shoot	31	5.87
9	Siliquae on main shoot	27	5.11
10	Siliqua length	15	2.84
11	Seeds/ siliqua	20	3.79
12	1000-seed weight	12	2.27
13	Seed yield / plant	25	4.73
14	Biological yield / plant	19	3.60
15	Harvest index	17	3.22
16	Oil content	26	4.92

** Maximum contribution; * Minimum contribution

and III (P-12) to get heterotic crosses for getting superior recombinants in early segregating generations.

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RESEARCH ARTICLE

Suitability of Indigenous Rice (*Oryza sativa* L.) Cultivars in Garhwal Hills of Uttarakhand Himalayas based on Seed Quality Parameters

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Hill agriculture systems of Garhwal Himalayas harbour wealth of rice landraces contributing to the agricultural biodiversity of the region. These landraces are an integral part of traditional cultivation systems for these have evolved against the adversities of agroclimatic conditions and have proven their suitability in the prevailing agro-climate of the region. However, intrusion of the high external-input intensive agriculture systems to increase crop productivity is posing great threat to the agro-biodiversity of the crop in the region. This wealth needs to be conserved for sustaining the traditional agriculture through implementation and integration of eco-friendly approaches suiting to the local conditions without harming the crop diversity. In the present study, the comparative suitability of certain widely cultivated landraces confined to mid Himalayan hills in the Garhwal region of Uttarakhand, India, was investigated on the basis of seed quality parameters. Local cultivars China-4, Lal dhan and Rikhwa showed considerably high germination (>90%) despite high levels of seed infection (>35%) like the improved varieties Prasad, PD-10, PD-11 and VL-62. Local cultivars China-4 and Lal dhan showed maximum germination to the range of >90% with high seedling vigour (1500- 2077). The study concluded that local cultivars/landraces showing higher seed vigour despite higher levels of discoloration and seed infection are better suited for cultivation in mid-hills of Uttarakhand.

Key Words: Indigenous, Landraces, Rainfed, Seed quality, Seedborne inoculum.

Introduction

Hill agriculture cropping systems of Uttarakhand falling under Indian Himalayan region, harbour rich crop biodiversity. Rice (*Oryza sativa* L.) traditionally being grown as a major food crop in the region under irrigated condition in the valleys along the river banks as well as under rainfed conditions on hilly terrains, possesses wide genetic variability of landraces cultivated since time immemorial It is locally termed as 'dhan' derived from the literary word 'dhanya' meaning prosperity and is an integral part of folk culture of the region. Under rainfed cultivation, wide array of traditional varieties/landraces is cultivated, making it the largest on-farm repository of traditional varieties in Garhwal Himalaya (Mehta *et al*., 2014). On-farm crop diversity and wild relatives constitute the genetic resources that are the basic requirement for crop improvement to deal with environmental stresses, plant diseases and pests under the climate change scenario. Traditional farming systems have played great role in preserving the crop biodiversity which operate through the human knowledge and cultural practices and have shaped the existing diversity worldwide (Bellon 1991).

This on-farm crop biodiversity of local germplasm, the landraces cultivated since ages, are potentially able to withstand the adverse conditions and are the valuable genetic resource from climate change point of view by virtue of their adaptability to the existing agricultural system. These genetic resources need to be integrated with improved eco-friendly agricultural technologies to improve their performance. Seed treatment is one such low cost technology with high impact on yield however, seed treatment must be recommended based on the level of seedborne inoculum particularly in case of seedborne diseases. Seed borne inoculum not only deteriorates the seed quality but also initiates many crop diseases in the

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field. In case of rice, blast, leaf spot and sheath rot are major seed borne diseases in rainy (*Kharif*) season rice crop in hill regions of Uttarakhand.

Most of the rice growing areas of hilly regions in the state are under local cultivars. Rice (*Oryza sativa* L.), mainly grown in *Kharif* season (April–October) in hilly cropping systems of Uttarakhand, shows tremendous genetic diversity in the region (Rana *et al.,* 2009; Mehta *et al.,* 2014; Agnihotri and Palni, 2007). It is mostly taken as upland rainfed crop in mid and high hills (1200-2500m asl). Natives of the hilly regions traditionally grow different rice cultivars (landraces) as mixed crop (Zhardhari, 2001). The farmers of the region cultivate several landraces of paddy evolved through centuries as a result of climatic selection pressure as well as the wisdom involved in farmers' selection. These landraces are preferred by the farmers of the region over modern high yielding varieties owing to their desirable attributes such as pest resistance, drought-tolerance, high nutritive value, flavour, medicinal properties etc. It is estimated that about three-quarters of the original varieties of agricultural crops have been already lost from the farm fields between 1950 and 1995 (FAO 1997). Therefore, maintaining the crop diversity, collection, characterization, and conservation of traditional indigenous germplasm/ landraces is very essential.

Though monocropping and line sowing of improved varieties is being popularized in some regions of the state, most of the area is still under landraces. The state has 0.3 million ha area under rice cultivation which covers irrigated as well as rainfed rice in hilly areas. The average productivity of the state is as low as 20.39 q/ha. The major constraints to paddy production and productivity in hills are non-availability of quality seed, inadequate irrigation, cold stress and diseases like blast, brown spot & leaf and sheath blight. The farmers keep the seed in their household seed stores and sow it without any seed treatment. Dominance by traditional varieties/landraces (about 65%) is reported under rainfed condition (Mehta *et al.*, 2014). The production technologies of these preferred landraces need to be refined to increase the production and productivity of the crop through adoption of eco-friendly agricultural technologies suitable for the sustainable crop production system without harming the agroecosystems. Improving the seed quality of local germplasm with use of suitable seed treatment chemical or agent is such a technology which advocates minimal use of chemical pesticides at the initial stage itself to restrict the pathogens at the beginning. Good quality seed with low seedborne inoculum load has a role in increasing the production as the seedborne inoculum not only plays important role in deteriorating seed quality but also initiates many seedborne diseases in field. However there is paucity of information on status and level of seed borne inoculum in local cultivars and popular varieties of paddy grown in the region. Therefore, a study was conducted with ten landraces popularly grown in the region and 17 paddy varieties some of which are recommended for the hill regions, to assess the level of seedborne inoculum and its effect on seed quality parameters under mid-hill conditions of Garhwal region of Uttarakhand. The locally grown varieties and landraces were assessed for level of seedborne inoculum and their effect on seed quality parameters. Several fungal plant pathogens like *Bipolaris, Drechslera, Curvularia, Pyricularia* and *Aspergillus* spp. were found associated with seeds.

Materials and Methods

The seed samples of different rice varieties recommended for hill regions were collected from Department of Genetics & Plant Breeding, G.B. Pant University of Agriculture & Technology, Pantnagar and *Vivekananda Parvatiya Krishi Anusandhan Sansthan*, Almora, Uttarakhand, The landraces were collected from fields of local farmers of villages Ranichauri, Jagdhar, Dargi, Weed, Maun, Sabli, Chamba and Nagani in Tehri Garhwal, Uttarakhand (Fig.1). The details of the collection sites and the study material are presented in Tables 1 and Table 2.

Table 1. Geographical location of the villages under the study

S. No.	Place/ Village	Geographical Coordinates		
		Latitude	Longitude	Altitude (m)
1	Chamba	30º 20′ 45.00′′ N	78º 23′ 38.00′′ E	1574
2	Dargi	30º 19′ 13.12′′ N	78º 24′ 30.55′′ E	1722
3	Jagdhar	30º 19′ 24.73′′ N	78º 23′ 55.68′′ E	1504
4	Maun	30º 18′ 12.64′′ N	78º 23′ 44.50′′ E	1651
5	Nagni Sera	30º 18′ 45.51′′ N	78º 20′ 30.00′′ E	1064
6	Sabli	30º 19′ 36.47′′ N	78º 23′ 19.00′′ E	1330
7	Weedh	30º 18′ 49.33′′ N	78º 24′ 56.17′′ E	1617
8	Ranichauri Campus	30º 18′ 44.00′′ N	78º 24′ 42.00′′ E	1815

Seed discolouration percentage was counted on the basis of number of discoloured seeds in each sample.

Fig. 1 Geographical location of seed collection villages of the area under study

Table 2. List of rice landraces and varieties collected for the study

S. No.	(a) Land Races	Cultivation condition
1.	China-4	Irrigated
2.	Bauna Dhan	Irrigated
3.	Garuda-Fine	Rainfed
4.	Garuda (Coarse)	Rainfed
5.	Gorakhpuri	Irrigated
6.	Hansraj	Irrigated
7.	Lal Dhan	Irrigated
8.	Raj	Rainfed
9.	Rikhwa (Coarse)	Rainfed
10.	Rikhwa (Fine)	Rainfed
	(b) Varieties	
1.	IR-8	Irrigated
2.	Govind*	Irrigated
3.	Manahar	Irrigated
4.	Prasad*	Irrigated
5.	PD-4	Irrigated
6.	PD-6*	Irrigated
7.	PD-10	Irrigated
8.	PD-11*	Irrigated
9.	PD-12	Irrigated
10.	Pant Sugandh Dhan-15 (PSD-15)	Irrigated
11.	PD-16	Irrigated
12.	Pant Sugandh-17 (PS-17)	Irrigated
13.	VL-62*	Irrigated
14.	VL-82*	Irrigated
15.	VL-206*	Rainfed
16.	VL-221*	Rainfed
17.	PRR-2	Rainfed

* Varieties recommended for hill regions

Seed infection was detected by standard Blotter Paper method and fungi associated with discoloured seeds were isolated by standard Agar Plate method (ISTA 1993) and percentage was calculated. Fungi associated with discolorations, observed in standard Agar Plate method, were isolated, purified and identified by observing the characteristics of fungal colony on PDA, sporulation, conidial characteristics, fruiting structures, etc. developed on the seed surface with the help of identification manuals (Mathur and Cunfer 1993). Seed germination percentage was estimated by using standard Rolled Paper Towel method (ISTA 1999). Test weight was taken for 1000 seeds. The seedling vigour index was calculated by the formula given by Abdul-Baki and Anderson (1973).

$$\text{Seedling Vigour Index} = \text{Germination (\%)} \times \text{Seedling length (cm)}$$

The data obtained was subjected to statistical analysis.

Results and Discussion

Germination percentage of locally cultivated landraces like China-4 (93%), Rikhwa (92%) and Lal dhan (91%), was recorded higher than varieties like VL-206 (91%), PD-10 (88%) and IR-8 (84%). Among varieties, the test weight (1000 seed weight) was recorded maximum for VL-82 (3.26g) followed by PD-4 (2.668). However among landraces, it was maximum for Bauna Dhan (2.56g) followed by Hansraj 2.509g (Table 3a and 4a). It was positively correlated with Seed Vigour (Table 5).

Table 3(a). Seed quality parameters of different rice landraces

S. No.	Landraces	Germination (%)	Root Length (cm)	Shoot Length (cm)	Test weight (1000 grain wt.) (g)	Seed Vigour Index
1	China-4	93	9.47	8.77	2.358	1691.19
2	Bauna Dhan	68	6.92	7.35	2.561	976.88
3	Garura (Fine)	82	8.55	9.52	2.284	1480.92
4	Garuda Coarse	62	11.38	11.90	2.237	1451.67
5	Gorakhpuri	82	8.82	7.90	2.457	1362.88
6	Hansraj	48	8.72	8.08	2.509	811.62
7	Lal Dhan	91	8.65	14.22	2.319	2077.53
8	Raj	68	6.17	8.10	1.693	966.88
9	Rikhwa (Coarse)	92	11.80	10.65	2.477	2065.77
10	Rikhwa (Fine)	35	6.25	5.83	1.516	421.51

Table 3(b). Seed health parameters of different rice landraces

S. No.	Landraces	Seed Discoloration (%)	Seed Infection (%)
1	China-4	21	35
2	Bauna Dhan	27	40
3	Garuda (Fine)	25	00
4	Garuda Coarse	14	10
5	Gorakhpuri	24	00
6	Hansraj	22	10
7	Lal Dhan	12	25
8	Raj	15	45
9	Rikhwa (Coarse)	11	15
10	Rikhwa (Fine)	6	35

Table 4(a). Seed quality parameters of different rice varieties

S. No.	Varieties	Germination (%)	Root Length (cm)	Shoot Length (cm)	Test weight (1000 grain wt.) (g)	Seed Vigour Index
1	IR-8	84	6.70	5.20	2.144	1015.69
2	Govind	79	10.61	8.58	2.005	1516.61
3	Manahar	77	11.08	7.48	2.032	1426.12
4	Prasad	95	4.55	7.53	1.913	1149.37
5	PD-4	72	7.08	8.60	2.668	1141.11
6	PD-6	72	6.30	8.15	1.793	1041.81
7	PD-10	88	7.27	8.12	2.234	1355.54
8	PD-11	81	10.20	8.25	2.437	1492.62
9	PD-12	77	6.99	9.55	2.440	1272.92
10	Pant Sugandh Dhan-15	66	7.93	7.78	1.843	1037.36
11	PD-16	67	8.92	7.38	2.319	1097.57
12	Pant Sugandh-17	58	7.40	5.25	1.948	724.53
13	VL-62	88	8.75	9.28	2.430	1587.10
14	VL-82	85	7.68	9.78	3.260	1488.20
15	VL-206	91	4.40	6.63	2.345	1004.71
16	VL-221	80	9.35	9.88	2.481	1532.12
17	PRR-2	66	11.56	10.86	2.021	1480.12

* Each digit is a mean of three replications

Higher root length in landraces / varieties of rainfed conditions was observed as compared to varieties. It was maximum in Rikhwa (11.8 cm.), followed by Garuda (coarse) and PRR-2 (11.6cm). Higher seed vigour was observed in Lal Dhan, Rikhwa, China-4 as compared to Govind, Manahar, PD-11, VL-62, Vl-221 etc. Seed vigour was maximum (2065.77) in Rikhwa followed by the most popular local cultivar under irrigated condition *i.e.* China-4 (1691.19).

Inoculum load, in terms of per cent seed discolouration and per cent seed infection, and seed quality parameters of all the varieties and landraces were determined to assess the impact of seed borne fungi on seed quality. Seed discolouration ranged from 6 per cent to 27 per cent resulting in 0 to 40 per cent seed infection in local cultivars. For rice varieties it ranged from 6 per cent (VL-221) to 51 per cent (Prasad) resulting in seed infection in the range of 0 per cent (IR 8 and VL-82) to 50 per cent (VL-62). Per cent seed discolouration and seed infection (Table 3b and 4b) was lower for varieties grown under rainfed conditions (VL 206 and VL 221) (Fig.2a and 2b).

Fig. 2(a). Types of seed discolouration in rice landraces

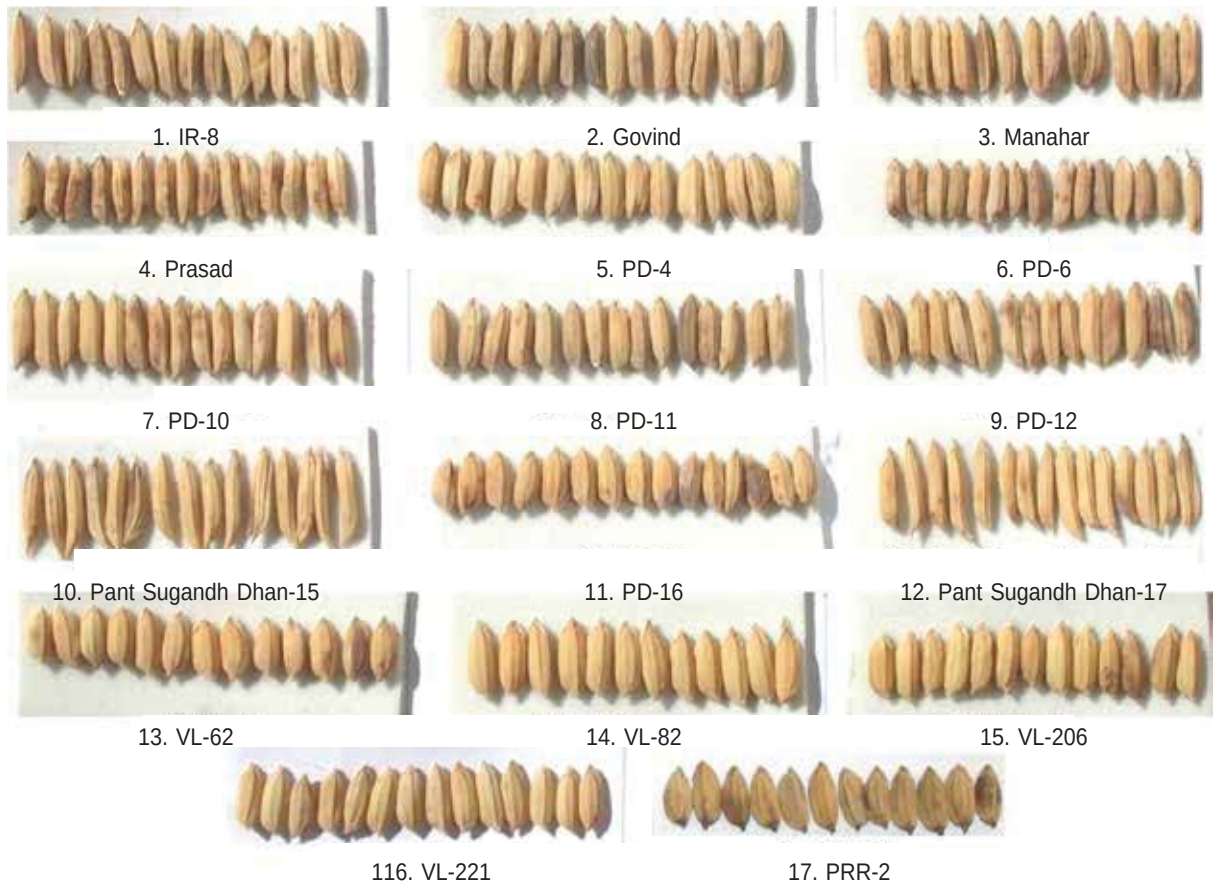

Fig. 2(b). Types of seed discolouration in rice varieties

Seed discoloration, an indicative of seedborne inoculum, showed positive correlation with seed infection as reported earlier (Querijero *et al.,* 1993) and infected seeds exhibited association of fungi like *Bipolaris* sp., *Drechslera* sp., *Curvullaria* sp., *Pyricularia* sp. and *Aspergillus* sp., in confirmation with earlier reports (Misra *et al.,* 1990; Ou 1985; Querijero *et al.,* 1993, Srinivas and Ramakrishna 2002; Srinivas and Ramakrishna 2005). Bhat *et al.* (2009) reported eight fungal genera associated with glume discolouration of paddy including species of *Helminthosporium, Curvularia, Pyricularia and Aspergillus* as reported in the study.

Highest seed vigour was recorded for landraces like Lal dhan (2077.53) followed by Rikhwa (course) (2065.77) and China-4 (1691.19) with high germination (>90 per cent) which was more than in case of the improved varieties like VL-62 (1587.1), VL-221 (532.12), PD-11 (1492.6). Among varieties, germination was observed in Prasad (95%), Vl-206 (91%) and VL-62 (88%). The seedling root length was recorded maximum for land races like Rikhwa-coarse (11.8 cm), Garuda coarse (1.38 cm), China-4 (9.47 cm) and Lal dhan (8.65 cm). The maximum root length was observed in case of PRR-1 (111.6 cm), Manahar (11.08 cm), Govind (10.61 cm) and PD-11 (10.2 cm) varieties. The extensive root system in case of landraces as compare to improved varieties makes them able to withstand water stress under rainfed conditions. Bisht *et al.* (2007) have reported extensive root system, grain yield and recycled biomass in case of one of the popular landraces Lal dhan.

The data presented in Table 6 indicated that per cent germination showed negative correlation to the per cent seed infection however, root length was negatively correlated to per cent seed infection and per cent seed discolouration, indicating that per cent seed discolouration an indicative of seed infection, was responsible for reducing the seed germination as reported earlier (Misra *et al.,* 1990). Similar observations were made by Ou, (1985) and Querijero *et al.* (1993). Per cent seed infection and per cent seed discolouration showed negative correlation with seed vigour. The results of genetic and phenotypic coefficient of variability and heritability estimation (table 6) showed minimum heritability of root length (2.59%) indicating that root length is influenced by environmental conditions *i.e.* moisture stress under rainfed condition and cannot be preferred for selection as it has been reported that characters with high heritability could be used as powerful tool in selection process as these characters are less influenced by the environment (Panse & Sukhatme, 1995). However, a few landraces cultivated since ages by the locals and improved varieties released for the rainfed agricultural system showed their adaptability to the changing climate in terms of root length and high seedling vigour.

The study revealed that seed discoloration, an indicative of seedborne inoculum, reduced the seed vigour. Germination percentage, root length and shoot length were adversely affected by seed discolorations (Fig. 3). Higher root length observed in landraces indicated their suitability for cultivation under rainfed conditions. Therefore, local cultivars with extended / deep root-system (e.g. Rikhwa-coarse, Garuda-coarse, China-4 and Lal dhan), can better withstand the water scarcity and show higher seed vigour despite higher levels of discoloration and seed infection and can be better suited for cultivation with appropriate seed treatment, under rainfed condition in mid-hills of Uttarakhand. The suitability and preference of indigenous landraces, owing to their tolerance/ resistance to existing biotic and abiotic stresses and superior nutritional traits has been well documented (Agnihotri *et al.,* 2007; Maikhuri *et al.,* 2001). The native microfloras are also likely to play an important role in naturally restricting the seed borne pathogens (Srinivas and Ramakrishna 2003; Srinivas and Ramakrishna 2005) in the crop micro-ecosystems.

Table 4(b). Seed health parameters of different rice varieties

S. No.	Varieties	Seed Discoloration (%)	Seed Infection (%)
1	IR-8	26	00
2	Govind	21	35
3	Manahar	26	20
4	Prasad	51	20
5	PD-4	15	15
6	PD-6	29	55
7	PD-10	40	20
8	PD-11	26	50
9	PD-12	41	25
10	Pant Sugandh Dhan-15	27	25
11	PD-16	26	35
12	Pant Sugandh-17	13	10
13	VL-62	10	50
14	VL-82	10	00
15	VL-206	7	05
16	VL-221	6	00
17	PRR-2	30	45

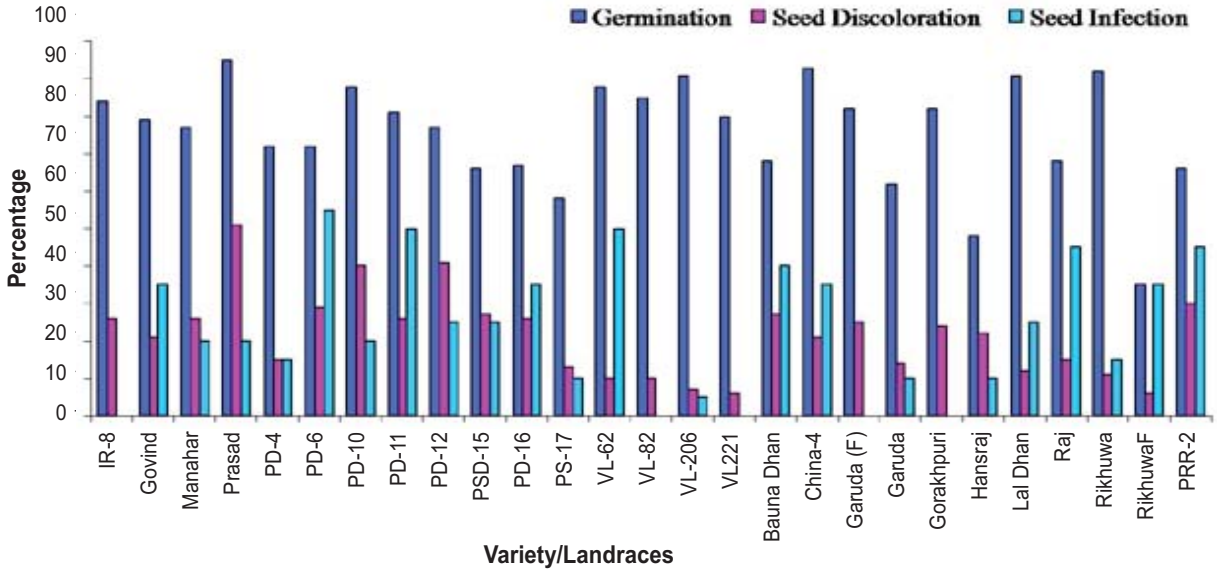

Fig. 3. Effect of seed discolouration and infection on seed germination

Table 6. Genetic and Phenotypic Coefficients of Variability in Rice

	Mean	Range	Genetic Coefficient of Variability	Phenotypic Coefficient of Variability	Heritability
Germination %	75.81	35-95	18.37	19.41	89.53
Root Length (cm)	9.75	4.4-11.8	18.17	22.83	2.59
Shoot Length (cm)	8.54	5.2-14.2	19.77	25.44	60.41
Test weight(g)	2.12	1.52- 3.26	53.16	56.86	87.41
Seed Vigour	1284.1	422- 2078	30.81	27.48	79.53

The rainfed agro-system of Garhwal Himalaya is well-known as the largest repository of traditional varieties. Furthermore, in view of rapid drop in the crop diversity over the past few decades, it is the need of the hour to conserve these landraces that are being protected by local farmers, to conserve crop diversity of the region.

Conclusion

The traditional landraces have shown better preferences owing to their inbuilt and adapted traits suitable to the agroclimatic situation of mid Himalayas. However, modern agricultural technologies which have been proved to improve the crop yields to great extent, at the same time have also resulted in considerable loss of agricultural biodiversity in the region for last three-four decades. Therefore, it is a cause of concern to conserve the existing crop diversity to stop genetic erosion and to maintain the sustainability of hill agroecosystems. The local germplasm, the landraces being cultivated for centuries, are well suited to rainfed hill agriculture. The higher root length in case of landraces as compare to improved varieties seems to be a desirable attribute to make them suitable to tolerate the moisture stress posed under rainfed agriculture system of hill region. However, the high level of seedborne infection in landraces needs to be managed and minimized to improve their performance. These landraces need to be conserved and brought to the forefront of sustainable hill agricultural system, with adoption of eco-friendly agricultural technologies like seed treatment with eco-friendly bio-fungicides, botanicals or safe chemical fungicides for management of the initial seedborne infection to improve the germination and overall performance.

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SHORT COMMUNICATION

A Note on the Collection and Conservation of Hedgehog Cucumber (*Cucumis dipsaceus* Ehrenb. ex Spach) Germplasm from Coimbatore, Tamil Nadu, India

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Hedgehog cucumber (*Cucumis dipsaceus* Ehrenb. ex Spach) (family Cucurbitaceae) is a native of Saudi Arabia which was introduced to India and is now reported to be occurring as a ruderal in some parts of peninsular India. The authors in a recent survey of Coimbatore in Tamil Nadu came across two populations of this species. Its germplasm was collected along with the passport data and conserved in the long term storage of the National Gene Bank at New Delhi. Herbarium specimens were also collected at source and deposited in the National Herbarium of Cultivated Plants at ICAR-NBPGR, New Delhi.

Key Words: Conservation, *Cucumis dipsaceus*, Germplasm collection, Hedgehog cucumber

The genus *Cucumis* L. (family Cucurbitaceae) includes 32 species (Kirkbride, 1993). Yadav *et al.* (2014) in a recent study have reported 14 species from India, out of which the germplasm of all those species except *Cucumis dipsaceus* Ehrenb. ex Spach have been conserved in the National Genebank at the ICAR-National Bureau of Plant Genetic Resources, New Delhi. Hedgehog cucumber is a native of Saudi Arabia which was introduced to India and is now reported to be occurring as a ruderal in some parts of peninsular India, especially in Tamil Nadu and Karnataka (Sarvalingam *et al.,* 2010; Sutar *et al.,* 2013). Geethakumary *et al.* (2015) reported the extended distribution of the species further southwards to Attappadi in Palakkad District of Kerala. It is easily distinguished from other members of the genus by its densely aculeate fruit. It is naturally distributed in West Indies, Africa, North and South America and Saudi Arabia. The authors in a recent survey of Coimbatore in Tamil Nadu came across two populations of this species, one from a wasteland at Vedappatti and the other from Karupanayaganpalayam, found climbing in a roadside fence. Sufficient quantity of mature fruits was collected from the second site (10.966^{o} N and 77.283^{o} E) along with the passport data and the seeds were extracted and conserved as IC619125 in both the long-term storage of the National Gene Bank, New Delhi and at the Medium-Term Storage of the ICAR-NBPGR Regional Station, Thrissur, Kerala. Herbarium specimens were also collected at source and deposited in the National Herbarium of Cultivated Plants (HS22566) at ICAR-NBPGR, New Delhi as well as at the ICAR-NBPGR Regional Station, Thrissur.

Ecology

Habit and habitat: Annual climber in deciduous woodland, wooden grassland and bush land, also in cultivated places; at elevations upto 2,000 m.

Phenology: Flowering and fruiting from April to June in Karnatic plains.

Morphology

Stem: Procumbent or climbing; sulcate; on the ridges hispid and in the grooves hispidulous; not deciduous; hairs 0.8-1.5 mm long; nodes not geniculate; internodes 2-6 cm long.

Leaves: Petioles 1.5-5 cm long; sulcate; not aculeate; weakly hispidulous to hispid. Leaf blades entire or trilobate; with the margin regularly serrate to entire; ovate to broadly ovate in outline; cordate at the base; with a basal sinus; broadly acute to obtuse at the apex.

Tendrils: Present; solitary; simple; 1.5-6 cm long; basally hispidulous and apically glabrate.

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Fig. 1. a. Plant growing under natural habitat; b. Fruits of hedgehog cucumber; c. Fruit in section; d. Seeds

Inflorescence: Unisexual.

Male inflorescence: Solitary flower or fasciculate; 1-5 flowered; sessile.

Male flowers: Pedicel terete; 5-20 mm long; hispidulous; without bracteoles. Calyx lobes narrowly oblong to linear; narrowly acute at the apex; 1.6-4 x 0.1-0.3 mm; sparsely hispidulous. Corolla infundibular; sparsely hispidulous outside; glabrous inside. Stamens separating from the hypanthium 2-2.8 mm from the base of the hypanthium.

Female inflorescence: A solitary flower, axillary.

Female flowers: Pedicel sulcate; 5-15 mm long; hispid and hispidulous; cylindrical. Calyx lobes linear; narrowly acute at the apex; sparsely hispidulous. Corolla sparsely hispidulous outside; glabrous inside. Style 1.5 mm long; 0.6 mm in diameter; subtended by a circular disc. Stigma 2.5 mm long; 2.8 mm in diameter; lobate; with five finger like projections on the margin.

Fruits: Maturing above ground and readily visible. Pedicel sulcate; 1-3 cm long; hispid; with non breakaway hairs; cylindrical. Fruit monocoloured; pale yellow; ellipsoid to globose; 3-6.5 cm long; 2.5-4 cm in diameter; single fruit weight range from 20-29.3 g (mean 26.5 g), densely aculeate; glabrous; blunt at the apex.

Seeds: Elliptic; 4-5 × 2 mm; 1 mm thick; wingless.

Economic Importance

Tender leaves and young shoots are chopped, cooked, added coconut milk or groundnut paste and served with a staple in Tanzania. Also, tender leaves are dried in

the sun and then pounded into powder, soaked in hot water, boiled and stirred. The vegetable is then eaten with a staple. Leaves and fruits are used for fodder (Ruffo *et al*., 2002). Leaves and roots are pounded and used as a poultice to treat wounds. The juice from fruit is used as an antidote for poisoning, but it has to be supplemented by drinking fresh milk. The authors have not come across with any report of its use in the sites of collection.

Evidence from chromosome pairing and pollen fertility of hybrids shows that *Cucumis dipsaceus* is closest to *C. prophetarum* and *C. zeyheri* (Singh and Yadava, 1984). However, it is quite different from *C. prophetarum*. *C. prophetarum* has longitudinally striped fruits (dark green) with sparse recurved aculei whereas *C. dipsaceus* has light green unstriped fruits with abundant aculeation forming an outer layer of the fruit. It is easily distinguished from other members of the genus by its densely aculeate, uncurved-doom shaped stalk and blossom ends of fruits, robust growth.

Mature fruits have prolonged shelf life and are of ornamental value. A ripe fruit produce 360 to 498 seeds (mean 472.5) and a well grown plant produces 23 to 48 fruits indicating its potential to become a weed in the absence of natural enemies. However, being an exotic, its spread needs to be closely monitored, lest it becomes an obnoxious weed in cultivated fields.

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SHORT COMMUNICATION

Morpho-agronomic DUS Characterization of Scented *Harinakhuri* Rice Landrace of Coastal West Bengal

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The agro-morphological characterization of aromatic *Harinakhuri* rice landrace was done at Bidhan Chandra Krishi Viswavidyalaya (BCKV), Kalyani, West Bengal, India during *kharif* seasons of 2013, 2014 and 2015. This landrace had medium plant height (scale 5, 120-130 cm) with late maturity (scale 7, 140-150 days). The lemma and palea of matured grain was straw coloured with purple spot at tip and sterile lemma in purple colour. The grains were short (7.90 mm) and occasionally awned, which had low test weight (15.2 g). The white-coloured kernels belonged to medium-slender group (length 5.76 mm and width 2.00 mm), which had low amylose content (15.6%), high-medium gelatinization temperature (medium alkali value, score 3.66) and medium aroma (score 1.70).

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

The South Bengal region consisting of *gangetic* plains is home to thousands of fine and coarse-grained rice landraces, which include small and medium-grained non-Basmati aromatic types as well. Among such scented rice landraces, *Harinakhuri* is traditionally cultivated in *lower gangetic* plains, particularly in the coastal saline zone of West Bengal since a long time. The name '*Harinakhuri*' paddy is composed of two Bengali words, '*Harina*' and '*Khuri*'; where '*Harina*' meaning deer in English probably representing the native deltaic Sunderban region and *'khuri*' meaning locally made small earthen bowl in rural areas. The earliest record of '*Harinakhuri*' could be found as *aman* paddy of Midnapur district in 'A Statistical Account of Bengal: Districts of Midnapur and Hugli (including Howrah)' (Hunter, 1876), and thereafter as a Burdwan variety having special virtues suited for ordinary paddy lands in the 'Handbook of Agriculture' (Mukherji, 1901).

With quick adoption of high-yielding rice varieties during the last 4-5 decades, *Harinakhuri* like other landraces was almost eroded except in few villages of coastal saline tracts of West Bengal, where it is under localized cultivation. Farmers cultivate *Harinakhuri* paddy following traditional practices during *kharif* (wet) season mainly for their domestic consumption especially for preparations of dessert (*payes*), *khichuri* (pulse-mixed rice), popped rice, *chira* (flattened rice), etc.

In the present-day agricultural system, it has become a necessity to register important landraces as farmers' varieties under PPV&FRA, 2001 to strengthen the right of the farming community to conserve, cultivate and protect the same against unauthorized utilization by multi-national seed companies at national and global level. Hence, morpho-agronomic characterization of *Harinakhuri* rice landrace has been taken up as a legal evidence to push for its registration with the plant authority.

The seeds of *Harinakhuri* paddy landrace was collected from Sagar Krishnanagar Swami Vivekananda Youth Cultural Society, South 24 Parganas, West Bengal. 25 days old seedlings @ single plant/ hill were transplanted in an open puddled field with five replications in the field of 'C' Block Farm (22º59′N, 88º27′E and 9.75 m above mean sea level) at BCKV, Kalyani, Nadia, West Bengal, India during *kharif* (wet) season of 2013, 2014 and 2015. Each experimental unit consisted of 6-metre row length comprising 30 rows including row to row distance of 30 cm and plant to plant distance of 20 cm. The morphological and

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related characteristics of *Harinakhuri* rice landrace were determined following 'DUS Test Guidelines for Rice' of PPV&FRA, Government of India (PPV&FRA, 2007). The grain quality parameters like size and shape of grain and kernel, amylose content, alkali spreading value and aroma were determined at Aromatic Rice Laboratory, Department of Agronomy, BCKV, Mohanpur, Nadia.

The *Harinakhuri* rice landrace was adapted to rainfed medium-low land in the coastal saline region of West Bengal, whose characteristics are described in Table 1. The summary of traits for plant, stem, leaf, inflorescence, flower and grain are as follow to get a overall picture of the ideotype of the landrace *Harinakhuri*.

Plant: *Harinakhuri* rice belonged to long-duration type with late heading (scale 7, 113 days) and late maturity (scale 7, 143 days).

Stem: Plants were medium-statured with average stem length of 126.4 cm excluding panicle. There was weak anthocyanin colouration (scale 2) (Fig. 1) on nodes and internodes. The attitude of the culm could be categorised as semi-erect (scale 3) at booting stage.

Leaf: Leaves were long, narrow and green. The colour of basal leaf sheath was light purple (scale 2), while the intensity of green colour of the leaf as well as anthocyanin colouration on inner surface of leaf sheath was medium (scale 5). The average length and width of leaf blade were recorded as 64.6 mm and 9.9 mm, respectively. Split-type ligule (scale 3) and sickle-shaped auricle at leaf base were observed in the leaf. The attitude of the flag leaf was erect (scale 1) at early, and semi-erect (scale 3) at late observation.

Inflorescence: The length of panicle was categorized as medium (scale 5, 26.6 cm) with the curvature of the main axis as deflexed (scale 5) (Fig. 2). The landrace produced medium (scale 3, mean 12.2) and well-exerted panicles in the field. The colour of lemma and palea was straw background with purple spot at the tip (scale 8) at ripening stage. The variations in size, shape and colour of grain between *Radhatilak* and *Harinakhuri* was reported by Ghosh (Personal Communication, 2015), which could be considered as an important parameter to differentiate these two traditional scented rice landraces.

Flower: The landrace produced bi-sexual flowers including six yellow coloured anthers, and an ovary with purple coloured feathery stigma.

Grain: The grains were short in size (mean length 7.90 mm and width 2.30 mm) and occasionally awned (Fig. 3). The weight of 1000 fully-developed grains was low (15.2 g). The colour of lemma and palea was straw background with purple spot at the tip (scale 8), while that of sterile lemma was purple (scale 4).

Fig. 1. Nodes and internodes of '*Harinakhuri*'

Fig. 2. Panicle of '*Harinakhuri*'

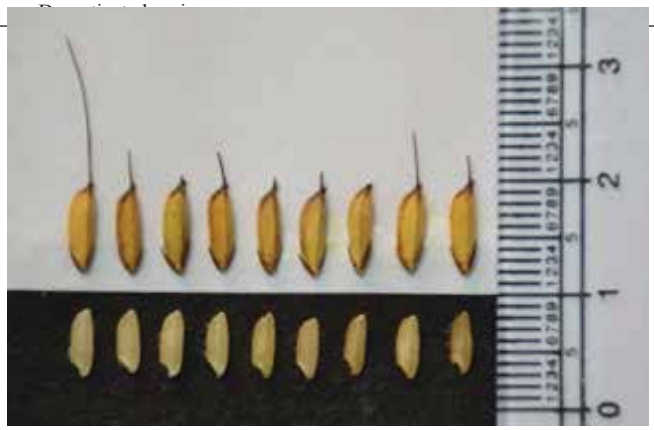
The kernels were medium slender in shape (length 5.76 mm and width 2.00 mm) and white in colour (Fig. 3), which had low amylose content (15.6%), high-medium gelatinization temperature (medium alkali spreading value, score 3.66) (Fig. 4) and medium aroma (score 1.70).

Acknowledgement

The authors are thankful to Sagar Krishnanagar Swami Vivekananda Youth Cultural Society, South 24 Parganas for sparing the seeds of *Harinakhuri* rice landrace for DUS characterization. The technical and financial assistance from RKVY Project 'Bengal Aromatic Rice' sanctioned by the Department of Agricultural Marketing, Government of West Bengal is gratefully acknowledged.

Table 1. DUS characters of *Harinkhuri* rice landrace of West Bengal

S. No.	Characteristics	Scale	Remarks / Measured values etc.
1	Coleoptile: colour	1	White
2	Basal leaf sheath colour	2	Light purple
3	Leaf: Intensity of green colour	5	Medium
4	Leaf: anthocyanin colouration	1	Absent
5	Leaf sheath: anthocyanin colouration	9	Present (inner side of leaf sheath)
6	Leaf sheath: intensity of anthocyanin colouration	5	Medium
7	Leaf: pubescence of blade surface	5	Medium
8	Leaf: auricles	9	Present
9	Leaf: anthocyanin colourations of auricles	1	Colourless
10	Leaf: collar	9	Present
11	Leaf: anthocyanin colouration of collar	1	Absent
12	Leaf: ligule	9	Present
13	Leaf: shape of ligules	3	Split
14	Leaf: colour of ligule	1	Green
15	Leaf: length of blade	7	Long (64.6 cm)
16	Leaf: width of blade	3	Narrow (9.9 mm)
17	Culm: attitude	3	Semi-erect
18	Time of heading (50% of plants with panicles)	7	Late (113 days)
19	Flag leaf attitude of blade (early observation)	1	Erect
20	Spikelet: density of pubescence of lemma	5	Medium
21	Male sterility	1	Absent
22	Lemma: anthocyanin colouration of keel	5	Medium
23	Lemma: anthocyanin colouration of area below apex	3	Weak
24	Lemma: anthocyanin colouration of apex	7	Strong
25	Spikelet: colour of stigma	5	Purple
26	Stem: length (excluding panicle)	5	Medium (126.4 cm)
27	Stem: anthocyanin coloration of nodes	9	Present
28	Stem: intensity of anthocyanin colouration of nodes	3	Weak
29	Stem: anthocyanin colouration of internodes	9	Present (weak)
30	Panicle: length of main axis	5	Medium (26.6 cm)
31	Flag leaf: attitude of blade (late observation)	3	Semi-erect
32	Panicle: curvature of main axis	5	Deflexed
33	Panicle: number per plant	5	Medium (12.2)
34	Spikelet: colour of tip of lemma	5	Purple
35	Lemma & palea : colour	8	Purple spot at tip on straw background
36	Panicle: awns	9	Present
37	Panicle: colour of awns (late observation)	8	Purple
38	Panicle: length of largest awn	5	Medium (12.0 mm)
39	Panicle: distribution of awns	3	Upper half only
40	Panicle: presence of secondary branching	9	Present
41	Panicle: secondary branches	2	Strong
42	Panicle: attitude of branches	5	Semi-erect
43	Panicle: exertion	7	Well exerted
44	Time of maturity	7	Late (143 days)
45	Leaf: senescence	5	Medium
46	Sterile lemma: colour	4	Purple
47	Grains: weight of 1000 fully developed grains	3	Low (15.2 g)
48	Grain: length	3	Short (7.90 mm)
49	Grain: width	2	Narrow (2.30 mm)
50	Decorticated grain: length	3	Medium (5.76 mm)
51	Decorticated grain: width	5	Medium (2.00 mm)
52	Decorticated grain shape	3	Medium-slender
53	Decorticated grain: colour	1	White
54	Endosperm: presence of amylose	9	Present
55	Endosperm: content of amylose	3	Low (15.6%)
56	Gelatinization temperature through alkali spreading value	5	High-medium (Alkali value /score 3.66)



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Plant Germplasm Registration Notice*

The Plant Germplasm Registration Committee of ICAR in its XXXVIIIth meeting held on June 2nd, 2018 at the National Bureau of Plant Genetic Resources, New Delhi approved the registration of following 46 germplasm lines out of 54 proposals considered. The information on registered germplasm is published with the purpose to disseminate the informationto respective breeders for utilization of these genetic stocks in their crop improvement programmes. Upon request, the developer(s)/author(s) is/are obliged to distribute the material for crop improvement programme of National Agricultural Research System.

1. RP Bio Patho-2 (IC0626002; INGR18001), Rice (*Oryza sativa)* with Broad-Spectrum Resistance for Leaf Blast (Pi-54) and Bacterial Blight (Xa21 & xa13). Present in the Elite Genetic Background of Improved Samba Mahsuri and 94.9% Recurrent Parent Genome Recovery. Moderate Resistance for Neck Blast, Sheath Blight, Sheath Rot and Brown Spot besides Blast Resistance.

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The Near isogenic line (NIL), RP Bio Patho-2 (RP Bio 5864 Patho-2-18-5) was developed at Indian Institute of Rice Research (ICAR-IIRR) by making a cross between Improved Samba Mahsuri as a recurrent parent with Tetep as a donor (*Pi-54*) through Marker assisted backcross breeding. This NIL contains two bacterial blight resistance genes *i.e.Xa21* and *xa13* from Improved Samba Mahsuri and one blast resistance gene *Pi-54* from Tetep.

Associated Characters and Cultivation Practices

Phenotyping for blast and bacterial blight resistance: RP Bio Patho-2 was screened for blast and bacterial blight resistance in Uniform blast nursery (artificial screening method) and leaf clip inoculation method (pot culture) respectively during the years 2009 & 2010. The result showed a mean resistance score of 1.0 for leaf blast and 1.0 for bacterial blight disease.

Phenotyping for multiple disease resistance in various screening nursery trials: RP Bio Patho-2was screened under donor screening nursery of AICRIP 2011 to 2016 in replication for multiple disease resistance. The result showed that the RP Bio Patho-2 has promising resistance for leaf blast with susceptibility index (SI) of 4.5, 3.4, 2.9, 3.3, 3.6 & 3.3 respectively. Further the line was screened under National Screening Nursery of AICRIP 2015 to 2016 in replication for multiple disease resistance.

The result showed that the RP Bio Patho-2 has promising resistance for leaf blast with susceptibility index (SI) of 2.8 & 3.0 and bacterial blight with susceptibility index (SI) of 3.7 & 3.6 during 2015 and 2016 respectively, whereas recurrent parent Improved Samba Mahsuri showed mean SI of 5.6 and 7.5 (for leaf blast), 3.0 and 3.0 (for BB) during 2015 and 2016 respectively.

The susceptible checks for leaf blast are HR-12 and TN_1 showed mean susceptibility index (SI) of 7.8, 8.5 and 5.0, 5.7 during 2015 & 2016 and susceptible checks for bacterial blight are TN1 and BPT5204 showed mean susceptibility index (SI) of 7.5, 8.3 and 6.0, 7.0 during 2015 and 2016 respectively. Based on various screening nursery trials conducted across country it is confirmed that NIL (RP Bio Patho-2) showed promising resistance against blast and bacterial blight diseases

Genotyping: The RP Bio Patho-2 has been checked for the presence of blast resistance gene (*Pi-54*) and bacterial blight resistance genes (*Xa21+xa13*) using linked markers Pi-54 MAS (*Pi-54*), pTA248 (*Xa21*) and xa13 promoter (*xa13*). Back ground selection with 118

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SSR markers revealed that, this line (RP Bio Patho-2) has 94.9 % recurrent parent genome recovery.

Morpho-agronomic characteristics: The Identified NIL (RP Bio Patho-2)was evaluated for their agronomic and grain quality traits as compared to the recurrent parent Improved Samba Mahsuri at the field farm Indian Institute of Rice Research (ICAR-IIRR), Hyderabad during *kharif* season with two replications. RP Bio Patho-2showed (13.5%) yield advantage over Improved Samba Mahsuri. The Identified NIL (RP Bio Patho-2) showed excellent blast and bacterial blight resistance, yet it is 10 days early maturing than Improved Samba Mahsuri and shorter than recurrent parent (Improved Samba Mahsuri).

This line could be used as elite genetic stock for resistance to rice blast and bacterial blight disease. It could be involved as donor parent for developing resistance varieties for rice blast and bacterial blight disease.

Important Agro-morphological Characteristics of RPBioPatho-2 & Improved Samba Mahsuri

Character	RPBioPatho-2 (NIL)	Improved Samba Mahsuri (RP)
Spad values	39.6	36.0
No.of tillers per plant	21	18
Plant height at maturity	80.0 cm	89
No.of panicles per plant	19	15
Time of heading	90 days (Medium duration)	110
Basal leaf sheath Color	Green	Green
Stem Length	Very short	Very short
Decorticated grain type	Medium Slender	Medium Slender
Decorticated grain color	White	White
Decorticated grain Aroma	Absent	Absent
Resistance genes	*Pi-54+Xa21+xa13*	*Xa21+xa13+xa5*
Resistance to	Rice blast isolate	Susceptible
Resistance to	Rice BB isolate	Resistant

2. RPBio4918-228S (IC0626001; INGR18002), Rice (*Oryza sativa)* Introgression Line Resistant to Brown Plant Hopper (BPH) *Nilaparvata lugens*. Possesses High Resistance in Vegetative and Reproductive Stages. Present in Background of Popular Commercial Variety Swarna (MTU 7029).

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Rice is the principal food crop in India and is grown in an area of ~ 44 Million hectares and with a production of ~100 Million metric tonnes. Rice production is limited by various biotic and abiotic stresses of which insects are estimated to cause 21-30% yield loss. Among the insect pests, rice brown planthopper (BPH) *Nilaparvata lugens* (Stal) (Delphacidae: Hemiptera) is very serious pest which causes severe yield losses upto 100% under severe hopper burn situations.

The large scale cultivation of dwarf, early maturing, photoperiod insensitive, high tillering and high yielding rice cultivars coupled with the increased use of nitrogenous fertilizers had become congenial for brown planthopper infestation in farmers' fields. All growth stages of rice plant right from seedling to maturity stage are affected. Nymphs and adults stay at the base of the plant and damage the crop by sucking phloem sap which results in yellowing of leaves, reduced tillers and plant height, unfilled grains and death of the plant. Damage is more severe in reproductive stage of crop wherein round yellow patches appear in the field which later turn brownish due to drying of plants called as hopper burn. The patches of infestation may spread in the entire field, coalesce together and in severe cases complete destruction of crop occurs.

Since 1970's several devastating BPH epidemics have been reported and now outbreaks of brown planthopper have become common and severe. Many insecticides are suggested for the control of this pest, but application of these chemicals interrupts the natural balance of rice ecosystem.Cultivation of resistant varieties is the better and environmentally safe substitute. Hence, breeding programme for development of BPH resistant varieties with different modes of host plant resistance

(such as antixenosis, antibiosis and tolerance) is extremely important.

More than 30 genes and QTLs have been identified from cultivated and wild species introgression lines. Most of the genes for BPH resistance identified so far area gainst biotypes 1, 2 and 3 and only few are resistant against biotype 4, which could be one of the reason for their ineffectiveness against BPH biotype which ismost destructive and is distributed over the Indian sub-continent.Many of the donors which showed stable resistance across the biotypes have one or two major genes along with QTLs associated with resistance. Hence,it is important to identify new donors, novel genes/major QTLs effective against BPH biotype4 to pyramid them for stable resistance.The genes from wild rices are reported to be robust and stable.

The wild species of *Oryza* representing AA, BB, CC, BBCC, CCDD, EE, FF, GG and HHJJ genomes are a rich reservoir of useful genes resistant to major biotic and abiotic stresses, more adapted to adverse and changing environmental conditions. The genetic variability for some of these stresses is restricted in the cultivated rice germplasm. Moreover, changes in insect biotypes are an enduring threat to increased rice production. There is thus a critical need to broaden the rice gene pool by introgressing genes for such traits from diverse sources. Therefore, it is important to discover these valuable genes hidden in wild rice species and use them in breeding programmes to develop resistant varieties. However, low crossability and limited recombination between chromosomes of cultivated and wild species limit the transfer of such genes. In addition, genes from wild rices are often linked with genes governing undesirable traits. So, development of advance back cross introgression lines is very essential, for selective transfer of only preferable genes and to avoid linkage drag. Creation of introgression lines using cultivated rice as recipient and wild rice as the donor is the most durable approach to explore primitive and broad genetic resources in rice breeding.

Most of the genes identified are based on the reaction at seedling stage but the maximum crop damage occurs at tillering and reproductive stage in farmer's field.Hence, it is necessary to know the effectiveness of donors/genes against BPH at later plant growth stages. Therefore, the present attempt was made to identify donors for BPH resistance from introgression lines derived from *O.nivara* at seedling stage and also at maximum tillering and reproductive stage.

Process of development of RPBio 4918-228S: With this background a set of backcross intogression lines were developed from one cross *viz.*, Swarna/ *O. nivara* (*accession no.* IRGC 81848 S) following single seed descent method. Swarna is a popular commercial variety susceptible to brown planthopper; *O. nivara* (*accession no.* IRGC 81848 S) is a wild rice accession with AA genome. A backcross strategy was followed to develop the line RP Bio 4918-228S. A single plant of *O. nivara* (*accession no.* IRGC 81848 S) was used as a male parent and crossed to Swarna to generate F1 plants. These F1 plants derived from the cross between Swarna and *O. nivara* (IRGC 81848) were backcrossed to Swarna to produce BC1F1 plants. These BC1F1 plants were again backcrossed to Swarna to develop the BC2F1 generation. These BC2F1 were selfed till five generations to produce BC_2F_6 backcross introgression lines (BILs). 250 number of BILs were selected which were more like Swarna phenotypically and were screened for BPH resistance in the greenhouse and field conditions, out of which RPBio4918-228S and five more BILs were found resistant to BPH.

Phenotyping for brown planthopper resistance: 250 number of BC_2F_6 backcross introgression lines (BILs) were screened for brown planthopper resistance by adopting mass screening test under controlled greenhouse conditions at Indian Institute of Rice Research. The method involved infestation of 12 day old young seedlings of the BILs grown in screening trays with BPH nymphs along with resistant (PTB 33) and susceptible (TN1) checks. When more than 90 per cent of TN1 plants were killed, the BILs were scored for the damage reaction, based on a 0-9 scale of Standard Evaluation System (SES). The lines were screened for three seasons in the DRR greenhouse during 2009-2010. In the repeated screening in the greenhouse, out of the 250 BILs from Swarna/O. *nivara* screened, six BILs were found to be resistant and RP BIO 4918 228S was one of the BILs with brown plant hopper resistance with damage score of 1.2. Swarna and the susceptible check (TN1) recorded a damage score of 9, the resistant check PTB 33 recorded a damage score of 1.2 (Jhansi Lakshmi *et al*., 2010). The RP bio 4918-228S was screened along with germplasm accessions in 2012 in DRR greenhouse and was found to be resistant with a damage score of 1.4 (Akanksha *et al.,* 2017).

Table 1. Reaction of RP Bio 4918-228S to BPH under natural and artificial infestation during 2009-2014

Year/located on		2009	2012 MRST								2013 MRST							2014 MRST					2012
		DRR	DRR	CBT	MTU	RPR	LDN	CTC	PNR	GGV	DRR	CBT	MND	LDN	GGV	PNR	DRR	LDN	CBT	MTU	GGV	DRR
Greenhouse	RP bio 4918-228S	1.2	2.3	3.0	5	0	1.1	1.0			3.4	5.0	3.0	1.3		1.7	2.5	3.2	5.0			1.4
	PTB 33	1.2									1.2	3	1.0	1.3		2.0	1.8		5.0			
	TN1	9	9	9	9	9	8	9			9	9	9	9		9	9	9	9			
	Swarna	9																				
Field	RP bio 4918-228S								4.8/ hill	1.0					3.0					3.0	1.0	
	PTB 33														0							
	TN1								5/hill	3.0					5					9.0	9.0	

DRR: Directorate of Rice Research, CBT: Coimbatore, MND: Mandya, RPR: Raipur, LDN: Ludhiana, MDR: Madurai and GGV: Gangavathi, PNR: Pantnagar, CTC: Cuttack, MTU: Maruteru.

Table 2. Morphological characters of RP Bio 4918-228S and Swarna

Name of the variety	:	RP bio 4918-228S	Swarna
Plant height	:	84.6	101.27
Plant type	:	dwarf	Semi-swarf
No. of tillers /plant	:	12	11.6
Days to 50% flowering	:	114	119.2
Seed to seed duration	:	145	148.15
Panicle type	:	compact	compact
No. of panicles /plant		11	10.64
Panicle exsertion	:	Well exserted	Well exserted
Panicle weight		2.4	2.65
Awning	:	present	absent
Yield/plant (g)		13.8	18.57
1000 grain weight	:	13.0 g	15.1 g
Kernel length	:	7.308333	6.3mm
Kernel breadth	:	2.116667	2.1mm
L/B ratio	:	3.46	3
Grain type	:	Medium slender	Medium
Kernel appearance	:	Very occasionally chalky	Very occasionally chalky
Milling recovery	:	63%	68.6%
Head rice recovery (HRR)	:	45%	56.3%
Alkali spreading value (ASV)	:	4	5
Amylose content (AC)	:	24.8	26.5
Gel consistency	:	24	43
Yield potential		4.6 t/ha	5.5-6.0 t/ha

This BIL RP Bio 4918-228S was screened in hotspot locations for brown planthopper resistance in the multilocation testing in 2012 in Multiple Resistant Screening Trial (MRST) and performed better and promising in 8 out of 8 tests/locations and recorded damage score of <3 in six locations and 5 in one location (total 8 *viz.,* DRR, Coimbatore, Cuttack, Raipur, Ludhiana, Maruteru, Pantnagar and Gangavathi). The susceptible check TN1 recorded a damage score of 9 and the resistant check PTB 33 recorded a DS of 1-5 (Ref: Screening nurseries 2012 AICRIP DRR and Progress report 2012 AICRIP DRR). In 2013, RP Bio 4918-228S was retested in Multiple Resistant Screening Trial (MRST) and was found to be resistant and recorded a damage score of <3 at 4 locations viz., Pantnagar, Ludhiana, Mandya and Gangavathi and <5 in two locations *viz.,* DRR and Coimbatore. The susceptible check TN1 recorded a damage score of 9 (Ref: Screening nurseries Entomology Pathology AICRIP 2013, DRR AICRIP Progress report 2013, DRR Annual report 2013-14). In 2014, RP bio 4918-228S was retested in Multiple Resistance Screening Trial (MRST) and was found to be resistant in five locations viz., DRR, Maruteru and Gangavathi with a damage score of <3 and in Ludhiana and Coimbatore with a damage score of <5. The susceptible check TN1 recorded a damage score of 9 (Ref: DRR Screening nurseries 2014 AICRIP DRR and DRR AICRIP Progress report 2014, Proceedings of the 49th Annual Rice Research Group Meetings, 2014, Proceedings of the 50th Annual Rice Research Group Meetings, 2015, DRR Annual report 2014-15). In the 49th Annual Rice Research Entomology Group Meetings, 2014, RP Bio 4918- 228S was identified as resistant to brown planthopper and recommended for registration with NBPGR (Ref. Proceedings of the 49th Annual Rice Research Group Meetings, 2014). In the 50th Annual Rice Research Entomology Group Meetings, 2015, RP Bio 4918-228S was identified as resistant to brown planthopper (Ref. Proceedings of the 49th Annual Rice Research Group Meetings, 2014).

Simultaneously, RP Bio 4918-228S was evaluated for agronomic and quality traits. It is an intermediate, mid duration culture (145 days) possessing medium slender grains. Qualitywise, it recorded medium head

rice recovery (HRR) of 45% and intermediate amylose content 24.8% (Table 2).

This line could be used as elite genetic stock for resistance to brown planthopper (BPH), *Nilaparvata lugens*. It could be involved as donor parent in hybridization program for developing resistant varieties for brown planthopper**.**

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3. NH686 (RP Bio 5477-NH686) (IC0626284; INGR18003), a Rice (*Oryza sativa*) Germplasm Tolerant to Low P Condition. Dark Green Leaves and Grain Yield Six fold more than N22 under Low P Condition. Early Duration, Broad Leaves and Medium Bold Grains.

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NH686 or SM686 is Ethyl Methane Sulphonate induced mutant of an upland rice cultivar Nagina 22 (N22). N22 is a well known early maturing, drought and heat tolerant aus variety giving yield of 12 to 15 g/plant. Mutants are valuable resources for causal gene discovery and use in crop improvement. The mutant was developed as a part of DBT funded project "Characterization and use of EMS induced mutants of rice variety N22 for yield, drought and phosphorus use efficiency" at ICAR-IIRR, Hyderabad. The M2 mutants were first screened along with wild type N22 and check varieties Rasi and Kasalath in low P field conditions (2Kg/ha P) and NH686 was identified as the best low-P tolerant mutant in terms of tiller number and grain yield. The grain yield of NH686 and N22 in normal soil is 23g and 14g per plant while in low P condition it was 7g and 1 g/plant respectively. Thus, NH686 is tolerant to low P condition showing 6 fold more grain yield than N22.

Table 1. AICRIP data of NH686

IET No. 23834 (RP Bio5477-NH686)				
			Concentration (ppm)	
Year	Number of locations	Grain yield (Kg/ha)	Zn	Fe
2013	12	3835	17.27	9.27
2014	14	3535	18.68	3.19
2015	19	3450	19.0	3.7

Table 2. Important agro-morphological characteristics of NH686

Characteristics	
Days to flowering (50%)	76
Plant height (cm)	70
Number of tillers	24
Plant type	Stay green
Flag leaf	Erect
Panicle	Well exerted
Stem thickness	Medium
Grain shape	Medium bold
Stem colour at nodes	Absent
Number of grains/ panicle	150
Spikelet fertility (%)	96
Yield / plant (g)	23

Apart from tolerance to low P condition, NH686 is tolerant to multiple abiotic stresses and also has high Zn concentration in polished rice as evaluated under AICRIP biofortification trial for 3 years (Table 1). The mutant is stable and breeding true as it was tested in normal (60Kg/ha) and low P (2Kg/ha) soil till M9 generation. NH686 has highest grain yield and per day productivity among mid early lines. Additionally, the mutant shows heat tolerance characteristics. The agro-morphological characteristics of NH686 evaluated under normal soil are shown in Table 2.

NH686 is a stable, elite rice genetic stock tolerant to low P condition in field. It can be used as a donor parent or as a check when screening for rice varieties with tolerance to low P.

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4. DBW-EMS 98 (IC0625990; INGR18004), a Wheat (*Triticum aestivum*) Chlorophyll Deficiency Mutant.

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Fast evolving field of functional genomics calls for use of effective tools and techniques for understanding the mechanisms underlying traits of importance. Study of functional basis of gene function through study of trait mutants is an effective tool in functional genomics. A mutant population of wheat cultivar DPW621-50 derived through 0.7 per cent Ethyl Methanesulfonate (EMS) treatment was screened for mutant phenotypes three stations of ICAR-IIWBR at Karnal, Hisar, Dalang Maidan during 2014-2016 (Table1).

Large numbers of altered phenotypes were observed along with variation for yield parameters. Lines showing stable altered phenotypes for various traits were isolated. Efforts are underway to study the genetics of these mutants. One of the mutants DBW-EMS98 is deficient in chlorophyll content at seedling stage and remained pale green throghout its life cycle compared to its wild type parent DPW 621-50. Physiological characterization of this mutant has showed that it consistently showed lower total chlorophyll content, Chlorophyll a&b,

NDVI, photosynthesis and lower stomatal conductance throughout its life cycle compared to its wild type parent DPW 621-50 (Table 2). Though the mutant has recovered greenness to some extent compared to seedling stage still the significant difference was seen between parent and mutant throughout its life cycle.

Table 1. Confirmation of mutant traits at three sites over two years

Year	Location	Line	Chlorophyll mutant
Year 1 (2014-15)	Karnal	EMS-98	Yes
	Hisar	EMS-98	Yes
	Dalang Maidan	EMS-98	Yes
Year 2 (2015-16)	Karnal	EMS-98	Yes
	Hisar	EMS-98	Yes
	Dalang Maidan	EMS-98	Yes

Thus, DBW-EMS98 is a stable mutant wheat line deficient in chlorophyll at seedling stage and will act as a valuable source in understanding functional basis of genes and pathways involved in chlorophyll synthesis and photosynthesis. This chlorophyll mutant may serve as an effective tool for functional genomics studies of wheat photosynthesis**.**

Table 2. Comparison of mutant and parent for different chlorophyll associated traits

Trait	DBW-EMS 98	DPW 621-50
Assimilation rate (µmol m^{-2} s^{-1})	8.3	26.5
Stomatal conductance (mmol m^{-2} s^{-1})	256.4	805.5
Chlorophyll Content Index	24.26	44.54
Total chlorophyll (mg g^{-1})	0.27	0.59
Chlorophyll a(mg g^{-1})	0.15	0.34
Chlorophyll b(mg g^{-1})	0.12	0.25
Normalized difference vegetation index (NDVI)	0.16	0.52

5. PHS 1108 (IC0624499; INGR18005), a Wheat (*Triticum aestivum*) Germplasm with High Protein Content, Bold Seeds and High Manganese Content.

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Indian wheat production brought food self-sufficiency ensuring the national food security. Now-a-days the quality of wheat grains and products is becoming important criteria for consumer's preferences. Wheat is recognized primarily as a source of energy and protein in human diets (Garvin *et al., 2006*). The micronutrients iron, zinc, copper and manganese (Pomeranz*, 1988;* Doisy, *1973*) are important components of nutritional quality in wheat.

The proposed genotype PHS 1108, as source of high protein content with bold seeds, was developed from the cross CMH83.2517/GW190 following pedigree method. Parental line CMH83.2517was obtained from CIMMYT nursery INDV 96. PHS 1108 was contributed in NGSN and evaluated for three seasons (2011-12 to 2013-14) along with check varieties DBW 17, Sonalika and durum variety HI 8498 at more than 25 centres across the country. It was also evaluated for quality traits including nutritional quality parameters.

The results (Table 1) indicated 9.6% superiority of PHS 1108 for protein content over best check variety DBW 17. PHS 1108 has protein content of 14.8% ranging from 14.3-15.8% during three years of evaluation. This genotype also has high 1000-grain weight (55g) with range of 52-57g which indicated higher content of protein per unit grain weight. In addition, the manganese (Mn) content of 44.2 ppm (mg/kg) was 36.4% higher as compared to best check variety Sonalika. The manganese content in PHS 1108 is even higher than the earlier reports of manganese availability in wheat grains to the tune of 25.23+3.86 mg/kg (Bawiec *et al*., 2014; NDL, 2016). PHS 1108 also has longer spikes (12 cm) and early in heading (73 days) and maturity (123 days) as compared to all the check varieties.

PHS 1108 is a promising source for high protein content with bolder seeds (high 1000-grains wt.) which may be useful as donor in wheat improvement programmes. High manganese content in grains is an additional feature for nutritional improvement. PHS 1108

Table 1. Performance of PHS 1108 in National Genetic Stock Nursery

Traits	Year	Entry	Check varieties		
		PHS 1108	DBW 17	Sonalika	HI 8498 (d)
Protein content (%)	2011-12	14.3	12.5	12.2	12.6
	2012-13	15.8	14.6	14.4	14.6
	2013-14	14.4	13.3	13.5	12.9
	Mean	14.8	13.5	13.4	13.4
1000-grains weight (g)	2011-12	57	39	44	50
	2012-13	52	39	43	48
	2013-14	55	41	43	48
	Mean	55	40	43	49
Manganese (ppm)	2011-12	44.2	30.2	32.4	25.7
Zinc content (ppm)	2011-12	43.5	29.6	29.7	27.1
	2012-13	38.4	31.4	31.6	36.7
	2013-14	36.7	36.4	35.3	37.1
	Mean	39.5	32.5	32.2	33.6
Days to heading	2011-12	74	88	79	84
	2012-13	74	86	76	83
	2013-14	71	82	71	79
	Mean	73	85	75	82
Days to maturity	2011-12	123	128	125	128
	2012-13	124	130	127	129
	2013-14	123	128	123	127
	Mean	123	129	125	128
Spike length (cm)	2011-12	12	9	9	8
	2012-13	12	9	9	8
	2013-14	12	9	9	7
	Mean	12	9	9	8

would add further un-exploited genetic variability for yield components and mineral nutrient content.

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6. DBW 129 (IC0624497; INGR18006), a Wheat (*Triticum aestivum*) Germplasm with Multiple Disease and Pest Resistance (Stripe, Leaf & Leaf Blight, Karnal Bunt, Flag Smut, Powdery Mildew and Shoot Fly).

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The disease and pest resistance is crucial to realize high yield potential in any variety under commercial cultivation. This necessitates development of high yielding genotypes with resistance to most of the prevailing diseases. The efforts made under warmer area programme of the ICAR-IIWBR, Karnal has resulted in identification of germplasm line DBW 129 which showed promise for multiple diseases and pest resistance and is being proposed for registration as genetic stock.

The proposed genotype DBW 129 (PFAU/ Milan/5/ Chen/*Ae. squarrosa* (Taus)//BCN/3/VEE#7 / BOW/4/Pastor) was developed by pedigree method at IIWBR, Karnal. It was cross of 21st ESWYT-44 (PFAU/ MILAN) used as female parent and 34th IBWSN-295 (Chen/*Ae. squarrosa* (Taus) //BCN/3/VEE#7/BOW/4/ Pastor) as male parent. The germplasm line DBW 129 was initially contributed in NIVT-5A in 2012-13 for evaluation under restricted irrigation conditions (Anonymous, 2013a). Based on its yield superiority and disease resistance, it was promoted to Advance Varietal Trial of the NWPZ in 2013-14 (Anonymous, 2014a). During its testing for yield under coordinated trials, it was also evaluated for disease resistance under artificial epiphytotic conditions at multi-locations in the Plant Pathological Screening Nursery (PPSN) during 2012-13 and 2013-14. Thereafter, it was evaluated in Elite Plant Pathological Screening Nursery (EPPSN), Multiple Disease Screening Nursery (MDSN) and Multiple Pest Screening Nursery (MPSN) for disease and insect pest resistance. DBW 129 was found promising for multiple diseases and pest resistance in all these nurseries.

The PPSN was conducted at 13 locations for screening against stripe rust (10 locations) and leaf rust (8 locations) during 2012-13 to 2013-14 in which DBW 129 showed highly resistant reactions to stripe (ACI: 3.7) and leaf (ACI: 2.5) rusts (Anonymous, 2013b, 2014b). Based on its performance in PPSN (Table 1), it was promoted to EPPSN during 2014-15 conducted at 13 locations (Anonymous, 2015) in which it again showed highly resistant reactions (Table.1) to all three rusts (ACI of 3.1 to yellow rust, 0.0 to leaf rust and 3.1 to stem rust).

Table 1. Response of DBW 129 against rusts in PPSN & EPPSN

Genotypes	Rust diseases			
	Stripe (North)	Leaf (North)	Leaf (South)	Stem (South)
	HS (*ACI*)	HS (*ACI*)	HS (*ACI*)	HS (*ACI*)
PPSN (2012-13 & 2013-14)				
DBW 129	20S (3.7)	20S (2.5)	-	-
WH1080 (C)	40S (18.2)	30S (9.9)	-	-
HD 3043 (C)	60S (20.2)	20MS(5.0)	-	-
PBW 644 (C)	80S (37.3)	20S (4.4)	-	-
EPPSN (2014-15)				
DBW 129	5S (3.1)	tR(0.0)	tR (0.0)	5S (3.1)
Infector (C)	90S (75.0)	100S (63.3)	60S (41.3)	100S (55.0)
MDSN (2015-16)				
DBW 129	5S (1.0)	tR(0.0)	5MS (1.6)	5X (1.7)
PBW 343(C)	80S (43.0)	20S(13.3)	60S (34.6)	5S(3.0)
Raj 4015(C)	40S (18.0)	20S(6.6)	60S (20.3)	40S(30.0)
Sonalika(C)	100S (48.0)	60S (20.0)	80S (55.0)	30S (15.1)

HS- Highest score ; ACI- Average coefficient of infection

Further, it was evaluated in MDSN at 15 locations during 2015-16 for disease response against rusts, leaf blight, Karnal bunt, flag smut and powdery mildew (Anonymous, 2016). DBW129 showed highly resistant response to all the three rusts, leaf blight, Karnal bunt, powdery mildew and flag smut (Table 2). The screening of DBW 129 against different insect pests in MPSN during 2015-16 also showed resistance to shoot fly with very low incidence of 7.81% and falling in the resistant category (Anonymous, 2016).

In addition, this germplasm showed mean yield of 47.2q/ha with 2.4% superiority over check WH 1080 with yield potential of 67.4q/ha under limited irrigation conditions (Table.3).

DBW 129 is a promising source for resistance to multiple diseases and pests in wheat with high yield under restricted irrigation conditions. These features make it very useful for wheat improvement programmes. DBW 129 would add further un-exploited genetic variability for yield component and resistance to diseases and insect pests.

Table 2. Performance of DBW 129 in MDSN & MPSN (2015-16)

Genotypes	Diseases in MDSN				Insect-pests in MPSN	
	Leaf blight (dd)	Karnal Bunt (%)	Powdery mildew (0-9)	Flag smut (%)	Shoot fly incidence (%)	Brown wheat mite(No./cm^2)
	Av (HS)	HS	Av (HS)	Av (HS)	Av (HS)	Av (HS)
DBW 129	35 (57)	5.0	2 (4)	3.3 (10.0)	7.8 (15.4)	10.3 (10.6)
Checks						
Raj 4015 for LB	67 (78)	18.0	4 (6)	15.6(20.0)	-	-
PBW 343 for PM	34 (45)	21.0	6(9)	42.5(76.5)	-	-
Sonalika for SF	68 (79)	17.0	-	-	24.6 (66.7)	-
IWP 72 for BWM	-	-	-	-	-	19.0 (22.3)

Av- Average score; HS- Highest score

Table 3. Performance of DBW 129 for yield in coordinated trials (2012-13 to 2013-14)

Traits	Entry	Check Varieties		
	DBW 129	WH 1080	PBW 644	HD 3043
Mean Yield (q/ha)	47.2	46.1	45.7	45.2
Yield potential (q/ha)	67.4	59.3	57.5	61.8

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7. DBW 246 (IC0625998; INGR18007), a Wheat (*Triticum aestivum*) Germplasm Highly Resistant to Yellow Rust.

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Yellow rust caused by *Puccinia striiformis* is one the important disease of the wheat crop grown in Northern India and the fungus causes significant yield losses to the wheat productivity in the region. The pathogen can cause 100% yield loss, but damage generally ranges from 10-70% in a single field depending upon the stage, duration and severity of infection during the growing season and susceptibility of the cultivar. Keeping pace with the varietal improvement for the rust resistance, the yellow rust pathogens evolve very fast into more virulent ones.A resistant cultivar becomes susceptible when a new pathotype evolves. During the year 2016, seven pathotypes were identified for the first time and designated as 46S117, 110S119, 238S119, 110S84, 110S247, 7S0 and 6S0 and they would change the resistance pattern of wheat cultivars in northern India.

Winter wheats act as source for biotic resistance in wheat. DBW 246 is a spring wheat line developed from winter x spring wheat hybridization (UP2556//ID13.1/ MLT/3/Danphe) and it has been selected for yellow rust resistance during its course of development. In 2016-17 it was tested in the coordinated trials and the genotype showed resistance against yellow rust during the course of evaluation.

In the field reactions, the genotype showed resistance response at all the locations under artificial epipytotic conditions. (Table 1) The highest reaction was tR (ACI : 0.6) recorded at Malan centre while at rest of the locations it was free from the disease. The released cultivars DBW 88, WH 1105 and HD 2967 showed high susceptible reaction against the yellow rust. In Seedling Resistance Test (SRT) carried out at IIWBR-RS Flowerdale, Shimla, DBW246 has shown resistant reactions against all the stripe rust races including the recently identified virulent races viz., 238S119, 110S119, 110S84 and 110S247 (Table 2)

Apart from highly resistant to yellow rust, the agronomic features of DBW 246 like heading duration (91 days), maturity (138 days), plant height (95 cm) thousand grains weight (38 g), easy threshability and amber coloured grains makes it suitable to be used in future hybridization programmes. This line would be useful for bringing diversity in the breeding material for yellow rust resistance.

Table1. Yellow rust reactions of DBW 246 and cultivars at hot spots

Entry	Hisar	Jammu	P'nagar	Durgapura	Karnal	Ludhiana	Delhi	Gurdaspur	Malan	Khudwani	HS	ACI
DBW 246	0	0	0	0	0	0	0	0	tR	0	tR	0.6
DBW88 (C)	10S	40S	20S	60S	80S	60S	40S	40S	20S	10MR	80S	37.4
HD2967 (C)	60S	60S	20S	80S	60S	60S	60S	60S	20S	30S	80S	51.0
HD3086 (C)	tMR	5MS	tMS	0	tMR	10S	tR	10MS	5S	10MR	10S	3.3
WH 1105 (C)	10S	20S	20S	40S	60S	60S	30S	10S	tR	10MR	60S	25.4

Table 2. Seedling Resistant Test of genotypes against yellow rust pathotypes

Variety /Line	Pathotypes																		Gene	Remarks
	110S119	110S247	110S68	238S119	110S84	111S68	46S119	78S84	6S0	79S4	79S68	T	P	K	L	38A	7S0	31		
DBW 246	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	-	Resistant
DBW88 (C)	S	S	R	MS	R	R	R	R	R	R	R	R	R	R	R	R	R	R	*YrA+*	
HD2967 (C)	S	S	R	S	R	R	MS	R	R	R	R	S	S	S	MS	R	R	R	*Yr2+*	
HD3086 (C)	S	S	S	S	R	R	MS	R	R	R	R	S	S	S	R	R	R	R	*Yr2+*	
WH 1105 (C)	S	S	R	R	R	R	R	R	R	R	R	MS	R	R	R	R	R	R	*Yr2+*	

8. IC-0624570 (IC0624570; INGR18008), a Wheat (*Triticum aestivum*) Germplasm Resistant Spot Blotch and Early Maturing.

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Spot blotch caused by *Bipolaris sorokiniana* is a major disease of wheat in warm humid regions. Average losses caused by this disease is 16% and expected yield loss form 9 million hectare is estimated around 3 million tons annually. Spot blotch resistant germplasm are late maturing hence trapped under terminal heat. Developing early maturing spot blotch resistant genotypes facilitate wheat crop to escape terminal heat and spot blotch.

In 2000 at Banaras Hindu University, Varanasi two diverse wheat germplasm for spot blotch Sonalika (Susceptible) and Yangmai 6 (Resistant) were crossed for developing recombinant inbred lines. RILs were developed following single seed descend method in successive generations at BHU and Wellington, Tamilnadu. This RIL population (YS) was extensively used for QTL mapping (Kumar *et al.,* 2009, 2015). From this population YS#24 and YS#58 were identified in Sonalika background similar for most of the phenological traits except spot blotch resistance (Yusuf *et al.,* 2016). YS#24 was highly resistant and YS#58 was highly susceptible against spot blotch. These two lines (YS#24 and YS#58) were crossed in the 2010–11, utilizing off-season facility and following SSD method a new RIL population with 211 lines was developed.

These 211 RILs, 2 parents (YS#24, YS#58) and Sonalika as a susceptible check were planted in 7 environments i.e., during 2014-15 and 2015-16 at 3 locations viz., BHU, Varanasi, BISA, Samastipur and UBKV, Coochbehar W. B. and in 2016-17 only at BHU. On the basis of pooled data of 7 environments, line#94 (IC-0624570) was highly resistant against spot blotch. Mean of important traits over 7 environments are as AUDPC (280), early days to heading (66.5 days), early maturity (110 days), 1000-grain weight (35.78g) and plant height (90cm). This line was superior for all the mentioned traits over resistant parent (YS#24) as well as Sonalika.

This genotype is important genetic resource and may be used as a variety directly or as a parent in a crossing programme for developing genotypes with spot blotch resistance and early maturing in order to escape the terminal heat.

9. HTW (IC0625994; INGR18009), a Wheat (*Triticum aestivum*) Germplasm with Heat Tolerance.

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Heat stress is a major environmental stress limiting wheat productivity in most cereal growing areas of the world.The optimum temperature for wheat during anthesis and grain filling ranges from 12 to 22°C (Tewolde *et al.*, 2006). Exposure to temperatures above this range can significantly reduce grain yield. In many parts of the Asian subcontinent, due to the rice-wheat cropping system, crop damage due to heat stress under late planting conditions has become a significant factor limiting wheat yields. To sustain the wheat production and productivity, identification of heat tolerant genotypes and simultaneously transfer of tolerance into high yielding backgrounds is of utmost importance. The genotype HTW 9 was identified to be highly tolerant to heat stress based on multi-location and multiyear evaluation.

The heat tolerant genotype, HTW 9 was developed from the cross RAJ 3765/P11632 following a pedigree breeding approach. HTW 9 was evaluated for heat tolerance for two years under irrigated timely, irrigated late, rainfed timely and rainfed late sown field conditions at four locations viz. Akola, Dharwad, Karnal and Powarkheda under NICRA project. This genotype was found to be highly tolerant to heat stress (HSI -0.08) as compared to the identified heat and drought tolerant checks AKAW 2862-1(HSI 0.66), C 306 (HSI 0.64), C306-M 10 (HSI 1.10), HINDI 62 (HSI 1.25), HTW 11(HSI 1.61) and HTW 6 (HSI 0.80) under field conditions (Table 1). The genotype was found to be tolerant under late sown heat stress conditions at all the locations during both years. Location-wise and year wise data is given in Table 2.

Another experiment was also conducted simultaneously under temperature controlled conditions to confirm its tolerance to heat stress. This genotype along with all checks was planted under controlled conditions during 2016-17. The genotypes were planted under normal temperature conditions in one chamber and high temperature conditions in another chamber. The genotype HTW 9 was found to be tolerant to heat stress. The heat susceptibility index (HSI) value for HTW 9 was 0.77 as compared to the checks AKAW 2862-1(HSI 2.61), HINDI 62 (HSI 2.34), HTW 11(HSI 0.96) and HTW 6 (HSI 0.69) (Table 1).

Table 1. Heat susceptible index (HSI) of HTW 9 and checks (Pooled over 4 locations and 2 years)

Entry	Field conditions	Controlled Temperature conditions
HTW 9 (J31-24)	-0.08	-0.03
Checks (Registered Genetic stocks for heat tolerance)		
AKAW 2862-1	0.66	2.61
HINDI 62	1.25	2.34
HTW11	1.61	0.96
HTW 6	0.80	0.69

Table 2. Heat susceptible index (HSI) of HTW 9 and checks at 4 locations during 2015-16 and 2016-17

	Heat susceptible index (HSI)				
Genotype	Akola	Powarkheda	Karnal	Dharwad	Average
2015-16					
HTW 9 (J31-24)	0.34	-0.11	0.23	-0.58	-0.03
Checks (Registered Genetic stocks for heat tolerance)					
AKW2862-1	0.95	0.00	-0.18	0.91	0.42
HINDI 62	0.93	1.45	0.44	0.76	0.90
HTW11	0.90	1.08	0.61	0.64	0.81
HTW6	0.88	1.04	-0.66	-1.21	0.01
2016-17					
HTW 9 (J31-24)	-1.7	0.41	-0.03	0.81	-0.13
Checks (Registered Genetic stocks for heat tolerance)					
AKW2862-1	0.44	1.49	0.93	-0.76	0.52
HINDI 62	1.45	0.45	1.07	0.83	0.95
HTW11	0.57	0.66	0.82	0.84	0.72
HTW6	0.10	0.70	-0.76	0.66	0.17

The performance of HTW 9 under irrigated late sown conditions with respect to other desirable agronomic features namely: days to heading (71 days), days to maturity (113 days), plant height (111cm) and 1000 grain weight (42.6g) has also indicated its suitability to be used in crossing programmes (Table 3).

This genotype is found to be highly tolerant to heat stress conditions showing potential to be used as a donor for enhancing heat tolerance in wheat.

Table 3. Agronomic characteristics of the drought tolerant genotype, HTW 9 pooled over two years under field conditions

Genotypes	Days to Heading		Days to maturity		Plant Height (cm)		1000 grain weight (g)	
	*IR-TS	IR-LS	IR-TS	IR-LS	IR-TS	IR-LS	IR-TS	IR-LS
HTW 9	68	71	119	113	86	111	44.1	42.6
Checks (Registered Genetic stocks for heat tolerance)								
AKW2862-1	70	67	120	112	101	109	49.5	40.5
HINDI 62	87	72	129	112	99	99	36.4	35.0
HTW11	82	64	124	108	94	114	41.6	41.0
HTW6	84	64	127	108	103	92	38.8	40.4

*IR-TS- irrigated timely sown conditions; IR-LS- irrigated late sown conditions

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10. DBW 218 (IC0625997; INGR18010), a Wheat (*Triticum aestivum*) Germplasm with High Sedimentation Value and Grain Hardness.

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In order to meet the food nutritional demands of the future population, the breeding programmes need to focus on developing cultivars possessing high yield coupled with good quality characteristics. Wheat genotype, DBW218 was identified as a promising genotype possessing high sedimentation value, grain protein content and grain hardness along with acceptable yield.

The new wheat genotype DBW218 (PRL*PASTOR// PBW343*2/KUKUNA) was selected from an international nursery and was evaluated over years at ICAR-IIWBR, Karnal. This genotype was found promising and contributed to the special trial (very late sown) for testing under AICRPW&B.This genotype was evaluated at total nine locations including five locations (Delhi, Karnal, Hisar, Ludhiana and Gurdaspur) of North Western Plains Zone (NWPZ) and four locations (Faizabad, Kanpur, Pusa and Sabour) of North Eastern Plains Zone (NEPZ) along with the three checks (DBW71, WR544, DBW14) during 2015-16 for grain yield, disease resistance and quality traits and this followed as criterion for selecting genotypes for end productpurpose.

The key determinants for making good quality chapatti are sedimentation value (30-60 ml), grain protein content (11-13%) and grain hardness (40-75). Sedimentation value gives an idea of gluten strength. DBW218 showed high sedimentation value of 57ml pooled over both 9 locations coupled with desired level of protein content (12.7%) and grain hardness (76) as compared to the check varieties (Table 1).

Besides quality attributes, it also showed better performance for other desired agronomic characteristics over checks in nine locations indicating its wider adaptability. DBW 218 had high grain yield (32.7 q/ ha) under very late sown conditions as compared to all three checks namely; DBW 71(32.5q/ha), WR544 (29.1 q/ha) and DBW14 (31.6q/ha). It also had a thousand grain weight of 33g (21- 42g), plant height 90cm (61-106 cm) and matures in about 101 days (79-114 days).

Therefore, DBW218 being high yielding genotype having exceptionally good for chapatti making traits (high sedimentation value, grain protein content and grain hardness). This promising genotype may be utilized as donor parent (quality, yield and short duration) in breeding programs to improve chapatti quality and thus would contribute significantly for food and nutritional security of the country.

Table 1. Mean performance of DBW 218 and checks for agronomic and quality traits pooled over NWPZ and NEPZ under AICW&BIP (2015-16)

Traits	Proposed genotype DBW 218		Check varieties					
			DBW 71		WR 544		DBW 14	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Quality traits								
Sedimentation Value	57.0	52-62	44.5	42-47	43.0	40-45	44.0	42-47
Grain Hardness	76.0	66-86	75.0	65-82	70.5	62-77	65.5	58-65
Protein Content (%)	12.7	10.8-15.4	12.9	11.3-15.1	13.7	11.7-15.5	13.1	11.8-15.5
Agronomic traits								
Grain Yield (q/ha)	32.7	22.2-45.8	32.5	24.4-41.5	29.1	25.8-35.2	31.6	23.8-42.9
1000 grain weight (g)	33.0	21-42	33.5	22-40	34.5	24-42	33.5	25-40
Plant height (cm)	90	61-106	80	64-94	88	58-105	76	58-85
Days to maturity	101	79-114	99	81-115	95	75-110	96	77-115

11. EC531185 (EC531185; INGR18011), aWheat (*Triticum aestivum*) Germplasm with Low DSI (Drought Susceptibility Index) <0.5 for 5-6 traits including yield/m2. High & Stable Grain/Spike under Irrigated and Non-Irrigated Conditions. Presence of Genes Associated with Various Drought Responsive Traits *viz.*, Thylakoid Membrane Stability (Fv/Fm), Grain Filling Duration (GYD), Grain Yield (GY) and Gene Maintaining Low Leaf Temperature under Drought Stress Condition.

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Drought is one of the most devastating phenomenon, which occurs in all the climatic regions leading to the great losses in agriculture. There are projections that drought events may intensify in future making farming exceedingly challenging for the farmers. Wheat (*Triticum aestivum* L.) is the major staple crop satisfying hunger globally. Wheat production may decline substantially in China, India and Russia due to climate variability (Knox *et al*., 2012). Yield losses due to drought depend on the growth stage and severity of stress (Daryanto *et al*., 2016). Breeding for drought tolerance using novel genetic resources is the most viable strategy to cope with the changing climatic conditions (Mwadzingeni *et al*., 2016). However, due to limited availability of resistance sources, progress in breeding drought tolerant cultivars is not satisfactory. Present study was conducted to identify drought tolerant wheat lines. In this study, field screening was done in multi-environment for four years followed by validation at molecular level to identify stable drought tolerant wheat genotypes. In field screening, based on ten quantitative traits including drought susceptibility index (DSI) under irrigated and non-irrigated conditions in two successive years, 44 wheat genotypes were selected from an initial set of 177 genotypes. These selected lines were further screened for two more years against drought stress. Stability analysis and AMMI biplot was also performed to analyze the performance of genotypes across the environment and years. Selected genotypes were further evaluated for the presence of six drought-linked molecular markers. Based on drought susceptibility index, other physiological parameters and molecular analysis, the genotypes namely, ET127225, ET127230, EC531185, ET127236, ET127267 and ET127269 were found drought tolerant. Among

these germplasm lines, EC531185, a cultivar named Kanchan derived from cross UP 301 X C306 is being proposed to be registered as genetic stock for drought tolerance. It is a semi –dwarf variety with plant height 90-100 cm, number of tillers 6-7, panicle initiation 60-80 days, days to maturity 106-112, 45-55 grain per spike, white grain, 1000 grain weight 45-48 gram. It is an ideal genotype for genetic and physiological studies and a potential genetic resource for drought tolerance, which can be used in wheat improvement programme.

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12. EC339604 (EC339604; INGR18012), a Wheat (*Triticum aestivum*) Germplasm with Resistance to Prevailing Leaf Rust Pathotypes. Presence of Leaf Rust Resistance Genes viz., *Lr22a, Lr46+, Lr67+* and additionally Carries Stripe Rust Resistance Genes *Yr5, Yr15 and Yr48*.

Sundeep Kumar[1*], S Archak[1], RK Tyagi[1], Jagdish Kumar[2], VK Vikas[2], Ashok Kumar[1], SR Jacob[1], K Srinivasan[1], J Radhamani[1], R Parimalan[1], Jyoti Kumari[1], BS Phogat[1], AK Singh[1], Manas Bag[1], Anirban Roy[1], TV Prasad[1], M Datta[1], KC Bansal[1], M Sivasamy[2], P Jayaprakash[2], SC Bharadwaj[3], P Parsad[3], OP Gangwar[3], MS Saharan[4], Indoo Bhagat[5], Madhu Meeta[5], NS Bains[5], JB Sharma[6], Robin Gogoi[6] and GP Singh[4]

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A comprehensive germplasm evaluation study of wheat accessions conserved in the Indian National Genebank was conducted to identify sources of rust and spot blotch resistance. Genebank accessions comprising three species of wheat – *Triticum aestivum, T. durum* and *T. dicoccum* were screened sequentially at multiple disease hotspots, during the 2011-14 crop seasons, carrying only resistant accessions to the next step of evaluation. Wheat accessions which were found to be resistant in the field were then assayed for seedling resistance and profiled using molecular markers. In the primary evaluation, 19,460 accessions were screened at Wellington (Tamil Nadu), a hotspot for stem and leaf rusts (Nagarajan & Joshi 1985). We identified 4925 accessions to be resistant and these were further evaluated at Gurdaspur (Punjab) a hotspot for stripe rust and Cooch Behar (West Bengal), a hotspot for spot blotch. A second round evaluation identified 498 accessions potentially resistant to multiple rusts and 868 accessions resistant to spot blotch. Evaluation of rust resistant accessions for seedling resistance against seven virulent pathotypes of three rusts under artificial epiphytotic conditions identified 137 accessions with multiple disease resistance. Molecular analysis to identify different combinations of genetic loci imparting resistance to leaf rust, stem rust, stripe rust and spot blotch using linked molecular markers, identified 45 wheat accessions containing resistance genes against all three rusts as well as a QTL for spot blotch resistance. Among identified 45 germplasm lines, EC339604 which showed the presence of resistance genes for leaf and stripe rust diseases viz., *Lr22a,Lr46+,Lr67+,Yr5,Yr15* and *Yr48*is a promising multiple disease resistant germplasm and could be included in wheat improvement program as one of the parents for developing durable multiple rust resistant cultivars.

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13. IC252459(IC0252459; INGR18013), a Wheat (*Triticum aestivum*) for Resistant to Stripe Rust Pathotypes K (47S102), P (46S103), L (70S69), 13 (67S8), I (38S102), 46S119 & 78S84 Carries Stripe Rust Resistance Genes viz., *Yr5*, *Yr15* & *Yr48* additionally also carries Leaf Rust Resistance Genes *Lr46+*, *Lr50* & *Lr24/Sr24*.

Sundeep Kumar[1*], Sunil Archak[1], RK Tyagi[1], Jagdish Kumar[2], VK Vikas[2], AK Sharma[3], Jyoti Kumari[1], Sherry Rachel Jacob[1], Kalyani Srinivasan[1], J Radhamani[1], BS Phogat[1], R Parimalan[1], Amit Kumar Singh[1], Manas Bag[1], Anirban Roy[1], TV Prasad[1], M Datta[1], KC Bansal[1], M Sivasamy[2], P Jayaprakash[2], SC Bharadwaj[4], P Parsad[4], OP Gangwar[4], MS Saharan[3], Indoo Bhagat[5], Madhu Meeta[5], NS Bains[5], AK Chaudhary[6], BC Saha[6], PM Bhattacharya[6], JB Sharma[7], Robin Gogoi[7], Deep Shikha[8], SP Singh[9], SS Vaish[10], PC Mishra[11], BK Honrao[12], IK Kalappanavar[13], VA Solanki[14], GP Singh[3]

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disease resistant germplasm and could be included in the wheat improvement program as one of the parents for developing durable multiple rust resistant cultivars. In addition, this germplasm line was also found resistant to all three rusts under NBPGR multi-location evaluation programme conducted during 2004-2009.

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14. IC564121 (IC0564121; INGR18014), a Wheat (*Triticum aestivum*) Germplasm Highly Resistant to Spot Blotch.

Jyoti Kumari[1*], Sundeep Kumar[1], Nidhi Singh[1], SS Vaish[2], Saikat Das[3], Arun Gupta[4], JC Rana[5], Amit Kumar Singh[1], Ruchi Bansal[1], Sherry R Jacob[1] and Ashok Kumar[1]

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Spot blotch disease leadsto significant yield losses ranging from 18 to 50% in warmer and humid regions of the world such as Eastern India, Bangladesh, the Terai of Nepal, Latin America, China and Africa (Saari 1998; Singh *et al*., 2007; Gurung *et al*., 2014). Besides, losses in grain yield, leaf blight causes seed discoloration, shrivelled seeds and loss in seed viability. In order to reduce the loss and stabilize the yield, spot blotch resistant wheat variety is priority of wheat breeders and therefore search of novel donor for spot blotch resistance is urgently required.

Under CRP-agrobiodiversity, one thousand four hundred and eighty three spring wheat germplasm (*Triticum aestivum* L.) lines comprising Indian as well as exotic lines were screened for resistance to spot blotch disease during winter 2014-15 at hot spot locations i.e., Banaras Hindu University, Varanasi and Uttar Banga Krishi Vishwavidyalaya, Cooch Behar. Severity of the disease at different stages beginning from tillering to dough stage was recorded. Twenty eight accessions were resistant or highly resistant at both locations. These 28 accessions were validated during the winter season (2015-2016). Out of 28 accessions, seven (IC564121, IC529684, IC443669, IC443652, IC529962, IC548325 and EC178071-331) were highly resistant across the locations and over the years of study. These accessions comprised one exotic and six indigenous accessions belonging to Uttarakhand and Haryana. These lines can be deployed for identification of resistance gene and breeding germplasm. The lines showing highly resistant reaction alongwith wider adaptability can be expedited for direct cultivation or for the development of high yielding and disease resistant cultivars.

The spot botch resistant accession IC564121, a landrace named as Lal Mundia was collected from Pauri district of Uttarakhand. It has red kernel colour, medium maturity and medium spike length (~8cm) and grains per spike (~50). This germplasm line can be used as a donor for spot blotch resistance in wheat breeding programme.

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Singh G, DP Singh, R Chatrath, BS Tyagi, GP Singh, SK Singh, J Shoran (2007a) Combating *Helminthosporium* leaf blight in wheat through resistance breeding. *Indian Journal of Genetics and Breeding* **67(3)**: 293-296.

Table 1. Spot blotch resistant germplasm with their disease reaction, maturity and yield

Accessions	Name/Source	Grain Yield (g/m row length)	Thousand Grain Weight (g)	Days to Maturity	Mean AUDPC value	DS (High)	DS (Av.)	Category
IC564121	Lal mundiya/Pauri, Uttarakhand	91.90±7.18	25.78±1.75	122	362±13	23	13	HR
IC529684	VWFW-2299/Almora, Uttarakhand	117.90±11.44	28.30±1.62	127	79±17	23	12	HR
IC443669	PHR-1025/Karnal, Haryana	177.51±12.65	39.81±0.66	119	88±19	23	12	HR
IC443652	HUW-549/Karnal, Haryana	121.23±4.18	39.75±0.71	117	77±16	24	13	HR
IC529962	Almora, Uttarakhand	105.13±6.05	25.78±1.71	121	92±20	12	12	HR
IC548325	Shimla, Himachal Pradesh	161.97±19.06	37.62±0.60	109	174±42	23	12	HR
EC178071-331	Mexico	104.06±8.29	32.73±1.23	114	325±19	24	13	HR
Sonalika	Susceptible check	70±5.46	36.87±1.49	123	2630±22	69	59	S
DBW39	Resistant check	114±6.69	37±1.34	125	583±12	35	24	R

15. IC443669 (IC0443669; INGR18015), a Wheat (*Triticum aestivum*) Germplasm Highly Resistant to Spot Blotch.

Jyoti Kumari[1,*], Sundeep Kumar[1], Nidhi Singh[1], SS Vaish[2], Saikat Das[3], Arun Gupta[4], JC Rana[5], Amit K Singh[1], Ruchi Bansal[1], Sherry R Jacob[1] and Ashok Kumar[1]

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Under CRP-agrobiodiversity, one thousand four hundred and eighty three spring wheat germplasm (*Triticum aestivum* L.) lines comprising Indian as well as exotic lines were screened for resistance to spot blotch disease during winter 2014-15 at hot spot locations i.e., Banaras Hindu University, Varanasi and Uttar Banga Krishi Vishwavidyalaya, Cooch Behar. Severity of the disease at different stages beginning from tillering to dough stage was recorded. Twenty eight accessions were resistant or highly resistant at both locations. These 28 accessions were validated during the winter season (2015-2016). These germplasm were also evaluated at four environments for agronomic traits. Out of 28 accessions, seven (IC564121, IC529684, IC443669, IC443652, IC529962, IC548325 and EC178071-331) were highly resistant across the locations and over the years of study. These accessions comprised one exotic and six indigenous accessions belonging to Uttarakhand and Haryana. These lines can be deployed for identification of resistance gene and breeding germplasm. Two lines, IC529962 and IC443652 had higher yield than the best check at all the locations. In addition, the line IC443669 was highly resistant to spot blotch and also high yielder with specific adaptability to UBKV, Cooch Behar. Thus germplasm line IC443669 showing highly resistant reaction can be expedited for the development of high yielding and disease resistant cultivars.

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Gurung S, S Mamidi, JM Bonman, M Xiong, G Brown-Guedira,TB Adhikari (2014) Genome-Wide Association Study Reveals Novel Quantitative Trait Loci Associated with Resistance to Multiple Leaf Spot Diseases of Spring Wheat. Xu M, ed. *PLoS ONE*. **9(9):** e108179. doi:10.1371/journal.pone.0108179.

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DBW39	Resistant check	114±6.69	37±1.34	125	583±12	35	24	R
HD2967	Agronomic check	105.45±6.39	36.65±0.74	118	-	-	-	-

16. QLD 49 (IC0626288; INGR18016), a Wheat (*Triticum aestivum*) Germplasm with Soft Grain (Very Low Grain Hardness Index) Suitable for Biscuit Making.

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Soft grain textured wheat produces tender and larger biscuits. The key determinants of good biscuits are soft texture, low protein content and weak gluten. Biscuit industry is India's largest industry amongst food industries with an estimated production of 70,000 tonnes and cost of USD 3bn.

QLD 49 was developed at ICAR-Indian Institute of Wheat and Barley Research (ICAR-IIWBR) by crossing 37th IBWSN 72/5th IAT. The genotype was evaluated at 12 centers in Quality Component Screening Nursery (QCSN) for three consecutive years (2014-15 to 2016-17). QLD 49 was found to be superior with 20.0 grain hardness index over the years and locations to all the testing genotypes and soft grain check variety HS 490 (27: grain hardness index) (Table 1).

QLD 49 recorded the lowest grain hardness index in all the 12 tested centers compared to the best check variety (HS 490) for low grain index. QLD 49 recorded lowest grain hardness index of 17.0, 21.0 and 21.0, respectively during 2014-15, 2015-16 and 2016-17. Whereas, the check variety HS 490 tested in 2015-16. QLD 49 was 25.93% superior over check variety HS 490. Low grain hardness index is very important factor to obtain high spread factor of biscuit and even better biscuit quality.

Thus, QLD-49 would be a potential source to be utilized in future breeding programs to develop bread wheat varieties suitable for better biscuit making.

Table1. Grain hardness index of QLD 49 at 12 locations during three years as compared to the best available check HS 490.

Zone	Location	QLD 49			HS 490 (Check)
		2014-15	2015-16	2016-17	2015-16
NHZ	Almora	23	14	19	24
NWPZ	Ludhiana	14	24	12	26
	Durgapura	13	21	13	33
	Delhi	17	20	21	27
	Pantnagar	10	21	26	29
	Karnal	9	26	23	29
NEPZ	Pusa	-	18	12	26
	Kanpur	18	20	-	21
CZ	Junagadh	18	23	22	25
	Vijapur	23	20	20	24
PZ	Pune	17	19	32	21
	Dharwad	30	24	30	37
Mean (National)		17	21	21	27
Three years mean		20			27
% Superiority of QLD 49 over best check variety HS 490		25.93%			

17. QLD 46 (IC0626289; INGR18017), a Wheat (*Triticum aestivum*) Germplasm with High Grain Protein Content.

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Grain protein is an important trait that determines the nutritional quality and baking properties of wheat. Protein is an essential component of cells and it supports muscle growth, immune and enzymatic reactions; protein-energy malnutrition causes marasmus and kwashiorkor.

QLD 46, a promising bread wheat genotype was developed at ICAR-Indian Institute of Wheat & Barley Research (ICAR-IIWBR), Karnal by crossing NL 655/ OPAT85//NL836. The genotype was evaluated at 12 locations along with high protein check variety (UP 2672) during 2014-15, 2015-16 and 2016-17 under Quality Component Screening Nursery (QCSN). QLD 46 found to be superior with 14.42% protein content over the years and locations to all the testing genotypes and check variety UP 2672 (13.59%) (Table1).

QLD 46 recorded the high grain protein content in all the 12 tested centers compared to best check variety (UP 2672) for high grain protein content. QLD 46 was recorded high grain protein content of 13.18%, 15.19% and 14.90%, respectively during 2014-15, 2015-16 and 2016-17. The check variety UP 2672 recorded 12.67%, 14.39% and 13.72%, respectively during 2014-15, 2015-16 and 2016-17.

Thus, QLD 46 would be a potential source to be utilized in future breeding programs to improve grain protein content of bread wheat varieties.

Table 1. Grain protein content of QLD 46 and UP 2672 (C) at 12 locations during 2014-15, 2015-16 and 2016-17.

Zone	Location	QLD 46			UP 2672 (C)		
		2014-15	2015-16	2016-17	2014-15	2015-16	2016-17
NHZ	Almora	11.6	14.9	14.2	10.77	13.27	12.64
NWPZ	Ludhiana	13.6	14.7	14.76	13.10	12.73	13.26
	Durgapura	13.5	15.8	14.24	13.33	15.67	13.64
	Delhi	13.2	15.7	14.68	13.20	14.70	14.24
	Pantnagar	13.7	14.7	12.73	13.23	12.90	11.84
	Karnal	13.2	15.0	14.86	13.07	14.00	12.85
NEPZ	Pusa	-	15.6	14.37	-	15.10	13.91
	Kanpur	13.7	15.4	-	12.03	13.83	-
CZ	Junagadh	13.2	16.3	15.68	12.73	15.33	14.99
	Vijapur	13.1	14.8	15.43	12.30	14.63	13.89
PZ	Pune	13.0	14.3	14.93	13.27	14.80	15.00
	Dharwad	13.2	15.1	14.72	12.37	15.70	14.63
Mean (National)		13.18	15.19	14.90	12.67	14.39	13.72
Three years mean		14.42			13.59		

18. DDW 32 (IC0626290; INGR18018), a Wheat (*Triticum durum*) Germplasm with Loose Smut Resistance.

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Loose smut disease can cause losses as high as 25 percent or more in severe cases. In general, percent yield loss from loose smut is approximately equal to the percentage of affected wheat heads. A durum genotype, DDW 32, developed at ICAR-IIWBR, Karnal was found to be having significantly higher resistance against loose smut with better pasta quality. DDW 32 is an indigenous durum line with PDW245/WH896// WH896 parentage. The spikes of DDW 32 along with check varieties were inoculated during 2014-15 with local isolates of loose smut pathogen using Go-go method of inoculation and disease infections were recorded in 2015-16 at hot spot locations like Almora, Durgapura, Hisar, Karnal and Ludhiana ICAR-IIWBR Annual Report 2016-17; AICW&BIP, Progress Report, Vol. III (Crop Protection), 2016).

The percentage of tillers showing loose smut infection in different entries is presented in Table 1. The genotype DDW 32 showed immune type of reaction at all five tested locations. On the contrary, the susceptible check variety Sonalika (*aestivum*) showed higher infection levels ranging from 31.3 (Ludhiana) to 87.2 (Almora) with an average score of 61.3. In North Western Plains Zone, all the checks showed higher score of loose smut infection. The released durum varieties of Central Zone showed the loose smut score from 10.5 (HI 8498) - 60.1 (HD 4728). While, the durum varieties of peninsular zone showed the score from 4.7 (UAS 446) to 61.3 (MACS 3927). DDW32 was also evaluated for yield in NIVT 4 during 2013-14. It yielded 54.4 q/ha and had significant superiority over the best check PDW314 (50.7 q/ha) and was promoted in AVT 1st year of North western plain Zone 2014-15. In AVT it yielded 48.4 q/ha. In addition to having loose smut resistance DDW 32 also has superior pasta quality by having Sedimentation value (ml) of 44.

Table1. Percent infection of loose smut in DDW 32 and check varieties during 2015-16 crop season

Entry Name	Almora	Durgapura	Hisar	Karnal	Ludhiana	HS	Avg.
DDW32	0.0	0.0	0.0	0.0	0.0	0.0	0.0
North Western Plains zone (Checks / released varieties)							
PDW 233	0.0	0.0	37.1	0.0	0.0	37.1	7.4
PDW 291	0.0	0.0	40.3	0.0	0.0	40.3	8.1
PDW 314	52.2	0.0	50.3	0.0	0.0	52.2	20.5
Central Zone (Checks / released varieties)							
HD 4728	0.0	0.0	60.1	0.0	0.0	60.1	12.0
HI 8498	0.0	0.0	10.5	0.0	9.0	10.5	3.9
HI 8737	0.0	0.0	51.6	0.0	5.0	51.6	11.3
MPO 1215	0.0	0.0	27.1	5.1	19.2	27.1	10.3
Peninsular Zone (Checks / released varieties)							
MACS3927	13.3	0.0	61.3	0.0	0.0	61.3	14.9
AKDW2997-16	0.0	0.0	10.1	0.0	7.5	10.1	3.5
UAS428	12.9	0.0	21.3	0.0	5.7	21.3	8.0
UAS446	4.1	0.0	-	0.0	4.7	4.7	2.2
Sonalika (Susceptible check)	87.2	73.9	80.4	33.5	31.3	87.2	61.3

Table 2. Additional traits in DDW 32: Quality Parameters

Variety	Grain Appearance	Test Weight (Kg/hl)	Protein Content	Grain Hardness Index	Sedimentation value (ml)
DDW32	6.4	76.6	11.6	82	44
Varieties / Checks					
PDW233	6.8	78.5	12.3	92	37
PDW291	7.2	80.1	11.9	94	31
PDW314	6.8	79.9	12.0	86	38

Thus, DDW 32 would be a potential source to be utilized in future breeding programs to improve loose smut resistance.

DDW32 is resistant to loose smut at 5 locations of NWPZ and had '0' reaction at all centres compared to check varieties having average infections ranging from 15% to 61% of spikes

DDW 32 was found resistant to loose smut against popular ruling varieties of all zones.

DDW 32 also has higher sedimentation value compared to check varieties

19. DWRB152 (IC-0624535) (IC0624535; INGR18019), a Barley (*Hordeum vulgare*) Germplasm Highly Resistant to Stripe Rust at Seedling and Adult Plant Stages.

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Stripe rust is one of the cereal devastating diseases and caused by the fungus *Puccinia striiformis* f. sp. *hordei* in barley. It is wide spread in different in northern upper and mid hills, foot hills, north western plains zone (NWPZ) and with medium to low incidences in north eastern plains zone (NEPZ). DWRB152 (DWRB73/ DWRB78) is a two row malt barley genotype, which is highly resistant to stripe rust at seedling and adult plant stages. DWRB152 was evaluated for disease reactions at multi-locations for consecutive two years *viz. rabi*, 2015-16 and 2016-17, under National Barley Disease Screening Nursery (NBDSN) and Elite Barley Disease Screening Nursery (EBDSN), respectively (Anonymous, 2016; Anonymous 2017). The genotype DWRB152 exhibited highly resistant response (0) for yellow rust under artificial disease epiphytotic conditions during both

the years. Whereas, the two-rowed malt barley check DWRUB52 and six-rowed feed barley check BH902 exhibited susceptible yellow rust reactions (HS) of 15S and 40S, respectively (Table 1).

Table 1. Multi-location adult plant resistance (APR) for stripe rust reactions (*artificial inoculation*) of DWRB152and checks over two years

Year	Location	DWRB152	Checks		Registered stock DWRB137	Infector
			DWRUB52	BH902		
First Year (2015-16)	Bajaura	0	0	20S	0	100S
	Ludhiana	0	10S	10S	0	60S
	Durgapura	0	TMR	10MS	0	100S
	Karnal	0	0	5MR	0	60S
	Jammu	0	5S	10S	0	80S
	Almora	0	0	20S	0	80S
	Durgapura	0	5MS	30S	TS	100S
Second Year (2016-17)	Ludhiana	0	0	40S	0	60S
	Bajaura	0	15S	40S	0	100S
	Jammu	0	10MS	10S	5MS	80S
	Karnal	0	0	40S	0	100S
Two years HS		0	15S	40S	5MS	100S

In seedling resistance test (SRT), DWRB152 showed resistant reactions for prevalent barley stripe rust races viz. 57 and M consecutively for two years *i.e.rabi*, 2015-16 and 2016-17. DWRB152 exhibited resistant response to all the barley stripe rust races and depicted mix response to the races 24 and 6S0 during *rabi*, 2015-16 and 2016-17, respectively.Average days to heading, maturity and 1000 grain weight for DWRB152 were recorded as 77 days (65-81), 114 days (102-136) and 50 g (44-56), respectively in IVT-LS-MB-NWPZ coordinated trial, during *rabi*, 2015-16.

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20. DWRB190 (IC-0624536) (IC0624536; INGR18020), a Barley (*Hordeum vulgare*) Germplasm Resistant to Spot Blotch.

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Spot blotch (*Bipolaris sorokiniana*) is devastating disease in barley and broadly resistance for the disease is not available in released cultivars of north western and eastern plains in India. The genotypic resistance is always desirable and eco-friendly over chemical control to protect environment and to enhance profitability of small and marginal farmers. DWRB190 is six-row barley genotype, which showed moderately resistance reactions for spot blotch under coordinated evaluation (*artificial inoculation*) in initial barley disease screening nursery (IBDSN) during 2016-17 (Table 1). The genotype DWRB190 was selected from segregating exotic material (M104/TOCTE//OROSUS/PETUNIA1) for spot blotch resistance and other morphological characters at Karnal during *rabi*, 2014-15 to 2015-16. During *rabi*, 2016-17, the genotype was evaluated in IBDSN at the centres namely, Pantnagar, Varanasi, Kanpur and Faizabad and showed resistance for spot blotch (Anonymous, 2017). The details of DWRB190 with checks are as hereunder-

Table 1. Multi-location screening for spot blotch (artificial inoculations) of DWRB190 and checks

Year	Location	DWRB190 (BK1633)	Zonal Checks			Infector
			BH902 (NWPZ*)	HUB113 (NEPZ)	BH959 (CZ)	
(2016-17)	Pantnagar	35	67	78	99	79
	Varanasi	35	68	89	89	89
	Kanpur	24	36	36	79	89
	Faizabad	35	57	57	79	78

**NWPZ=north western plains zone, NEPZ=north eastern plains zone, CZ=central zone*

Average days to heading, maturity and 1000 grain weight for DWRB190 were observed as 88 days (84-90), 130 days (127-135) and 37 g (35-41), respectively.

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21. DWRB174 (IC0626008; INGR18021), a Barley (*Hordeum vulgare*) Germplasm with Extra Early Heading and Short Plant Height.

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The genotype DWRB174 is six-row early heading barley genotype coupled with higher grain yield, short plant height, which was selected (single plant selection) and purified from segregating exotic material (GIJA 121/ CI 06248/4/APM/IB65//11012-2/3/API/CM67//DS/ APRO/5/ATHS) for earliness, plant height and other morphological characters. DWRB174 has an extra early heading (54 days) coupled with desirable plant height (81-85 cm). The genotype was evaluated at Karnal and Hisar (Anonymous, 2017; Kumar *et al*., 2016) and heading details are presented in Table 1.

DWRB174 is six row feed barley genotype with desirable yield attributes, higher grain yield, short plant height than earlier registered genetic stock DWRB173 and can be directly utilized in rainfed breeding programmes with-out grain yield penalty. The botanical comparison and extra traits than DWRB173 are presented below in Table 2.

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Table 1. Heading days details of DWRB174 with check BH902 and earlier registered genetic stock DWRB173

Location	Year	DWRB174 (six-rowed awned)	BH902 (six-rowed check)	DWRB173 (two-row hooded)
Karnal	2015-16	55	89	55
Karnal	2016-17	54	91	54
Karnal	2017-18	54	89	54
Hisar	2015-16	54	87	53
Hisar	2016-17	54	88	-
	Mean	54	89	54

Table 2. Advantage of DWRB174 over DWRB173

Trait	DWRB174	DWRB173
Spike type	Six-row	Two-row
Awns type	Present	Hooded
Plant height (cm)	81-85	110-115
Grain yield (q/ha)	42-45	26-30
Grains/spike	55-75	22-27

22. IC568489 (IC0568489; INGR18022), Scented Sorghum (*Sorghum bicolor*) Germplasm.

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Sorghum [*Sorghum bicolor* (L.) Moench] is originated from North-East quadrant of Africa. India is secondary centre of diversity (Singh, 2002). The first FAO-IBP report on the survey of crop genetic resources in their centers of diversity spotted out in India and important among is the river valley of Madhya Pradesh (Singh, 1973). The first major effort on the collection of sorghum genetic diversity was made under the auspices of the Rockefeller Foundation and the Indian Council of Agricultural Research (ICAR); and a total of 16,138 collections were assembled by 1970 (Rockefeller Foundation, 1970).

The present exploration was to collect the traditional cultivars of sorghum especially the unexploited basmati (scented) jowar in the Bundelkhand regions of Madhya Pradesh. Prasada Rao (1979) reported the collection of Basmati jowar in the Chattarpur district of Madhya Pradesh. Scented sorghum with the local name "Basmati" was collected for the first time from Karri and Sarwa villages near Bijawar (Prasada Rao and Murthy, 1979). Its grains are dimpled, chalky white in colour with a characteristics aroma resembling that of Basmati rice. Five scented sorghum germplasm viz., IS 18674, IS 19907, IS 19908, IS 19910 and IS 19912 has been reported in the ICRISAT list of promising sorghum germplasm, which are the collections from Madhya Pradesh (Elangovan et. al. 2006).

Morpho-agronomic Characteristics: The basmati jowar plant is very tall in height, semi compact panicle, white caryopsis, which are very bold, straw glume with dark red colour at the basal part of the glume and grains with 75% coverage and belong to *durra guinea* race. The whole panicle gives aroma like basmati. Even the flowering panicles smelled like basmati. The results of the search were encouraging. The basmati (scented) jowar was not only re-collected from the first ever collection location but explored in two more locations viz. Katia (E 228) and Kerwan (E 254) villages of Chattarpur district. In the process of natural crossing, basmati jowar also has been collected in the name of *chatkul basmati* (E 213), *basmati sabet* (E 220), and *zunku basmati* (E 219) where the aroma was identified.

Associated Characters and Cultivation Practices: The collected basmati jowar was characterized through marker assisted selection (Rahul Zanan *et al*., 2016). Fragrance is one of the most important and valued quality characters in sorghum and other foods and attracts a premium price in local and global trade. The allele of the SbBADH2 gene in fragrant sorghum cultivar E228 was characterized. A 1441 bp deletion extending from exon 13 to 15 was found rather than a deletion from exon 12 to 15 as had been reported earlier. This allowed the development and validation of a new perfect PCR-based marker for identification of fragrant sorghum accessions in breeding. The concentration of 2-acetyl-1-pyrroline (2AP) in the grain of this cultivar was estimated to be 6.5 ± 0.4 ppb using headspace solid-phase microextraction (HSSPME) coupled with GC-MS. Flavor components of fragrant sorghum accession E228 (IC 568489) were analyzed and compared with the non-fragrant M35-1 cultivar. PCA analysis revealed that 2AP, benzothiazole, 2,3,5-trimethylpyridine, (1E)-1-ethylidene-1H-indene, cedrene, 2,4-bis(2-methyl-2propanyl)phenol, 2-hexyl-1-octanol, and 2-butyl-1octanol were among 25 compounds that were found in sorghum grain that may be contributing toward the aroma of fragrant sorghum. Proline and methylglyoxal contents were found to be higher in E228 than in M35-1, while SbBADH2 expression in E228 was half that in M35-1, suggesting a similar 2AP biosynthetic mechanism to that found in fragrant rice and soybean.

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23. *Corchorus aestuans* (WCIN179); KC/AK/DS-54 (IC0558459; INGR18023), a Jute (*Corchorus aestuans*) Germplasm Highly Resistant to Jute Hairy Caterpillar.

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The uniqueness and novelty of *C. aestuans* (accession WCIN-179) is confirmed on the basis of unique biochemical and VOC profile which makes it resistant against jute hairy caterpillar through multiple mechanisms *i.e*. antibiosis and non-preference recording least suitable for egg laying, biology and growth of insects. As *C. aestuans* is crossable with the cultivated species, *C. olitorius,* it is quite practical that this accession can further be used for insect resistance breeding program in jute.

Till date there is no source of resistance against hairy caterpillar which is a regular pest of jute. Occasionally its infestation becomes alarming due to outbreak in large area causing extensive damage to jute crop. India is harboring nine species (7 wild and 2 cultivated) of *Corchorus* namely, *C. aestuans, C. depressus, C. fascicularis, C. pseudo-olitorius, C. tridens, C. trilocularis, C. urticifolius, C. capsularis and C. olitorius* (Mahapatra *et al.,* 1998). Few accessions of wild species germplasm were evaluated to find out the source of resistance against this pest and the associated mechanism of resistance. Among these species, *C. aestuans* accession namely, WCIN-179 was found to be highly resistant to jute hairy caterpillar, *Spilosoma obliqua* during the four years of study at ICAR-Central Research Institute for Jute and Allied Fibres, Barrackpore, Kolkata and ICAR- Central Tuber Crops Research Institute, Regional station, Bhubaneswar. This accession was confirmed to be resistant as evidenced through the biology, feeding preference and oviposition behavior of *S. obliqua* recorded on *C. aestuans* (Accession: WCIN-179) as compared to other *Corchorus* species. The wild species (*C. aestuans* accession number,WCIN-179) showed resistance to hairy caterpillar and had recorded 71% less oviposition and 52% less preference for feeding by *S. obliqua* compared to cultivated variety, *C. olitorius* (cv. JRO-204).

The plants have cylindrical stem with red color with a plant height of 149 cm and basal stem diameter of 0.76 cm. The leaf posess green lamina with red margin and a cordate shape with yellow petal color. The color of the stigma is green; fruit (Pod color) is brown, cylindrical in shape with prominent horns on the fruits. Shattering behavior of fruit with Chocolate brown seed and 1000 seed test weight is 0.532 grams. Oviposition and feeding by *S. obliqua* on *C. aestuans* (WCIN-179) was also significantly less compared to other wild species. The larval growth on WCIN-179 was significantly impaired and the larvae failed to pupate and complete the life cycle. Normal growth of the immature stages on this accession *i.e.* WCIN-179 was heavily impaired compared to the other jute species (Gotyal *et al.,* 2013).

Biochemical studies revealed that the adverse effect on fecundity, feeding, biology recorded on WCIN-179 is imparted by the specific compositions and level of biochemical constituents which make it a unique and novel line of jute highly resistant to *S. obliqua* because of non-preference and antibiosis mechanism of resistance. The phenol content was also estimated in other wild and

cultivated *Corchorus* species and it has been found that the phenol content of WCIN-179 was significantly higher than JRO-204 *(cv. C. olitorius)* and other two wild species *i.e., C. fascicularis and C. trilocularis*. Higher level of phenol generally has adverse effect on insect biology and causes pre-mature mortality. Such type of resistance is due to antibiosis mechanism. This accession number (WCIN-179) also contains significantly less protein and higher phenol over *C. olitorius* and other wild species which may be the cause of imparting the resistance to *S. obliqua* (Gotyal *et. al*., 2015). Besides this accession has very specific profile of volatile organic compounds (VOCs) because of which it is not preferred by the adults for egg laying and feeding as these compounds cause repellent effect on the insects. Other accessions of wild species have different set of VOC profile. The GC-MS analysis of VOCs from WCIN-179 indicated that *S. obliqua* repellent compounds, benzene-1 ethyl-3 methyl, dodecane and tridecane were eluted at 6.70, 12.13 and 14.17 min. respectively. Other jute species have different set of VOCs and not as unique as it is present in WCIN-179 that makes it resistant due to non-preference mechanism resulting in less preference for egg laying and feeding. The uniqueness and novelty of this accession of *C. aestuans* is confirmed on the basis of very unique biochemical and VOC profile which makes it resistant against hairy caterpillar through multiple mechanisms i.e. antibiosis and non-preference recording least suitable for egg laying, biology and growth of insects. As *C. aestuans* is crossable with the cultivated species, *C. olitorius* (Choudhary *et al.,* 2015), it is quite practical that this accession can further be used for insect resistance breeding program in *tossa* jute. The seeds of the accession (WCIN-179) obtained from the institute germplasm unit and multiplied in research farm of ICAR- Central Research Institute for Jute and Allied Fibres, Barrackpore.

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24. GGPS 7x-1 (IC0625987; INGR18024), Novel Cytotype (heptaploid) (2n=7x=56) Member of Ploidy Series in Guinea Grass (*Panicum maximum*).

and

25. GGPS 11x-1 (IC0625988; INGR18025), a Novel Cytotype (eleven-ploid) (2n=11x=88) Member of Ploidy Series in Guinea Grass (*Panicum maximum*).

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Polyploidy is an important feature of plant evolution as many agriculturally important crops are polyploids (Udall and Wendel 2006). Naturally available or artificially synthesized ploidy levels within taxa were used to understand features amenable to modification by ploidy changes. Ploidy modifications by enhancing or reducing the ploidy levels were achieved by chemical treatments (eg. colchicine, oryzalin), haploid cell and tissue culture (eg. anther and ovule culture), and utilizing genes interfering with cell divisions (Crismani *et al*., 2013). Recently, a Hybridization-supplemented Apomixis-components Partitioning Approach (HAPA) has been reported to successfully manipulate the ploidy without using any chemical treatment. This method relies on utilization of uncoupled apomixis components for enhancing or reducing the ploidy levels in apomictic crops (Kaushal *et al*., 2009).

Guinea grass (*Panicum maximum* Jacq.) is a high yielding perennial multicut forage grass, widely grown in tropical and sub-tropical countries. Tetraploid cytotypes (2n=4x=32) with obligate apomictic mode of reproduction are most predominant (Savidan 1980, Jain *et al.* 2003). We have identified eight pathways of seed development arising from recombination between the three apomixis components in this crop (Kaushal *et al.* 2008). These recombination events were utilized to generate a ploidy series represented by 3x, 4x, 5x, 6x, 8x and 9x cytotypes from a single 4x progenitor following HAPA (Kaushal *et al.,* 2009). Such exhaustive ploidy series offers advantages to study incremental effect of addition of monoploid genome for the traits of interest. The above members of ploidy series are already registered with PGRC (INGR 09039 – 09044). In present communication, we report further enrichment of this series with two new novel cytotypes viz., representing 7x and 11x ploidies. Including these two novel cytotypes, the ploidy series in guinea grass is most exhaustive with 8 different ploidy levels and represent worlds' largest ploidy series in a crop plant.

A facultative aposporous 8x (2n=64) plant (identity GGPS 8x-1) was used as a maternal parent alongwith a 6x paternal plant (identity 3/29/2) to generate plants with novel ploidies. The cross pollinated progeny was initially screened using flow cytometry to identify plants with desired ploidies. One plant each representing 7x (designated as GGPS 7x-1) and 11x (designated as GGPS 11x-1) were recovered and established from a total of 209 plants tested. These selected plants were subjected to detailed characterization including growth habit, cytology and reproductive behavior. We have used reproductive diversity of GGPS 8x-1 maternal plant in generating these ploidy levels. Origin of 7x plant is from fertilization between both reduced female and male gametes. Egg cell in sexual embryo-sac (ES) of facultative aposporous GGPS 8x-1 was expected to be 4x which when fertilized with male gamete (3x) from the 6x parent generated the 7x progeny through n+n hybridization (BII) pathway. The 11x plant would have a B_{III} origin, whereby the unreduced egg cell (8x) from an aposporous ES of GGPS 8x-1 got fertilized with reduced 3x male gamete from the 6x parent. Reproductive diversity in seed development arising from uncoupled apomixis components in guinea grass had been described (Kaushal *et al.,* 2008).

GGPS 7x-1: 7x cytotype (2n=56): This plant has originated GGPS 7x-1 contained 2n=56 chromosomes, showing $2_I+13.4_{II}+2.6_{III}+3.2_{IV}+0.6_V+0.6_{VI}$ as an average chromosome confirmation. Hexavalents were the largest chromosome association observed. About 48% chromosomes were involved in bivalent formation whereas 49% chromosomes were involved in trivalents or higher associations. The plant was male fertile with 68% pollen stainability, though variable sized pollens were of common occurrence. This plant was measured to have 3.2 pg sporophytic DNA content, matching with the chromosome numbers (DNA ploidy). It was facultative aposporous in mode of reproduction, exhibiting 72% aposporous ES (four nucleated Panicum type). Multiple ES were seen in 38% ovules of this plant. Well filled seeds could be obtained upon self-pollination of this plant.

GGPS 11x-1: 11x cytotype (2n=88): The plant GGPS 11x-1 had 2n=88 chromosomes with $3.75_I+15.25_{II}+3.75_{III}+4.75_{IV}+2.25_V+1.75_{VI}+0.25_{VII}$ average meiotic chromosome configuration, and 5 pg sporophytic DNA content. The plant was semi-sterile showing 57% pollen stainability and high variability in pollen size. The plant was obligate aposporous with high frequency of occurrence of multiple embryo-sacs (72%). In general, this plant exhibited larger size of gynoecium and ovary, and characterized with occurrence of trifid stigma (70% expressivity), a feature rarely reported in guinea grass. This plant was a shy seed setter upon self pollination.

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26. P. ori 6x-1 (IC0625989; INGR18026), a Novel Hexaploid (6x) Cytotype with 2n=54 Chromosomes of Pennisetum (*Pennisetum orientale*) Recovered through BIII Hybridization of a 4x (2n=36) Cytotype.

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Pennisetum L. Rich is one of the largest genera (tribe Paniceae) of Gramineae (Poaceae) family and encompasses several economically important crops species. *P. orientale* belongs to tertiary gene pool and is a valuable pasture grass with high palatability, nutritive value and drought tolerance. It is a tall rhizomatous, deep rooted and perennial grass known for its excellent soil binding qualities (Bor 1960). *P. orientale* has a basic chromosome number x=9 with tetraploid cytotypes (2n=4x=36) as most predominant forms. Other ploidy levels, such as 2x, 3x, 5x, and 6x are rarely reported (Jauhar 1981). Being apomictic in mode of reproduction, gene transfer through hybridization approach is difficult. However, owing to recent knowledge on uncoupled apomixis components, fertilization of unreduced egg cell with reduced pollen (known as 2n+n or B_{III} hybridization) (Rutihauser 1948) is one of the possible approaches to obtain gene transfer. Uncoupling of the apomixis components (apomeiosis, parthenogenesis and functional endosperm development) has been recently reported in several agamic species (reviewed in Ozias-Akins and van Dijk 2007).

Tetraploid accessions (2n=4x=36) of *P. orientale* are being maintained at Indian Grassland and Fodder Research Institute, Jhansi, Indiai. In one of our experiments on progeny analysis of apomictic crops, self-pollinated seeds from one of these tetraploid accessions (identity P. ori 4x-2) were collected and raised in the nursery. Fifty five germinated seedlings were tested for their ploidy levels, utilizing flow cytometric measurement of DNA contents. All the progenies were found to have 4x DNA-ploidy, except one off type plant which was confirmed to have a 6x ploidy level. This plant was separated from other siblings and was characterized for its morphological, cytological and reproductive traits, *vis-à-vis* its 4x progenitor (Kaushal *et al.* 2015).

The 6x plant (P. ori 6x-1) had 2n=54 chromosomes exhibiting $22.3_{II}+2.3_{IV}$ average chromosome configuration. The sporophytic DNA content was estimated as 4.25 pg that matched with the 6x ploidy level, as the tetraploid accession had 2n=36 chromosomes and 2.83 pg DNA content. P.ori 6x-1 was perennial in life cycle similar to its 4x parent, however, was less vigourous. Higher ploidy in *P. orientale* seemed not to affect perenniality but had modified some morphological, biochemical and reproductive attributes. Most of the metric traits were similar in both the plants except for less number of tillers and more compact spike in P. ori 6x-1 than 4x plant. Both of them were male fertile, though the 6x cytotype showed occurrence of sterile pollens (about 27%), however set seeds on self-pollination. Mode of reproduction, as estimated by embryo-sac (ES) studies was found to be apomictic (four nucleated aposporous ES) in both the cytotypes, though frequency of multiple ES was higher in 4x (90%) as compared to 6x (74%). In general, ES were observed to be larger in size in the 6x plant.

The recovery of 6x cytotype from a 4x maternal plant suggested the 2n+n (termed as B_{III}) hybridization pathway of its origin, through fertilization of an unreduced egg cell (2n) with reduced male gamete (n) in the 4x cytotype (Kaushal *et al.,* 2008) and had provided the first direct experimental evidence of uncoupling (partitioning) of apomixis components (viz. apomeiosis, parthenogenesis and functional endosperm development) in *P. orientale*.

In addition, these uncoupling events had also generated variability in an otherwise apomictic species. The system may be used to elucidate mechanisms of uncoupling events in *Pennisetum* genus in general, andin *P. orientale* in particular. Such material where the ploidy is raised by uncoupling events offer advantages to manipulate ploidy at will and also offer a system to study regulation of morphological, physiological, reproductive and molecular traits in response to or as modulated by the ploidy levels.

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27. IC11613 (IC011613; INGR18027), a Blackgram (*Vigna mungo*) Germplasm Resistant to Mungbean Yellow Mosaic Virus.

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Yellow mosaic disease (YMD) of blackgram is an important production constraint in India. Yellow mosaic disease of blackgram, mungbean, cowpea and soybean cause yield losses even upto 100% under severe conditions. YMD is caused by at least four different species of whitefly transmitted begomoviruses collectively called legume yellow mosaic viruses. Use of resistant varieties is the feasible, effective, economical, environment friendly and sustainable approach to alleviate occurrence of YMD in areas where it is major constraint to grain legume cultivation. Search for new resistant lines is a continuing process. No specific large scale systematic screening has been conducted in India to identify resistant source against yellow mosaic viruses by combining field screening, vector inoculation and agro-inoculation. 344 germplasm accessions were grown in augmented block design (ABD) during kharif 2010. Susceptible check Barabanki local was usedas infector line. The response of the virus was assumed based on percent disease incidence and disease severity. Germplasm accessions while showed resistance response in three consecutive years under natural disease pressure were subjected to further screening through artificial inoculation using viruliferous whiteflies. Germplasm accessions which showed resistance response after whitefly inoculation were further challenged by infectious virus clones through agro-inoculation with the infectious cloned DNA-a and DNA-B components of a new Delhi isolate of MYMV. The IC-11613 germplasm collected from Punjab was found as potential source of resistance to MYMV Along with the virus response the resistant germplasm i.e IC-11613 was also evaluated for agronomic traits viz., plant ht(32.8cm), branches/Plant (7.1), number of pods/plant (35.8) pod length (4.99), number of seeds/pod (5.7), 100 seed wt.(4.3g) and seed yield/ plant (9.8g). IC-11613 germplasm was found superior in above morphological traits also in comparison to T-9 used as check. In the present investigation combined repeated field screening, whitefly transmission and agro-inoculation has shown that this particular accession is resistant to MYMV and hence it can be used for YMD resistant breeding programme or for cultivation after testing its adaptation to various zones and accepting quality traits.

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28. IC485638 (IC0485638; INGR18028), a Blackgram (*Vigna mungo*) Germplasm Resistant to Mungbean Yellow Mosaic Virus.

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Grain legumes are next to cereals due to their nutritional importance as food for human consumption. Yellow mosaic disease (YMD) mostly accounts for low harvest index of the present day blackgram cultivation. Although many resistant varieties of blackgram and mungbean have been developed using conventional breeding but durability of resistance does not hold good for a long time due to narrow genetic base of the cultivars as well as rapid emergence of new begomoviruses. Hence there is a need to book for new sources of resistance.A part of the germplasm collections of blackgram (344 accessions) was taken from The National Bank Gene and evaluated against yellow mosaic disease (YMD) and attempt was made to identity potential sources of resistance that could serve as valuable genetic resource for further disease resistance breeding programme. Field experiments were conducted on germplasm screening during the years Kharif 2010, 2011, 2012 at NBPGR experimental farm (28' 64' or 77' 15 E) using barabanki local as highly susceptible check.Germplasm accessions which showed resistance under field conditions for three years were subjected to artificial inoculation using viruliferous white flies. Accessions which showed resistance were further tested for validation of resistance through agro-inoculation with the infectious cloned DNA- A and DNA-B components of a New Delhi isolate of MYMV. A germplasm collection IC 485638was found resistant to MYMV some morphological traits of agronomic importance were also recorded like plant height (30.8 cm), primary branches (7), pods/plant(23) pod length (3.8 cm) sub/pod5.7), 100 seed wt. (2.9 gm) seed yield /plant (6.5g). This germplasm can be used as one of the parents in YMD resistant breeding programme. After verifying its stability and wider adaptation it can be recommended for further cultivation and utilization.

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29. IC420405 (IC0420405; INGR18029), a Cucumber (*Cucumis sativus*) Germplasm with High Carotenoid Content. Orange Flesh Colour.

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The pulp colour of common cucumber (*Cucumis sativus* var *sativus*) is white to greenish with low carotenoid content. A unique accession namely IC420405, showing orange flesh (mesocarp and endocarp) colour and lemon yellow skin colour was identified in 2012 at NBPGR farm, New Delhi (Ranjan *et al*., 2013; Ranjan *et al*., 2015). Due to difficulty in adaptaion during initial years, the evaluation and regeneration of enough quantity of seeds were conducted during 2013 to 2017 at NBPGR farm and Vegetable Research farm, IARI, New Delhi. This unique accession was collected long back in 2004 from Mamit district of Mizoram. The carotenoid content is found as high as 27.5 µg/g as compared to 1.44-2.2 µg/g in the best check variety Pusa Uday at Delhi conditions and 29.2 µg/g as compared to 1.06 µg/g in check variety Pusa Uday at Varanasi conditions. The detailed morphological description is presented in Table 1. This unique genotype has also got some more distinct features. Unlike common cucumber, it has five carpels and delayed flowering with short day requirement. Interestingly, it has a tendency of parthenocarpy at high temperature and fasciation during low temperature resulting clustered flowers.

The mature fruit can be used in making vegetable recipe or as salad. It can be best utilized in mixed salad as the appealing orange colour makes the salad more attractive to the consumers with enhanced nutritional value. Hence, this unique line may be used directly or as a donor parent for developing carotenoid rich cucumber to combat with the problem of vitamin A deficiency prevalent in our country.

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Table 1. Morphological and biochemical characters of IC420405

Trait Description	IC420405	Check variety (Pusa Uday)
Vine length	2.5-3.5	2-2.5
Day length requirement	Short day	Day neutral
Parthenocarpy	Present (at high temperature)	absent
No. of carpel	5	3
Fruit length (cm)	17-24	20-22
Fruit skin colour at market stage	Creamish green	Creamish green
Fruit skin colour (mature stage)	Lemon yellow	Brown netted
Spine colour	White	Black
Spine density	Sparse	Medium
Flesh colour (mature stage)	orange	white
Number of seed per fruit	100	400-600
Shelf life (days)	7-14	6-7
TSS (ºB)	4.0-4.6	5.2-6.0
Ash (%)	0.56	0.34
Total Carotenoid (µg/g)	27.5-29.2	1.06-2.2

30. IC257296 (IC0257296; INGR18030), a Cucumber (*Cucumis sativus*) Germplasm with Two Female Flowers Per Node. Earliness. Small Fruit.

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Cucumber (*Cucumis sativus* L.), one of the important indigenous cucurbitaceous vegetables, is grown all around the world for fresh vegetable market as well as for processing industry. Cucumbers are generally monoecious, bearing separate staminate and pistillate flowers on a single plant. Generally, each leaf axil produces either a cluster of staminate flowers or one pistillate flower. However, evaluation of cucumber germplasm conducted during Spring and *Kharif* season of 2011-2013 and *Kharif* 2017 at NBPGR, New Delhi, has identified an unique accession, IC257296 having two female flower per node (on upper nodes i.e. after 7th node), rare and unique character with very small fruits. Besides this, it is very early in bearing with first female flower at 4th or 5th node. This accession was collected in 1999 from Khurda district of Orissa. This variant was selected and grown for four years at Delhi conditions. The morpho-agronomic characters are presented in Table 1. The number of fruits per vine is significantly higher as compared to check variety Pusa Uday. Thus, variety with two female flowers per node can produce more fruits than varieties with single female flower per node within a short period as reported earlier for pickling cucumbers in glasshouse (Nandgaonkar and Baker,1981; Hikosaka and Sugiyama, 2004) and common cucumber in open (Ranjan *et al*., 2015).

This unique accession of cucumber having small fruit in large numbers may be used for processing industry in place of long fruited type. Due to earliness and two female flowers/ node, this accession bears small tender fruits in succession, suitable for salad purpose. This accession offers an advantage to organic growers who aim to harvest large numbers of fruits before plants are severely damaged by pests and diseases. Moreover, due to its short vine length, the accession may also be suitable for growing in high density, kitchen gardens as well as for pot cultivation.

Table 1. Morpho-agronomic features of IC257296

Trait	Description (IC257296)	Check variety (Pusa Uday)
Plant growth habit	Short viny	Long viny
Node bearing first female flower	4.25	7.20
Fruit length (cm)	7.88	13.87
Fruit diameter (cm)	4.44	5.28
Seed cavity length (cm)	6.5	12.0
Diameter of seed cavity (cm)	3.55	3.42
Fruit colour	Green	Green
Number of fruits/plant	14.27	9.2
Sex type	monoecious	monoecious
Number of female flower/ node	02	01

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31. Allohexaploid (H2) (IC0626000; INGR18031), an Indian mustard (*Brassica juncea*) Germplasm with Heat Tolerance. Resistant to *Alternaria brassiceae.*

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Crop Brassicas are cultivated throughout the world and serve as important sources of vegetables, oilseed and spices. The blackspot disease caused by the fungus *Alternaria brassicae* is a major problem for which no source of resistance is available in cultivated Brassica germplasm. In particular, the losses in India due to black spot disease have been estimated between 35-47% under epidemic conditions (Kolte *et al*., 1987). The disease not only causes reduction in seed yield but also leads to lowering of oil and meal quality.Wild relatives of Brassica are rich reservoir of genes that are invaluable in improvement of cultivated species. *Sinapis alba* is a close relative of Brassica (Warwick and Black 1991) and is known to possess many desirable traits besides *Alternaria* resistance, tolerance to high temperature and drought (Primard 1988, Brown *et al*., 1997), resistance to shattering (Chandler *et al*., 2005; Wang *et al.,* 2007), beet cyst nematode (Lelivelt and Hoogendoorn, 1993) and club root (Lelivelt *et al.,* 1993). However, successful transfer of genes for blackspot resistance from wild species has not been achieved so far. We have developed somatic hybrid between *Brassica juncea and S. alba* through protoplast fusion with the ultimate goal of transferring genes for resistance to *Alternaria brassicae* and heat stress, the traits that are lacking in cultivated Brassica. Here we have reported vary high frequency of regeneration after protoplast fusion.

Morpho-agronomic Characteristics: Somatic hybrid shows mixed features of the parents. The hybrid show vigorous vegetative growth and attains more than 3.0 m height in contrast to its parent *S. alba* (2m) and *B. juncea* (1.57m). Days-to-flowering is intermediate (115-135 days) to parent (*S. alba*-166 days; *B. juncea*-145 days). Leaves of the hybrid are lobed with dentate margins and bear prominent trichomes on upper and lower surfaces. Stem is highly grooved, angular, thick and lacks wax, features similar to *S. alba*. Flowers are bright yellow and are larger than the parent. However hybrid bears fewer seeds per siliques as compared to *B. juncea* parent. The siliqua show characteristics beak as found in *S. alba.*

Associated characteristics and cultivation practices: Mitotic count of somatic hybrids reveals that hybrid (H2) carry 2n=60 chromosomes (Fig. 1C), a sum of the parental chromosomes of *B. juncea* (2n=36) and *S. alba* (2n=24). Hybrid shows mostly 30 bivalents at meiosis and even distribution of chromosomes at anaphase/telophas. Nuclear genome constitution of the hybrid is determined by using 10 SSR markers showed polymorphism between the parents. Hybrid shows the bands corresponding to the parents indicating the presence of nuclear genomes of both the parents. We use primers corresponding to the *orf108* upstream region and the *atpA* region to determine the mitochondrial genome constitution of hybrid. *S. alba* mitochondrial genome carries the male sterility inducing *orf108* upstream of the *atpA* gene (Kumar *et al*., 2012) which is also detect in somatic hybrid suggesting that the hybrid might carry recombinant mitochondrial genome. *In vitro* leaf assay and filed inoculation studies reveal that the hybrid is highly resistant to *A. brassicae* (1D). Besides, the hybrid set seeds at temperature of >38°C while the parents fail to produce seeds indicating that hybrid possess heat tolerance. These stable hybrids provide a reliable genetic resource for transfer of genes from *S. alba* into cultivated *Brassica* species.

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32. IC265495 (IC0265495; INGR18032), an Indian Mustard (*Brassica juncea*) Germplasm with White Rust Resistance (PDI = 0).

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White rust is a highly destructive disease of oilseed *Brassica*s; thus, it is essential to identify useful sources of host resistance in *B. juncea* as breeding and/or selection for resistance is the most cost-effective method of delivering control for farmers.The germplasm accession IC265495 collected from Aizwal, Mizoram was a part of2000 accessions of *B juncea* which were screened at three locations viz. Ludhiana, Pantnagar, Hissar during 2014-15 and 2015-16 crop season. This showed 0% disease severity with no infection at all the locations for two years. This was further evaluated under artificial inoculation in the pathological laboratory of Division of Plant Pathology, IARI, New Delhi against DELHI isolate of *A. candida* during 2016-17 as per the method described by Fox and Williams (1984). Observations for per cent incidence of white rust on leaves were recorded 20 days before the maturity of crop as per disease scoring scale adopted by Conn *et al.* (1990) and AICRIP R&M (2011). IC265495 showed resistant reaction (PDI = 0) under artificial inoculations indicating the presence of strong genetic mechanism in the expression of resistance. Apart from resistance to white rust, the mean performance of IC265495showed appreciably high values for different traits like days to maturity, silique length and 1000-seed weight. Thus it can be used for breeding Indian mustard varieties with resistance to white rust and high yield It is assumed that genetic stocks having high level of resistance and superior agronomic background may yield better progenies than those having poor agronomic performance while using them in breeding programmes.

Table 1. Mean of performance of IC265495 and National check- Kranti for various agronomic traits

Accessions	PB	DF	PH (cm)	DM	SMS	SL (cm)	SS	YP (g)	SWT (g)	OC (%)
IC265495	4.9	42.5	180.3	112.0	31.8	4.4	15.2	14.6	4.7	34.6
National Check (Kranti)	6.3	46.0	176.4	143.6	48.9	3.6	12.7	20.7	3.5	41.7

Primary branches/plant (PB), days to 50% flowering (DF), plant height, cm (PH), days to 80% maturity (DM), silique on main stem (SMS), silique length,cm (SL), seeds/silique (SS), yield/plant,g (YP), 1000-seed weight,g (SWT) and oil content, % (OC)

Table 2. Disease resistant reaction of Indian mustard accessions to white rust resistance over the locations

Score	Total no. of accessions			Promising accessions over the location	Resistant accessions under artificially inoculated controlled
	Ludhiana	Pantnagar	Hissar		
0	168	46	185	IC597932, IC267703, IC313380, IC20167, IC265495, EC657030, EC699003, EC766091, EC766133, EC766134, EC766136, EC766144, EC766145, EC766148, EC766152, EC766164, EC766191, EC766192, EC766193, EC766230, EC766232, EC766272, EC766311, EC766313, EC766315, EC766316, EC766402	IC 265495, IC313380, EC766091, EC766133, EC766134, EC766192, EC766230, EC766272

33. IC313380 (IC0313380; INGR18033), an Indian Mustard (*Brassica juncea*) Germplasm with White Rust Resistant (PDI = 0).

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White rust is a highly destructive disease of oilseed *Brassica*s; thus, it is essential to identify useful sources of host resistance in *B. juncea* as breeding and/or selection for resistance is the most cost-effective method of delivering control for farmers. The germplasm accessionIC313380 collected from Pithorgarh, Uttarakhand was a part of2000 accessions of *B juncea* which were screened at three locations viz. Ludhiana, Pantnagar, Hissar during 2014-15 and 2015-16 crop season. This showed 0% disease severity with no infection at all the locations for two years. This was further evaluated under artificial inoculation in the pathological laboratory of Division of Plant Pathology, IARI, New Delhi against DELHI isolate of *A. candida* during 2016-17 as per the method described by Fox and Williams (1984). Observations for per cent incidence of white rust on leaves were recorded 20 days before the maturity of crop as per disease scoring scale adopted by Conn *et al.* (1990) and AICRIP R&M (2011).

IC313380 showed resistant reaction (PDI = 0) under artificial inoculations indicating the presence of strong genetic mechanism in the expression of resistance. Apart from resistance to white rust, the mean performance of IC313380showed appreciably high values for various traits like days to flowering, silique on main stem, silique length, seeds per silique and oil content (Table 1). Thus it can be used for breeding Indian mustard varieties with resistance to white rust and high yield It is assumed that genetic stocks having high level of resistance and superior agronomic background may yield better progenies than those having poor agronomic performance while using them in breeding programmes.

Table 1. Mean of performance of IC313380 and National check-Kranti for various agronomic traits.

Accessions	PB	DF	PH (cm)	DM	SMS	SL (cm)	SS	YP (g)	SWT (g)	OC (%)
IC313380	4.6	40.0	181.0	136.0	52.6	4.2	17.2	12.0	2.1	41.5
National Check (Kranti)	6.3	46.0	176.4	143.6	48.9	3.6	12.7	20.7	3.5	41.7

Primary branches/plant (PB), days to 50% flowering (DF), plant height (PH), days to 80% maturity (DM), silique on main stem (SMS), silique length (SL), seeds/silique (SS), yield/plant (YP), 1000-seed weight (SWT) and oil content (OC)

34. IC0096539; NC-212 (IC0096539; INGR18034), a Linseed (*Linum usitatissimum*) Germplasm with Early Maturity.

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Linseed is an important multipurpose crop known for seed oil, stem fibre, paper, wax, nutraceutical and food processing industries. Indian sub-continent is a center of origin and domestication for this crop and this region is known to have high biological diversity of genus Linum (Fu, 2005). India is the sixth largest producer in the world with contribution to global linseed area and production 13% and 5.5%, respectively. Early maturity is one of the important breeding objectives for linseed as it protects crops from stresses such as disease, heat, drought, and frost (*Kaur et al., 2017)*. Short duration crop varieties are expected to prove boon to rain-fed farmers as it could give bonus income by growing linseed crop on residual moisture. In the above mentioned perspective, one landrace accession from Akola district of Maharashtra *viz.* IC0096539 was identified for early maturity (average 102 days). This accession is having semi-erect growth habit with funnel shaped, white colored flowers showing valvate aestivation. The stamen color is white while anthers are yellow. It produced medium sized lustrous brown seeds in non-dehiscent capsules. The oil content estimated was 40.36 %.

The identified accessions (IC0096539) is significantly early for flowering and maturity compared to early maturing released cultivar, RLC76 (Table 1).In addition, this accession is also early flowering and good for other agro-morphological traits such as large boll size with more number of seeds per boll and high thousand seed weight which are desirable agronomic traits. This accession has potential to be used in linseed breeding program for earliness/short duration which is an important breeding target in India owing to linseed cultivation in rain-fed and *utera* conditions. In addition this accession is expected to prove valuable genetic resource to unravel candidate genes/markers associated with flowering time and maturity to assist genomics/markers assisted breeding as well as to understand regulatory network involved there in.

Table1. Year-wise data of linseed accession IC0096539 and check variety RLC76 pertaining to different phenophases

Trait	Days to 50% flowering					Days to 95% flowering					Days to maturity				
Year	2014-15 (IARI farm)	2015-16 (IARI farm)	2016-17 (IARI farm)	2016-17 (Issapur farm)	**Mean**	2014-15 (IARI farm)	2015-16 (IARI farm)	2016-17 (IARI farm)	2016-17 (Issapur farm)	**Mean**	2014-15 (IARI farm)	2015-16 (IARI farm)	2016-17 (IARI farm)	2016-17 (Issapur farm)	**Mean**
IC0096539	60	58	57	59	**58****	77	70	71	78	**74****	103	102	102	99	**102****
RLC76	77	78	76	83	**79**	97	101	99	96	**98**	118	116	122	115	**118**

**indicates significantly superior at $P_{0.001}$

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35. LG 05817 (IC0627273; INGR18035), a Sugarcane (*Saccharum officinarum*) germplasm Moderately Resistant to 4 Pathotypes of Red Rot. 18.8 % Sucrose. Better Ratoonability.

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Red rot caused by *Colletotrichum falcatum,* is considered to be cancerous disease of sugarcane resulting in substantial economic loss to crop in Indian condition (Alexander and Vishwanathan, 1996). Many sugarcane varieties namely Co 213, Co 312, Co 419, Co 997, Co 1148, Co 62175, CoS 510, CoJ 64, CoS 767, CoC 671 and BO 54 succumbed to this disease after extensive cultivation. In sporadic incidences, the yield loss by red rot can be up to 30% in red rot prone area whereas cv CoJ 87 exhibited 100% incidence by red rot disease. Durable resistance in a plant is the kind of field resistance that remains effective for a long period while cultivars containing it are grown in diverse areas. It is also used as synonym of partial resistance or horizontal resistance/ or race non specific resistance. The name, although has no specific relation to its genetic control of resistance or degree of expression or its race-specificity. Sugarcane varieties such as CoC 671, Co 86032, Co 1148, CoJ 64, CoLk 8102, BO91 and CoS 767 are grown over large area and exhibit durable resistance but for race specific resistance only. Donors of durable resistance like commercial varieties BO 91 and CoLk 8102 grown in red rot prone area were used in breeding programme to develop two population of CoLk 8102 × ISH 176 and BO 91 × ISH 100.

LG 05817 is progeny of cross between CoLk 8102 (a susceptible and widely adapted variety of north east zone) and Interspecific hybrid ISH 176 (Co6806 × Khakai) (moderately resistant to Cf 08 and moderately susceptible to Cf 09). LG 05817 possesses moderately resistant (MR) reaction to four important red rot pathotypes of north west zone i.e. *Cf* 01, *Cf*08,*Cf* 09 and resistant (R) reaction to *Cf* 11 indicating durable resistance (Pandey *et al*., 2012, 2014, 2015; Anonymous, 2010). This clone showed horizontal resistance over past 10 years. It has cylindrical shape of inter node with greenish colour. This clone is characterized with more than 18.8 % sucrose and cane yield of > 88 t/ha. It has good ratoonabilty. It has good flowering at Coimbatore but do not flower in Lucknow condition. In Coimbatore climatic conditions, the clone shows 36% pollen fertility.

When it was used as recurrent parents with susceptible clone Co 62198, it produced high number of moderately resistant progeny indicating its high breeding value for this trait. High inheritance of the trait was attributed to cumulative effect of all four genes present in the clone. The clone is being constantly used as parent in breeding sugarcane clones for resistance against red rot. The most recent one is LG 05817 x LG 97050. The first cycle of recurrent selection of the cross has produced clones with desirable combination of resistance to red rot and high sucrose content.

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36. NBIHT-3 (IC0625991; INGR18036), an Opium poppy (*Papaver somniferum*) Pure Breeding Line Rich in Thebaine content >10%.

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Thebaine is one of the non-narcotic alkaloid among the diverse array of alkaloids in opium latex, which is extensively used in synthesis of pharmaceutical drugs (Fist *et al*., 2011). Though thebaine is not directly used in therapy but it is an important component for manufacturing of number of opiates. Nowadays, thebaine is industrially converted into a number of compounds like oxycodone, oxymorphone, buprenorphine, nalbuphine, naloxone, naltrexone and etorphine (Mishra *et al*., 2013). The international demand for specific alkaloids especially of thebaine has increased multifold in recent past due to its non-narcotic and non-addictive nature and ease in conversion for industrial preparation of morphine and codeine (Report of INCB, 2014). The global demand of thebaine increased from 2009 (241 tons) to 2013 (348 tons) but India's share is only 3 tons of the total global production of thebaine. Owing to such intense pharmacological application, production of thebaine in India is quite less in comparison to its demand. Although India is one of the largest producer of licit opium but has to import thebaine and its derivatives due to the lack of thebaine rich lines/varieties. Indigenous germplasm as well as developed varieties of opium poppy are primarily rich in morphine (9.20-20.86%) and narcotine (4.79-8.97%) in latex while content of codeine (1.69-2.48%), thebaine (1.78-2.80%) and papaverine (0.00-2.07%) remain substantially low. To trap this ever increasing global demand of thebaine, Govt. of India was keen for the development of thebaine rich opium poppy lines which could suffice the national and international demand. In this context, CSIR-National Botanical Research Institute, Lucknow for the first time developed a number of thebaine rich lines through rigorous selection from advance generations of interspecific population derived from cross between *Papaver somniferum* L. and *Papaver setigerum* DC (Shukla *et al*., 2015). The developed lines had thebaine content upto 15% in comparison to the existing indigenous varieties/lines having 1-2% thebaine content. A total of 11 high thebaine yielding lines developed through interspecific hybridization were evaluated along with two checks in randomized block design with three replications for eight years for thebaine content, other alkaloids, yield traits and other unique agronomic features at experimental field of Genetics and Plant Breeding Section, CSIR-National Botanical Research Institute, Lucknow. Standard cultural practices throughout crop season was followed which included pre sowing addition of farmyard manure at the rate of 10 t/ha (Approx.), 5–6 t/ha neem cake and 30, 50, 40 kg/ha N, P, and K respectively as basal dressing. 60 kg/ha N in two split after 30 and 60 days after sowing as top dressing and spray of the fungicide diethelene biscarbamate (Dithane M-45 0.2%) at 45, 60 and 90 days after sowing as per requirement was provided. The field was irrigated as and when required. Out of these, one most promising high thebaine pure breeding line NBIHT-3 (Thebaine content ~15.97%) was identified suitable for commercial cultivation based on overall performance in terms of seed, raw opium, other major alkaloids beside high content of thebaine and unique agronomic features (Tables 1 & 2). As per the calculations based on per hectare yield of each components of economic benefit (opium, seed and major alkaloids), the benefits from the cultivation of the developed thebaine rich line is at par or either higher than the cultivated varieties beside meeting national and international demand of thebaine.

Table 1. Economic features of developed high thebaine pure breeding line NBIHT-3 of opium poppy (Average over eight years)

Traits	Developed high thebaine pure breeding line	Checks	
	NBIHT-3	Madakini	BROP-1
Thebaine %	15.97	2.00	1.80
Morphine %	16.06	13.90	12.61
Codeine %	3.66	2.17	1.94
Narcotine %	0.33	9.70	11.27
Papaverine %	0.00	0.28	0.31
Opium yield (kg/ha)	30.36	39.68	38.37
Seed yield (q/ha)	11.39	12.12	12.35

Table 2. Morphological features of developed high thebaine pure breeding line NBIHT-3 of opium poppy (Average over three years)

Traits	Developed high thebaine pure breeding line	Checks	
	NBIHT-3	Madakini	BROP-1
Plant height (cm)	124.55	121.39	122.14
Peduncle length (cm)	23.35	24.38	23.87
Flower color	White	White	White
Leaves / plant	22.90	21.09	21.05
Branches / plant	2.95	3.19	3.06
Stem diameter (cm)	1.33	1.18	1.20
Capsule size (cm^2)	8.70	12.29	11.69

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37. Jor Lab L-9 (IC0625982; INGR18037), a Malabar Lemon Grass (*Cymbopogon khasianus*) Germplasm with High Essential Oil 0.80 %. Methyle Eugenol Rich>75%. High Herbage Yield 242.5 qtl/ha/year.

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Genus Cymbopogon is a valuable medicinal and aromatic grass of the world. The objective of the experiment was to identify methyl eugenol rich genotype of *Cymbopogon khasianus*. Methyl eugenol is widely used as a perfumery chemical as well as starting material for the synthesis of methyl dopa, an important hypertensive medicine (Kumaran *et al.,* 2013). Moreover it can also be used in confectionery, condiments, non-alcoholic beverages etc. In this experiment total 72 accessions of *Cymbopogon khasianus* were collected from different parts of North Eastern India and planted in complete randomized block design with three replications at experimental farm of CSIR-NEIST in the year of 2013.Morphological data recording and its oil constituents were analyzed during the year 2013-14 and 2014-15. On the basis of two years selection trial data a new and distinct methyl eugenol rich strain of *Cymbopogon khasianus* was identified and named as Jor Lab L-9. This new genotype was planted in multi-location trials in four different locations and found the results were stable and contented. The average oil yield was 0.8% which comprises of 74-75% methyl eugenol. Essential oil was extracted through hydro distillation method and the quantification was done by GC and GC/MS instrument (Lal *et al.,* 2016a; Baruah *et al.,* 2017; Dutta *et al.,* 2016., Dutta *et al.,* 2017; Lal *et al.,* 2018). In GC-MS analysis it was found that methyl eugenol was the major component of essential oil while geraniol, myrcene, elemicin, citral, linalool, geranyl acetate, methyl isoeugenol were the minor compounds. Till date none of the methyl eugenol rich germplasm of lemon grass is reported and we for the first time identified a novel methyl eugenol rich germplasm.

The oil yield % was significantly higher in the variety Jor Lab L-9 than the check variety Jor Lab L-2. The metyl eugenol content is significantly higher in the essential of identified germplasm (Table 1.). The higher oil yield is a desired character in *C. khasianus* as it is more economically beneficial. Plants of this variety are vegetatively propagated through slips and the plants are stable for commercial cultivation.

Our present study revealed that major portion of methyl eugenol in identified germplasm of *C. khasianus*, occupying 75% of the total essential oil. Methyl eugenol can be used commercially for various purposes like insect trapping. It can be used for further crop improvement

Table 1. Multilocation data of methyl eugenol rich germplasm (Jor Lab L-9) with check variety (Average of four locations)

Variety	Vegetative plant height (cm)	Flowering plant height (cm)	Herbage yield (tones/ ha/year	Oil %(w/w)	Methyl Eugenol (%) in the essential oil
Jor Lab L-9	111.75	239	27.50	0.82	75.13
Jor Lab L-2 (check variety)	121.75	160	22.7	0.44	4.78

Table 2. Essential oil analysis of *C. khasianus* genotype Jor Lab L-9

Chemical Compounds	Percentage of compound in the essential oil
Methyl Eugenol	74.94
Myrcene	4.2
Geraniol	4.9
Elemicin	3.1
Methyl isoeugenol	2.4
Geranyl acetate	1.6
Citral a	0.7
Citral b	0.3
Linalool	0.8
Unidentified	4.6

programme of lemon grass. For the first time a novel strain of methyl eugenol rich lemon grass identified through pure line plant breeding method is reported. This novel variety can be used for commercial cultivation by the farmers, agro industries and pharmaceuticals industries.

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38. Jor Lab K-1 (IC0625983; INGR18038), a Galanga (*Kaempferia galanga*) Germplasm with Higher Essential Oil Yield 2.31 %. Higher Rhizome Yield 6.75 tones/ha/year. Higher Dry Rhizome Recovery 27.50 %.

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Kaempferia galanga, known as Chandramula in Hindi is a rhizomatous herb of Zingiberaceae family cultivated mainly for its aroma and other medicinal importance. It is widely cultivated in tropics and subtropics of Asia including India, China, Bangladesh, Japan and Indonesia.In India it is grown in the states of Tamil Nadu, Kerala, Karnataka, North East states and West Bengal (Raina *et al.,* 2015). Essential oil of *Kaempferia galanga* rhizomes gives yellow colour oil on distillation with a yield of upto 2.5 percent. The oil is a complex mixture of volatile monoterpenes, oxygenated terpenes and sesquiterpenes as well as non–terpene hydrocarbons, ester, fatty acids and ketones (Amberkar *et al.,* 2011). *Kaempferia galanga* is famous for its medicinal uses due to presence of many important bioactive compounds like ethyl-p-methoxycinnamate which is a good constituent for antimicrobial and antineolplastic activity, ethyl cinnamate is used as vasorelaxant; luteolin and apigenin are good anti oxidant agents (Mustafa *et al.,* 2010). Some of these active compounds are used in the medicinal as well as perfumery industry that holds a great promise in ancient as well as modern era. The rhizomes also have a good nutritive value and are quite rich in protein and carbohydrate but low in fat (Hatano *et al.,* 1989; Jiang, 2013). In India the present price of dry rhizome is Rs 300 to 400 per kg and the price of essential oil is Rs

15000-17000 per litre which can be helpful for both farmers and enterpreneurs in increasing their earning (Lal *et al.,* 2017). Due to the limited improved varieties there is need of superior varieties of *Kampferia galanga* for high oil yield and rhizomes yield. Therefore, in this regard thirty-five germplasms were evaluated during the year 2013-14.After two years of evaluation of high oil and rhizome yielding genotype of *K. galanga* was identified and named "Jor Lab–K -1" (Fig 1) which was propagated through vegetative means using the rhizome as explant.This elite new genotype was evaluated in four different locations of North East India during the year, 2015 and 2016.

As per data from Table-1, the new variety Jor Lab K-1 contains higher oil yielding capacity of 2.37% with high rhizome yield of 7.156 tonnes/hectare compared to the check variety. One promising variety earlier released by the Kerala Agricultural University named"Rajani" was used as check variety.

The major chemical constituents identified in the rhizome of essential oil are ethyl trans-p-methoxycinnmate

Table 1. Quantitative and qualitative data of *Kaempferia galanga* (Jor Lab K-1) (Average of four locations)

Variety	Rhizome yield (tons/ ha)	No. of Mother Rhizomes	No.of Primary rhizomes	Dry rhizome recovery %	Oil % (w/w)	Days to maturity
Jor Lab K-1	7.156	5.62	12.25	28.50	2.37	273.75
Rajani(Check variety)	4.931	4.00	10.00	30.00	1.825	279.12

(31.12%),ethyl trans-cinnamate(14.3%),1,8-Cineol(10.57%), Delta -3- Carene (5.12%) and n- pentadecane (4.8%) which is responsible for its pharmacological properties.

Cultivation of this high yielding germplasms will open new prospects for utilizing underutilized sloppy land.This will create avenues for employment of rural population in India. Cultivation of superior variety of *Kaempferia galanga* may give necessary boost to the pharmaceutical industry through higher value of essential oil which will lead to high income generation of the rural and entrepreneur development.

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39. Jor Lab L-10 (IC0625984; INGR18039), a Malabar Lemon grass (*Cymbopogon khasianus*) germplasm with High Elemicin content 70%. High Herbage yield 24.26 tones/ha/year.

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Cymbopogon Spreng is a genus belonging to family Poaceae. It is characterized by its different species having great variation in morphology and chemotypes (Bor 1960; Soenarko 1977). It is a valuable medicinal and aromatic grass comprising of 140 species all over the world out of which only 45 species are reported in India and tropical Asia (Dutta *et al.,* 2016; Lal *et al.,* 2016a, Dutta *et al.,* 2017). This medicinal and aromatic oil bearing grass plays an important role mainly in flavours, fragrances, cosmetics, soaps, perfumery, detergents and

pharmaceuticals (Lal *et al.,* 2016b) throughout the world and also gives essential oil upon steam distillation (Lal *et al*., 2016a; Baruah *et al*., 2017, Lal *et al.,* 2018). It is a vegetatively propagated perennial crop basically found in Khasi and Naga Hills and some lower part of Assam of North East India. Elemicin is a natural organic compound which is known as phenylpropene (Villanueva *et al.,* 1993 and Leela *et al.,* 2008), also a constituent of oleoresin. In this experiment total forty eight accessions of *Cymbopogon khasianus* were collected from different parts of Meghalaya and planted in complete randomized block design with three replications at experimental farm of CSIR-NEIST in the year 2013.Morphological data recording and its oil constituents were analyzed during the year 2013-14 and 2014-15. On the basis of two years selection trial data a new and distinct elemicin rich strain of *Cymbopogon khasianus* was identified and named as Jor Lab L-10. This new genotype was planted in the year 2015-16 and 2016-17 in multi-location trials at four different locations and the results were found to be stable and contented.

Till date none of the elemicin rich germplasm of lemon grass is available in the public domain. So we have made an effort to identify high elemicin rich germplasm of lemon grass from the collected germplasm. Therefore, development and cultivation of the elemicin rich strain is very much beneficial to the farmers as well as pharmaceutical industries and net return will be higher as compared to the other lemon grass variants.

Table1. Average multi-locational evaluation of quantitative and qualitative data of Cymbopogon khasianus (Jor Lab L-10)

Variety	Vegetative Plant height (cm)	Flowering Plant Height (cm)	Herbage yield (tones/ha/year)	Oil %(w/w)	Elemicin %
Jor Lab L-10	98	201	24.26	0.40	70.35
RRLJ-021 (check variety)	118.85	159.23	22.33	0.44	25.56

As per data from Table-1, the new variety Jor Lab L-10 has average oil yield of 0.40 % which comprises of 70% elemicin content and 175% superior in the term of elemicin content from the check variety.

Table 2. GC-MS data of some of the identified components present in the essential oil of *Cymbopogon khasianus* (Jor Lab L-10)

Chemical Compound	Percentage of compounds in the essential oil
Elemicin	70.475
Cis-Asarone	8.235
β-ocimene	5.632
Methyl eugenol	3.145
Limonene	2.269
Germacrene-D	1.569
Neral acetate	1.241
Camphene	0.982
α-pinene	0.891
Geranyl acetate	0.720
Caryophyllene	0.490
Trans-β-farnesene	0.312
Unidentified	4.039

In GC and GC-MS analysis it was found that elemicin was the major component of essential oil while cis-asarone, β-ocimene, methyl eugenol, limonene, germacrene-D, transneral acetate, camphene, α- pinene, geranyl acetate, caryophyllene and trans-β-farnesene etc. were the minor compounds. Till date none of the elemicin rich (>70%) variety of lemon grass is reported and we first time identified a novel elemicin rich variety (Lal *et al.,* 2018).

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40. Jor Lab L-14 (IC0625985; INGR18040), a Malabar Lemon Grass (*Cymbopogon flexuosus*) Germplasm with High Essential Oil 1.25 %. High Herbage yield 28.32 tones/ha/year. Citral Content 76 %.

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A new and distinct high essential oil and high herbage yielding variety of lemongrass (*Cymbopogon flexuosus*) was developed named Jor Lab L-14. After evaluation of four locations with two year 2015-16 and 2016-17 the novel germplasm contains 1.25 per cent essential oil and 28.32 tonnes /ha/year herbage yield. This novel variety Jor Lab L-14 of lemongrass is developed through mutation breeding. This variety is propagated through vegetative means using the slips and is stable for commercial cultivation.Lemongrass (*Cymbopogon flexuosus* L.), family Poaceae is an aromatic grass species and is commonly known as 'East Indian Lemongrass'. It is a vegetatively (also, seed) propagated perennial, multicut crop in the tropics Lal *et al.,* 2016., Saikia *et al.,* 2015., Baruah *et al*., 2017, Lal *et al.,* 2018). The prefix 'Lemon' owes to its typical lemon-like odor, released from the leaves on maceration, which is mainly due to the presence of citral as a cyclic monoterpene. The lemongrass oil as such is widely used in perfumes, soaps and cosmetics to obtain typical lemon notes (Lal *et al.,* 2016a; Lal *et al.,* 2016b; Lal *et al.,* 2018. Besides, it's an important source of citral, which is used in perfumes and medicine (Saikia *et al.,* 2015). While citral forms a significant raw material for confectionery and beverages, it is the principal source of β-ionone which is extensively used for the synthesis of vitamin A and a number of chemicals including synthetic violet perfumes (Lal *et al.,* 2016). In future, thus generating new varieties with higher oil yield and citral content in lemongrass is of utmost necessity.

In the view of above demand it is necessary to evolve essential high essential oil yielding varieties for respective locations. Due to the limited improved varieties for high oil yield and herbage yield, its cultivation is not popular among farmers. Therefore, there is a need to develop superior varieties of lemongrass for high oil yield and herbage yield. In CSIR-NEIST farm there is more than 534 accessions of *Cymbopogon* species. As per record this germplasm banks is first position followed by Oddakali germplasm bank (Dutta *et al.,* 2017). We screened the genotypes based on high oil yielding and high herbage yield from Gamma mutant progenies and identified a high oil yielding and high herbage yielding genotype named as Jor Lab L-14. Then, these superior selected clones were evaluated in four locations (Jorhat Assam, Imphal Manipur, Pasighat Arunachal Pradesh and Lakhimijan Assam) where these were compared to all the checks and found stable and high essential oil yield.

As per data from Table 1 the tillers per plant for Jor lab L-14 was significantly higher than the checks. As well as the oil yield is higher than the both check varieties. The oil yield per kg/ha/ year was significantly higher in the variety Jor lab-14. The characters plant height and herbage yield/tons/ha/year are non significant difference between the elite line and checks. The higher oil yield is a desired character in lemon grass as it more economically beneficial. This elite clone Jor Lab L-14 maintained its superiority over all the checks for herb and oil yield. Plants of this variety are propagated through only vegetative means using the slips, and the plants are stable for commercial cultivation.

Morpho-agronomic characterises

Characters	Range
Plant height (cm)	124-139
Growth habit	Semi compact
No of tillers/plant	70-92
Herbage yield /Qt/ha/year	275 to 302
Oil content %	1.25
Citral %	76

Table 1. Average morphological and quality data of Jor lab L-14 lines with checks in four Locations (2016-17)

Variety	Plant height (cm)	Tillers/plant	Herbage yield/tones/ha	Oil % w/w	Oil yield/kg/ha/year	Citral %
Jor LabL-14	124-139	86	28.32	1.25	350.4	76
Jor Lab L-2	118-132	44	27.80	0.42	116.76	79
Krishana	102-126	58	26.90	0.89	239.41	80

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41. Jor Lab P-1 (Patchouli) (IC0625986; INGR18041), a Patchouli (*Pogostemon cablin*) Germplasm with High Essential Oil Yield and High Herbage Yield 3220 kg /year.

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Patchouli (*Pogostemon cablin* (Blanco) Benth) is a medicinal and aromatic plant which has natural essential oil that can be extracted by steam distillation from both fresh and dry leaves. It is a perennial aromatic herb/ shrub, belonging to family Lamiaceae. Patchouli is native to Philippines 1 and other cultivating countries which include Indonesia, Malaysia, Thailand, West Africa, Brazil, China and India (Jadhav *et al.,* 2002). The Indian demand for patchouli oil is 220tones/annum and a major quantity of oil is imported (Kumar *et al.,* 2004). Patchaulol and α-patchoulene are the major components and many sesquinterpenes hydrocarbon such as α, β, γ-patchoulenes, α-bulnesene, α-guaiene and seychellene, cycloseychellene are the minor compounds of the essential oil (Donelian *et al*., 2009). The oil contains unique aroma and flavour, products made from its oil such as soaps; detergents, etc. have be-come successful commercial products in global business industries.It is also blended with sandal wood oil which is one of the finest attars widely used for scenting soaps, perfumes, body lotions, after shave lotion, detergents and cosmetics. In aromatherapy, it is used to calm nerves, control appetite and relieve depression and stress. It also possesses insecticidal, antibacterial and antifungal properties (Swamyand *et al.*, 2015). At present, the most important concern with commercial cultivation of *P. cablin* is availability of a cultivar that can produce high oil yielding along with high patchouli alcohol content. In the view of above needs and great prospects of cultivation of this crop successfully in various region of India, it is necessary to evolve high yielding genotypes for respective locations. Due to the limited improved varieties for high oil yield and herbage yield, its cultivation is not popular among farmers. Therefore, in this regard mutant populations were developed through gamma radiation to create the artificial variability and selection was done to identify the high yielding germplasm of patchouli named as Jor Lab P-1 which would be helpful in doubling the net income of farmers of India (Lal *et al.,* 2018). Then, these superior selected clones were evaluated in four different locations of NE region, where these were compared to the check variety.

As per data from Table 1, the oil yield kg/ha/year was significantly higher in the germplasm Jor Lab P-1 than the check variety Johor. The higher yield is a

desired character in patchouli as it is more economically beneficial. Plants of this variety are propagated through cutting and plants are stable for commercial cultivation. The variety Jor Lab P-1 (fig. 1) thus increases earnings of farmers as well as meet the demand of its essential oil in the country.

Table 1. Multilocation data of Patchouli variety (Jor Lab P-1) with check variety (Average of four locations)

Variety	Plant height (cm)	Leaf length (cm)	Leaf width (cm)	Fresh herbage yield (kg/ha/year)	Dry herbage yield (kg/ha/year)	Oil (%)	Oil yield (kg/ha/ year)	Patchouli alcohol (%)
Jor Lab P-1	112	9.4	7.26	11393.84	3220	3.10	80.5	38.00
Check variety (Johor)	101	7.3	6.89	10798	2446.5	2.15	52.60	32.8

Morpho-agronomic Characteristics

Characters	Range
Plant height (cm)	106-116
Fresh herbage yield (kg/ha/year)	9870-12340
Dry herbage yield (kg/ha/year)	2800-3251
Oil content %	2.85-3.251
Patchouli alcohol %	32-43

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42. EC338785 (EC338785; INGR18042), a Sweet Basil (*Ocimum basilicum*) Germplasm with High Methyl Chavicol Content (> 88.81±2.34 %) in Essential Oil Isolated from Aerial Parts.

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Sweet basil (*Ocimum basilicum* L.) is a pleasant smelling perennial herb of high industrial importance for the essential oil and aroma chemicals. The plant possesses wide range of genetic variability as observed through plant morphology, phenology and oil composition. Traditionally, basil has been extensively utilized in food as a flavoring agent, and in perfumery and medical industries. The basil essential oils are usually extracted from the leaves and flowering tops of basil plants. Through the centuries basil was cultivated for culinary and medicinal purposes in many countries, which created a great diversity of species within the *Ocimum* genus. The genus *Ocimum* comprises more than 150 species and is considered as one of the largest genera of the Lamiaceae family. The *O. basilicum* essential oils exhibited a wide and varying array of chemical compounds, depending on variations in chemotypes, leaf and flower colors, aroma and origin of the plants (Pushpangadan and Bradu, 1995).

The chemical composition of basil oil has been the subject of considerable interest due to extensive diversity observed in the constituents of the basil oils. The plant has the propensity to exhibit chemotypes i.e. morphologically indistinguishable plants of the same *Ocimum* species very often differ clearly in their chemical constituents. According to the chemical composition and geographical origin, *Ocimum* species were classified into three large groups: European type, Exotic or Reunion type and African type. Lawrence (1988) established

four essential oil chemotypes (methyl chavicol, linalool, methyl eugenol and methyl cinnamate) and also numerous subtypes. The chemical composition of basil is highly variable depending largely on the source, and can vary by extraction method and with developmental stages. Variations in chemical composition of basil oil play an important role in evaluating their utilization and value for industrial applications (Raina *et al*., 2017)

The present exotic germplasm collection of *O. basilicum* EC338785 was imported from USDA, USA in 1992. The germplasm was grown in experimental fields of ICAR-NBPGR for characterization and evaluation of agro-morphological and quality traits for four years. It was evaluated for the economically important quality trait of essential oil obtained from aerial parts of plant. Detailed chemical profiling of essential oil isolated from aerial plant parts of EC338785 by GC/MS exhibited methyl chavicol rich chemotype was present in this collection. Validation of this value-rich superior accession of *Ocimum* germplasm was performed over consecutive four years for major aroma compound and identified trait-specific value-rich germplasm accessions EC338785 (methyl chavicol 88.81 ± 2.34%). Methyl chavicol is an important compound highly prized for its use in perfume and flavor industry. The essential oils of basil extracted from leaves and flowering tops are used in food flavours, dental and oral products, in fragrances and in traditional medicines.Essential oils derived from *O. basilicum* offers a bright future for perfumes and flavours industry.

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43. Solan Selection (IC626004; INGR18043), a Malaxis (*Malaxis acuminata*) Germplasm with Yellow Coloured Flowers without any Purple Tinge. Yellowish Green Floral Buds. Greenish Basal Sheath at the Base of Shoot.

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Malaxis acuminata D. Don (syn. *Microstylis wallichii* Lindl.) is a terrestrial, perennial, medicinal orchid, distributed in India in temperate to subtropical Himalayas at an altitude of 1200-2100m (Clarke, 1985). It is an important ingredient of Ashtavarga drug and is used in Ayurveda for the preparation of Chyawanprash, Astavarga churna, Chitrakadi taila, Vachadi Taila, Mahakalyan Taila, Jivaniya ghrita, Mahamayura ghrita, Vajikarma taila, Brahini gutika and Himvana agada (Anonymous, 2006; Balkrishna *et al*., 2012). It is also used to cure tuberculosis and is a potent aphrodisiac (Chauhan, 1990). Due to unscientific harvesting, overexploitation and habitat destruction the existence of *M. acuminata* is under threat and is listed in CITES Appendix-II for ensuring its conservation. Genetic diversity assessment studies conducted at Shilly Farm (altitude 1550 amsl, latitude-N 30º54'30" and longitude E 77º07'30") under Dr YS Parmar Universityof Horticulture & Forestry, Solan, Himachal Pradesh resulted in isolation of one unique & stablemorphotype **(**named as Solan Selection) having distinct colour of floral buds, flowers, sheath on base of shoot (rhizome) and pseudobulbs.

Morpho-agronomic Characteristics: The plants of Solan Selection are characterised (Fig.1) by greenish basal sheath (at the base of the shoot in contrast to purplish in wild type), yellowish green floral buds (purplish in wild type), yellow coloured flowers without any purple tinge on their surface (purple tinge in wild type) and pseudobulbs covered by green sheath (purplish in wild

type). All theses distinct morphological characters were found consistently stable in successive growing seasons during the years 2013, 2014, 2015, 2016 & 2017.

Associated Characters and Cultivation Practices: *Malaxis acuminata* is successfully regenerated by vegetative propagules i.e. rhizomes and pseudobulbs (Sharma *et al.,* 2014). Each plant produce single rhizome and 2-4 pseudobulbs in a growing season. The basal swollen stem of the plant on senescence develop to single rhizome, which is the economic part and is used in drugs and pseudobulbs give rise to new plants during next growth cycle.

Investigations are continuing at Dr YS Parmar University of Horticulture & Forestry Nauni, Solan to assess the growth & yield performance in respect of total biomass yield with a view to develop a high yielding strain/variety fulfilling the requirement of DUS criteria.

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44. Rama Tulsi/ DOS-1 (IC0627270; INGR18044), a Basil (*Ocimum sanctum*) Germplasm with Number of PGs in leaf. Dry Leaf Recovery (23.10 %). Essential Oil Contents (0.65 %) in Green Herbage and Leaves.

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The holy basil (*Ocimmum sanctum* L. syn. *Ocimum tenuiflorum*) (Family Lamiaceae), is the most sacred herb. It is an excellent herb known as queen of herbs, native of India and is cultivated largely as a house hold species. The most common uses of the holy basil include preparation of herbal tea, healing remedies, cosmetics and as a preservative.

Each and every part of plant are economic parts used in the treatment of various diseases in Indian systems of medicine. India exports large quantity of leaves, plant extract and essential oil in the world market and earns crores of revenue every year. Germplasm constitute the basic quality materials required for the improvement of this crop. Increasing demand of leaves and essential oil in the national and international market calls for developing high yielding varieties with desired marker traits. For the first time, a selection with maximum no. of PGs per leaf and rich in essential oil content (DOS-1) was identified at the Directorate of Medicinal and Aromatic Plants Research, Anand, Gujarat. DOS-1 was also suitable for ratoon crops. DOS-1 was having a stem girth (1.3 cm), plant height (85 cm), plant spread (0.30 m^2), dry leaf recovery (23.10 %), plant spread (52 cm) and essential oil content from green herbage (0.65 %) as compare to Angna (check) which was having stem girth (1.6 cm), plant height (119 cm), plant spread (0.32 m^2) dry leaf recovery (21.0 %) and essential oil (0.35 %) content (Table A). The plant growth parameters and essential oil content could be some important parameters in tulsi which may be used to develop high yielding cultivars. Leaf, petiole, new branches and inflorescence are green in colour at the full flowering stage as compare to check (purplish). The maximum no. of PGs per leaf was observed in DOS-1 (8478) while minimum in Angna (4330) in leaf. The number of PGs were observed just double in DOS-1. The number of PGs and oil content having positive relationship in DOS-1/tulsi. The selection is stable, uniform and distinct and hence, may be used as an important allelic source to unravel genetics of growth, yield and quality variations. Further, these traits can be used as DUS character for identification of elite germplasm lines and cultivars

45. Sweet basil/ Rudra-2 (IC0627271; INGR18045), a Dwarf Basil (*Ocimum basilicum*) Germplasm with Early Flowering (27.75 days after transplanting).

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Basil (*Ocimum basilicum* L.) is one of the most important aromatic herb belongs to the family Lamiaceae. It originated from India and then reached to warm regions worldwide. In India, basil have been cultivated in Gujarat, Rajasthan, M.P. and U.P. in patches under approximately 2,160 ha area. Basil is cultivated extensively in France, Egypt, Iran, Hungary, Indonesia, Morocco, Greece and Israel, most in Mediterranean countries and in various regions with temperate and hot climates.

Now it is commercially grown for its essential oil, which are rich in three important essential oils *viz*., linalool (20-40 %), methyl chavicol (20-25 %) and eugenol (0-12 %). It also contains tannins and flavonoids. As per geographical origin and major constituents of basil, they are divided into four parts viz., European chemotype (linalool), reunion chemotype (estragole), tropical chemotype (methyl cinnamate) and eugenol chemotype (eugenol). Morphological and chemotypic characterization are the main criterion for selection of suitable morphotype for herbal industry. A wide variation was observed in Rudra-2 and GAB-1 of sweet basil based on their morphological traits. Two main morphotypes cultivated in India and adjoining regions are bunch type panicles and single panicles. The inflorescence was individual type (loose) and purplish green coloured in Rudra-2 as compare to GAB-1 which had bunchy (compact) and light purple coloured inflorescence. The variants with distinct characters' leaf size, plant height, spreads, canopy compactness and branches are available. The minimum growth parameters, especially plant height (53 cm), plant spread (37 cm), stem girth (1.10 cm), internodal space (3.26 cm), number of branches (10), leaf size (8.72 cm^2), leaf petiole length (1.08 cm) and earliest on set of flowering (27.75 days after transplanting) were observed in Rudra-2 as compare to variety GAB-1 which had plant height (105 cm), plant spread (64 cm), stem girth (1.89 cm), minimum internodal space (4.57 cm), number of branches (23), leaf size (12.45 cm^2), leaf petiole length (3.48 cm) and earliest on set of flowering of 39 days after transplanting.Reduced plant height is an important agronomic trait in basil breeding that has potential to be used for proper utilization of resources. Moreover, such traits permit higher plant populations under vertical and horizontal resources utilization. The basil, has an increased plant height as compared to a relatively restricted root system. Therefore, when it was grown in field where strong winds blew during the flowering period, plants suffered from lodging. The reduction in plant height could lead to enhanced lodging resistance. The selection is stable, uniform and distinct and hence, may be used as an important allelic source to unravel genetics of growth variations. Further, these traits can be used as DUS character for identification of elite germplasm lines and cultivars.

46. Sweet basil/ DOB-1 (IC0627272; INGR18046), a Basil (*Ocimum basilicum*) Germplasm with Upper leaf (Adaxial Surface) Puckering. Light Green Leaf Colour and. Maximum Number of PGs in Mature Leaf.

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Basil (*Ocimum basilicum* L.) known as sweet basil and one of the most important aromatic herb belonging to Lamiaceae (Labiatae) family, comprises perennial herbs and is native to tropical and sub-tropical regions of Asia, Africa and Central-South America. Medicinal plants' genetic resources with enormous diversity are

global assets of incalculable value to the present and future generations, and sufficient genetic variability is a prerequisite for efficient selection for crop improvement.

Basil, an economically important herb due to its essential oil, is used in hygiene and cleaning products, perfumes, cosmetics as a local anesthetic and antiseptics. Plant extracts are widely used in traditional medicines for its biologically active constituents that have insecticidal, nematicidal, fungistatic and antibacterial properties. The diverse signatures of basil in appearance, flavors, fragrances, industrial traits, edible and drying oils and natural pigments provide great opportunities for developing new culinary, ornamental and industrial crop with marker traits. Therefore, present registration was undertaken to elucidate the morphological markers with essential oil production in sweet basil. The inflorescence is individual type (loose) and purple in colour in DOB-1 as compare to GAB-1 which has bunchy (compact) and light purple in inflorescence colour. The variants with distinct combination of characters' upper side leaf (adaxial surface) puckering, light green upper leaf colour and maximum number of PGs (15.6/mm^2 leaf area) for high essential oil production in DOB-1 as compare to GAB-1 which had no puckering, green leaf colour and minimum number of PGs (6.4/mm^2 leaf area). The selection is stable, uniform and distinct and hence, may be used as an important allelic source to unravel genetics for morphological marker and quality traits. Further, these traits can be used as DUS character for identification of elite germplasm lines and cultivars.

GUIDELINES TO AUTHORS

GENERAL

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

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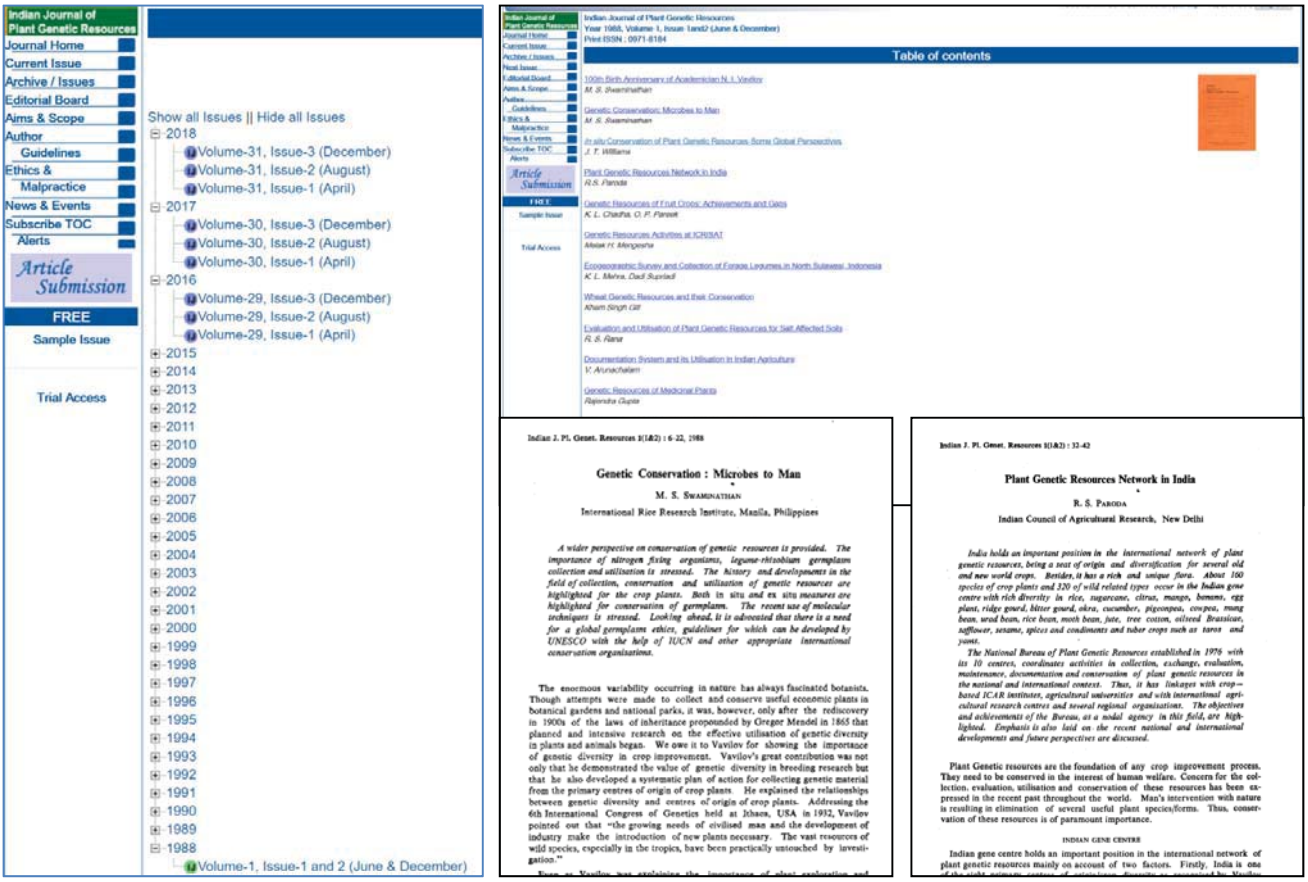

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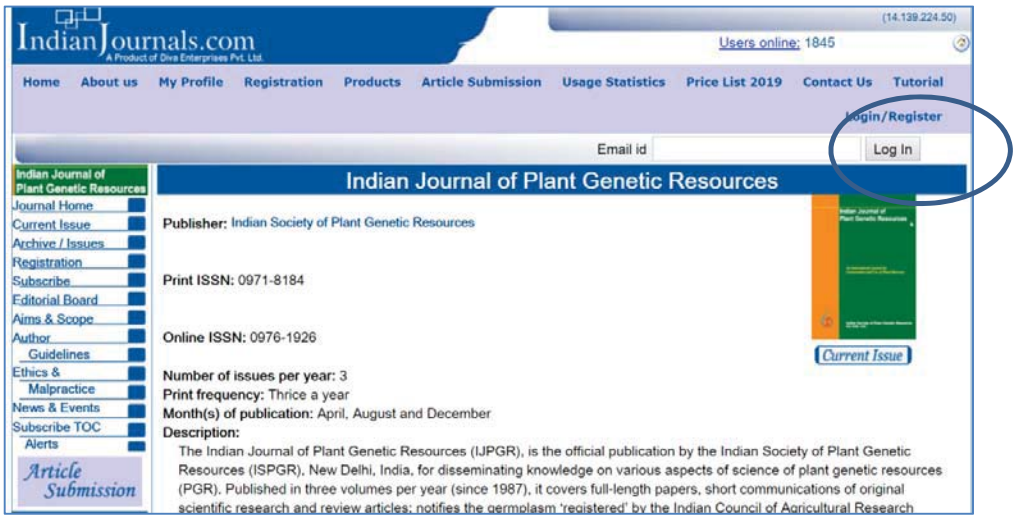

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