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- To provide a forum for organizing symposia/conferences with a view to develop close relationship among the scientists engaged and interested in plant genetic resources activities.

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INDIAN SOCIETY OF PLANT GENETIC RESOURCES

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CIMMYT's Seeds of Discovery Initiative: Harnessing Biodiversity for Food Security and Sustainable Development

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A visionary investment by Mexico's Secretariat of Agriculture, Livestock, Rural Development, Fisheries and Food (SAGARPA) has enabled the Seeds of Discovery (SeeD) initiative to assemble a platform of technologies to facilitate the effective use of genetic resources in breeding to address challenges of climate change and evolving consumer demands to ensuring universal food security. The platform consists of 1) high-density genotypic data and extensive phenotypic data characterizing maize and wheat germplasm bank accessions, 2) software tools to enable bioinformatics analyses of these and relevant germplasm bank data, and 3) maize and wheat lines incorporating novel diversity for priority traits from exotic germplasm into breeder-preferred, elite genetic backgrounds. Each of these platform components was co-developed with partners bringing unique expertise and resources to the project. Sustained impact, and equitable access to the platform and the maize and wheat genetic resources it describes, are pursued via multi-pronged capacity development and proactive intellectual property strategies described herein. After describing the SeeD initiative and some of its products, we conclude by sharing comments and insights from eminent scientists and partners who attended a special session on 'Harnessing Biodiversity for Food Security and Sustainable Development' during the 1st International Agrobiodiversity Congress (IAC), in New Delhi, November 2016.

Introduction

Humankind will soon face unprecedented challenges to ensuring its food security. Recent estimates by the United Nations (2017) project that global population will reach 9.8 billion by 2050, and 11.2 billion by 2100, and the world community is committed to ensuring that every person has access to a sufficient, nutritious diet (United Nations, 2015). Of course, the food for our growing population must be produced sustainably, to ensure the wellbeing of future generations. Making agriculture sustainable will require that we reverse trends that today are common in too many of our production areas, including depletion of aquifers, inefficient use of fertilizers, and degradation of soil health. Finally, the added challenges from changing climate, particularly rising temperatures and associated increasing frequency of extreme weather events (IPCC, 2014), make the goal of ensuring sustainable food security more daunting.

Although several actions will be crucial for meeting future food demand (Godfray *et al*., 2010), including reducing the current 30-40 percent global rates of food waste, sustainably enhancing agricultural productivity must be prioritized. Numerous examples have shown the value of crop biodiversity for developing improved varieties (Pimentel *et al*., 1997; Fig. 1). Similarly, and perhaps critically, genetic resources or biodiversity of crop plants will be valuable for the development of varieties that thrive in the emerging environments of the future.

Paradoxically, plant breeders are often reluctant to use wild relatives or unimproved collections of crop plants in their variety development programs. This reluctance is first because it is generally difficult to identify genetic resources that contain outstanding alleles for genes of interest, like searching for a needle in a haystack. Secondly, unimproved crop plants that contain such outstanding alleles also generally contain many undesired alleles for traits that the breeders have worked long and hard to improve in their elite lines. The International Maize and Wheat Improvement Center (CIMMYT) and the Mexican Government established the Seeds of Discovery (SeeD) initiative to address these

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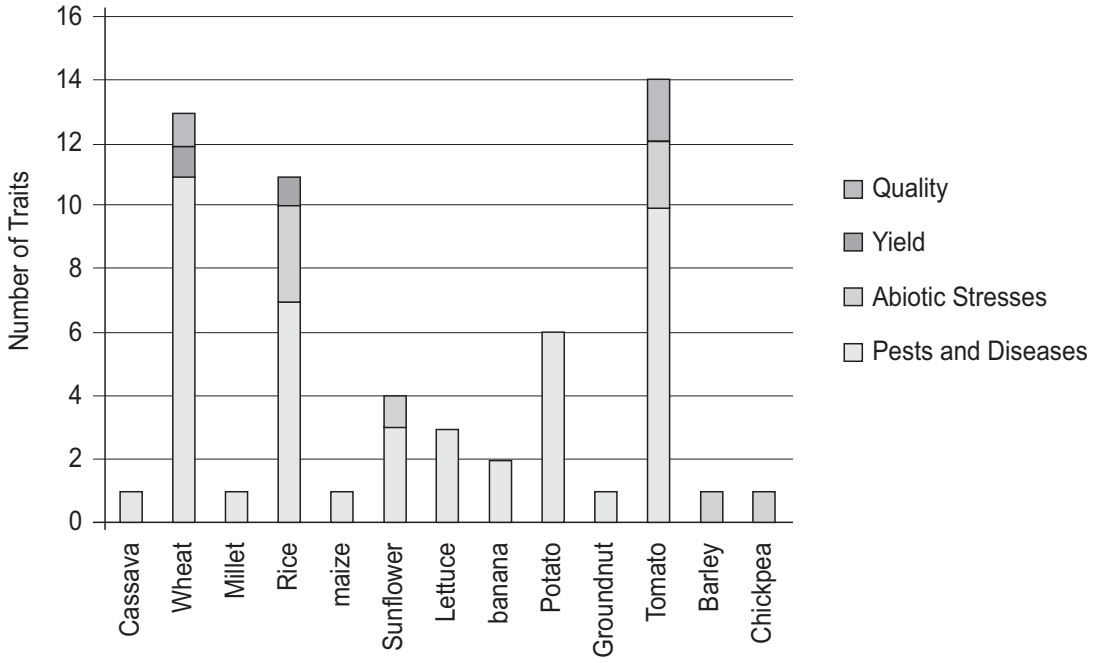

Fig. 1. Use of crop wild relatives in the past 20 years in released cultivars for 13 crops of international importance. Adapted from Hajjar and Hodgkin (2007)

important constraints to the use of maize and wheat biodiversity. The SeeD initiative aims to assemble a platform of technologies that facilitate the effective use of genetic resources to accelerate the development of improved crop varieties in breeding to address challenges of climate change and evolving needs of farmers and consumers.

This manuscript describes the SeeD initiative as a model that can inform other initiatives with similar objectives for other crops. Specifically, we will describe the SeeD strategy to enable more effective and equitable use of maize and wheat biodiversity, present selected results, and share feedback received from diverse partners and stakeholders.

Methods

Impact Pathway

The goal of the SeeD initiative is to enhance the use of novel genetic diversity held in germplasm banks to accelerate the development of maize and wheat varieties that meet the demands and contribute to food security of a growing population in a changing climate. SeeD develops products that when used by researchers will result in varieties grown by farmers to produce the food consumed by all. The SeeD initiative has five main components, described in this section.

- Genotyping, which produces data describing the genetic composition of germplasm bank collections;
- Phenotyping, which produces data describing the attributes of germplasm bank collections;
- Informatics, which produces software tools to capture, analyze and interpret the data;
- Pre-breeding, which produces improved wheat and maize germplasm, incorporating novel diversity from germplasm bank collections; and
- Capacity development, which produces public information, and provides training through various mechanisms including workshops and thesis projects for students.

The main users of SeeD's products are plant breeders, researchers, academics, and students. The beneficiaries of the SeeD initiative, however, include farmers, consumers, food and feed industry, advocates of equitable use of biodiversity, advocates of increased sustainability of farming systems, supporters of agricultural development, and society at large.

To achieve greater and equitable positive impacts in the shortest possible time, SeeD has applied "social network analysis" (Borgatti *et al*., 2013) to study the Mexican maize and wheat breeding and genetic resources

conservation systems. Using this approach, it has been possible to identify and collaborate with key players with greatest influence on the dissemination of knowledge and germplasm.

Genotyping

A cost-efficient genotyping service laboratory was established at CIMMYT headquarters and is available to SeeD partners. The laboratory uses the DArTSeq next generation sequencing technology (DArT, 2017), with which genotyping of CIMMYT's and some partner maize and wheat collections is underway. This platform produces several hundred thousand markers, both single nucleotide polymorphism (SNP) and presence absence variance (PAV) markers, for each genotype sequenced. This density of markers is ideal for studies of diversity, selection imprints, or to identify target genomic regions associated with traits of interest. About 28,000 maize and 60,000 wheat accessions in CIMMYT's germplasm bank have been sequenced, as have about 30,000 accessions from ICARDA's wheat and wild relatives collection.

Phenotyping

Phenotyping of wheat and maize germplasm bank accessions and pre-breeding lines seeks to identify useful donor alleles for traits prioritized by breeding programs (Table 1). The characterization of these prioritized traits is done mostly by SeeD partners who grow collaborative trials or nurseries; for example, a network of scientists in Mexico, India and Kenya are involved in the exploration of the wheat germplasm for resistance to yellow rust, stem rust, powdery-mildew and leaf spot diseases.

Informatics

Several partners have contributed to the suite of data storage, data management and data analysis tools assembled by the SeeD initiave (Fig. 2). The large volumes of genotypic and phenotypic data generated and

Table 1. Partial list of important breeding traits that have been evaluated by SeeD partners for various sub-sets of wheat or maize germplasm bank accession and pre-breeding lines

Wheat	Maize
Grain yield	Grain yield
Drought tolerance	Drought tolerance
Heat tolerance	Heat tolerance
Low soil phosphorus tolerance	Low soil nitrogen tolerance
Tan spot resistance	Tar spot complex resistance
Karnal bunt resistance	Turcicum blight resistance
Spot blotch resistance	Stalk rot resistance
Powdery mildew resistance	Ear rot resistance
Yellow rust resistance	Cercospora leaf spot resistance
Zinc concentration in grain	Carotenoid concentrations in grain
Iron concentration in grain	Root lodging resistance
Grain quality traits	Stem lodging resistance
Phenology	Phenology

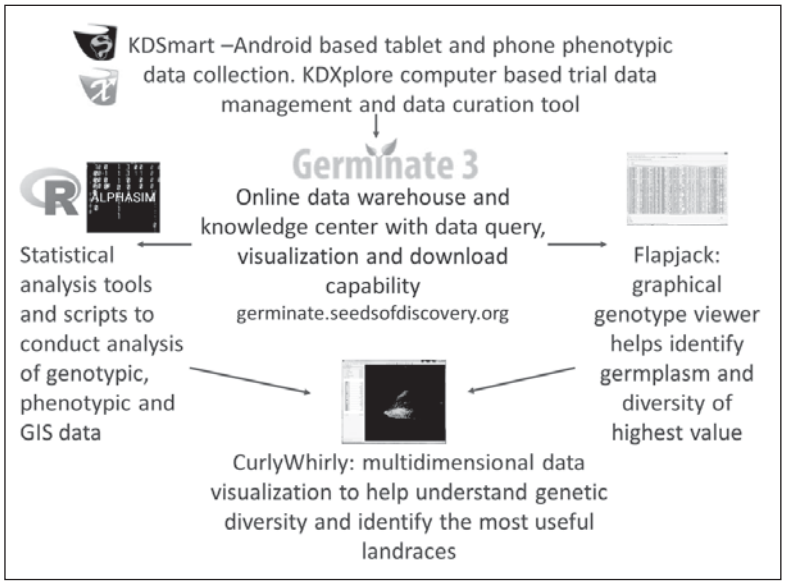

Fig. 2. Informatics components being assembled into a molecular atlas. KDSmart and DKXplore are co-developed with Diversity Arrays Technology (DArT, Pvt) and are useful for field collection, and data management and curation, respectively. Germinate is a data warehouse developed by the James Hutton Institute (JHI), while Flapjack and CurlyWhirly are genotypic data visualization tools co-developed with the JHI. R and AlphaSim are data analysis packages used to conduct the combined analysis of genotypic, phenotypic, GIS and other data. Figure credit: Sarah Hearne.

used in this platform exceed the capacities of common data spreadsheets, and necessitated the development or adaptation of specialized databases and software tools. In addition, the electronic collection and real-time curation of experimental data are important time-saving, cost-saving and error-reducing steps for which specialized tools were developed. All of these software tools are freely available (www.seedsofdiscovery.org). Work is ongoing to develop user-friendly interfaces that facilitate the use of this suite of tools. As a whole, these data and tools, referred to as a molecular atlas, can help a plant scientist to identify which accessions in the germplasm bank are most useful in breeding for a trait of interest. This suite of tools is analogous to a satellite navigation system that integrates diverse data – from maps, satellite imaging, user feedback, service providers, etc. – to assist the driver of a car to navigate efficiently from her current location to an unknown desired destination.

Pre-breeding

Wheat pre-breeding lines (PBLs) are developed using a three-way topcross strategy in which an exotic germplasm bank accession is crossed to an elite line, and the resulting F1 is crossed to a second elite line. The resulting populations are first advanced in bulk, and later by individual head selections with evaluation for grain yield and yield components under drought and heat at Obregon, Mexico, and mild selection for rust resistance and agronomic traits at El Batan or Toluca, Mexico. The resulting PBLs are shared with interested partners who evaluate them for biotic stresses (yellow rust, stem rust, powdery mildew and leaf spot diseases) and agronomic performance.

The maize pre-breeding strategy consists of identifying promising germplasm bank accessions for priority traits, especially drought and heat tolerance, and tar spot disease complex or maize lethal necrosis. The accessions are crossed with elite CIMMYT Maize lines (CMLs), and line development is either from these F1s or from back-cross populations using the elite line as recurrent parent. Line development then proceeds with selection for the primary trait plus general agronomic performance, and includes selection for combining ability by evaluating testcross hybrids of the experimental lines crossed to elite testers.

Capacity Development and Open Access for Equity in the Use of Genetic Resources

SeeD pursues sustained impact and equitable access to its genetic resources utilization platform and the maize and wheat genetic resources it describes, via multifaceted capacity development and proactive intellectual property strategies. Capacity development, including graduate student thesis projects, technical workshops, visiting scientist projects, and publically available software tools is essential to ensure that the current and next generations of researchers are able and keen to use SeeD's products. Capacity development is a cornerstone of SeeD's strategy to achieve equity in the use and benefit sharing from use of genetic resources. Empowering scientists from a broad range of public and private organizations to apply cutting-edge technologies and tools in the use of genetic resources to benefit their diverse range of client farmers and consumers, will increase equity in the access to and benefit from agrobiodiversity.

A proactive intellectual property strategy ensures that the data and tools developed by SeeD are freely accessible to all under a "one-click" agreement that prevents users from seeking intellectual property that would restrict others from using them. This policy forms a second cornerstone of SeeD's strategy in pursuit of equitable access and benefit sharing from genetic resources. The strategy seeks to facilitate widespread sharing of SeeD outputs and the benefits arising from their use by addressing two main objectives: 1) stimulate the innovative use of genetic resources in a pre-competitive and equitable environment, and 2) equitably share the benefits arising from the use of wheat and maize genetic resources with researchers, farmers and with the original germplasm custodians.

Results and Discussion

The SeeD initiative has produced a large number of products and services including germplasm, data, informatics tools, technical workshops, and publications; many of these are viewable in an on-line products catalogue (www.seedsofdiscovery.org). More than twenty international, refereed journal publications on methods or bioinformatics (Faux *et al*., 2016; Shaw *et al*., 2017; Swarts *et al*., 2014), wheat diversity and genetics (Crossa *et al.*, 2016; Li *et al*., 2015 and 2016; Lopes *et al*., 2015; Saint Pierre *et al.*, 2016; Sehgal *et al.*, 2015; Singh *et al*., 2016), and maize diversity and genetics (Brandenburg *et al.*, 2017; Chen *et al*., 2016; Figueroa *et al.*, 2013a and 2013b; Gorjanc *et al*., 2016; Hellin *et al*., 2014; Hickey *et al.*, 2014; Romero *et al*., 2017) are beginning to document the knowledge gained from SeeD collaborative work.

Partnerships have been crucial to every component of SeeD, for example, as described below by Dr. Anthony Hall of the Earlham Institute in the United Kingdom:

"Huge advances have been made over the last few years in our understanding of the wheat genome (IWGSC *et al.*, 2014; Clavijo *et al.*, 2017). Fortunately, these advances have been paralleled by huge investments by UK funding agencies, specifically the BBSRC (Biotechnology and Biological Sciences Research Council), which recently invested £35M in a cross-UK-institute program, "Designing Future Wheat". The SeeD initiative offers open access to genotype and phenotype data describing germplasm bank accessions, which are also publically available. These data are critical material as the global wheat research community moves from a single reference genome to exploring population scale genome diversity and developing tools capable of identifying genes playing major roles controlling important traits (Borrill *et al*., 2015). Within the SeeD initiative, a role of the Earlham Institute is to integrate new genomics technologies. By adding new genomic approaches, the global wheat research community can use SeeD material to ask fundamental and applied biological questions; for example: what genes have been selected during domestication and adaption to specific agricultural mega-environments? How much genetic variation exists for important genes within germplasm collections? Which allelic combinations deliver robust and sustainable yield under changing climate? In addition to these important questions, practically we can generate imputation methods to add more depth to existing genotypes (IWGSC et al., 2015), and we can also identify candidate genes and markers underlying the important traits brought into elite material from the SeeD initiative's diverse material (Gardiner *et al.*, 2016)."

Characterization of Wheat Genetic Resources

Nearly 100,000 wheat accessions from CIMMYT's germplasm bank have been evaluated in field trials and laboratory tests for different traits. This includes phenotypic evaluation of 70,000 accessions for heat and/or drought, 20,000 for grain quality traits, 10,000 for disease resistances, and 2,000 for phosphorus use efficiency. As expected, the accessions exhibited huge variability and encompass potential trait donors for drought tolerance, heat tolerance, grain quality, nutritional traits (e.g. zinc and iron content in grain), disease resistances and yield-related traits.

The genotyping and phenotyping efforts enabled various and ongoing bioinformatics analyses to understand and mobilize valuable diversity into breeding programs; for example, diversity analyses (Sehgal *et al*., 2015; Lopes *et al*., 2015) and the development of a wheat genetic map for use in further research (Li *et al*. 2015). Another study identified rare and potentially valuable allelic variations among more than 8,000 Mexican wheat landraces (Vikram *et al*., 2016). SeeD scientists subsequently formulated a unique strategy for selecting core sets of germplasm bank accessions. The approach incorporates genotypic and phenotypic variability and can capture up to 89% of rare allelic variation. This approach is the only published method that captures so much rare allelic variation (Vikram *et al*., 2016; Singh *et al*., 2016). Core sets of wheat germplasm bank accessions are available to the global wheat research community:

1. Mexican bread wheat landrace core set: 1,100 accessions selected to represent the approximately 10,000 Mexican wheat landraces held at CIMMYT. These landraces are descendants of wheat varieties brought to Mexico by the Spaniards during the colonial period. The core set was developed using 20,000 DArTSeq markers as genotypic data, combined with phenotypic data for several traits including days to heading, days to maturity, plant height, grain yield under heat, drought and irrigated conditions, and grain quality measures (Vikram *et al*., 2016).
2. Iranian wheat landrace core set: 249 accessions selected to represent the approximately 2,000 Iranian wheat landraces available from CIMMYT's germplasm bank. The core set was formed as described above for the Mexican bread wheat landrace core set.

Wheat and Maize Pre-breeding Germplasm

Wheat pre-breeding efforts have focused on developing drought and heat tolerant lines for use in wheat improvement. An array of pre-breeding germplasm, including about 15,000 pre-breeding lines (PBLs), has been developed, and partners in India, Mexico, Iran and Pakistan have evaluated about 2,000 PBLs for traits of interest to them (yellow rust, crown and root rot, powdery mildew, leaf spot diseases). The large-scale, multi-location characterization of diverse PBLs developed from a broad array of germplasm

bank accessions, is identifying valuable germplasm for current and future wheat improvement. Several wheat pre-breeding products are available from SeeD to the global wheat research community:

1. Wheat Drought Tolerant Pre-breeding Lines: These are wheat pre-breeding lines derived from top-crosses using one germplasm bank accession, crossed to an elite line, and then crossed to a second elite line. The accessions were selected as promising sources for use in breeding for drought tolerance, while the elite lines were recommended as the best available lines by CIMMYT wheat breeders. The experimental lines were selected based on field trials under drought stress and well-irrigated conditions in Mexico, with mild selection to eliminate material susceptible to yellow rust or with serious agronomic weaknesses.
2. Pre-breeding Wheat Heat Tolerant Pre-breeding Lines: These are wheat pre-breeding lines derived from top-crosses using one germplasm bank accession, crossed to an elite line, and then crossed to a second elite line. The lines were selected based on field trial results under heat stress and well-irrigated conditions in Mexico, with mild selection to eliminate material susceptible to yellow rust or with serious agronomic weaknesses.

Wheat Pre-breeding Lines with High Grain Yield: These are wheat pre-breeding lines derived from top-crosses using one germplasm bank accession, crossed to an elite line, and then crossed to a second elite line. The experimental lines were selected based on field trials under well-irrigated conditions in Mexico, with mild selection to eliminate material susceptible to yellow rust or with serious agronomic weaknesses. The lines may be useful in breeding for improved grain yield following further testing to confirm their performance in additional environments.

Maize pre-breeding efforts have focused on drought and/or heat tolerance, resistance to tar spot complex (TSC) disease, and more recently also on resistance to maize lethal necrosis (MLN). A small effort has also been invested to increase anthocyanin content of grain (for its nutritional value). The initial work on TSC identified two landraces in the germplasm bank that have subsequently been evaluated by smallholder farmers in the Mexican State of Oaxaca. The farmers are now using these landraces directly and in farmer-participatory improvement projects to enhance the TSC resistance of their landraces that are susceptible to this disease. Some of the maize pre-breeding products that are or will soon be available to the maize research and breeding community are described below (Terence Molnar and Sarah Hearne, personal communication, 2017).

1. Drought tolerant landraces: Germplasm bank landrace accessions that are promising for use in research and pre-breeding for drought tolerance at flowering and grain fill stages of the crop life cycle. Adaptation-based, e.g. lowland tropical or highland, panels have been developed comprising 500, 250, 100 and 50 landrace accessions that maximize and maintain representativeness of the diversity of the landrace collection.
2. Drought tolerant semi-inbred lines: Early generation lines developed through backcross breeding, which have demonstrated enhanced levels of drought tolerance at flowering and grainfill stages of crop growth. Drought tolerant landrace accessions were used as donors, and elite CIMMYT Maize Lines (CMLs) were used as recurrent parents.
3. Tar spot disease complex (TSC) tolerant semi-inbred lines: Early-generation lines developed through backcross breeding with demonstrated levels of TSC tolerance. Landrace accessions with useful levels of disease tolerance were used as donors, and elite CMLs were used as recurrent parents.
4. Heat tolerant landraces: Germplasm bank landrace accessions that are promising for use in research and pre-breeding for heat tolerance at flowering and grain fill stages of the crop life cycle. Adaptation-based, e.g. lowland tropical or highland, panels have been developed comprising 1000, 500, 250, 100 and 50 landrace accessions that maximize and maintain representativeness of the diversity of the landrace collection.
5. Maize blue kernel inbred lines: Lines developed through backcross breeding that have grain with enhanced levels of anthocyanin content. Landrace accessions with useful and stable levels of anthocyanin content were used as donors, and elite CMLs were used as recurrent parents.

Capacity Development for Equity in Access and Benefits from Genetic Resources

By the time of the 1st International Agrobiodiversity Congress (IAC), in November 2016, 238 researchers, faculty and students had participated in SeeD workshops,

13 bachelor, 8 MSc and 13 PhD students were enrolled or had graduated conducting research projects with SeeD, 5 Mexican scientists were working with SeeD scientists on specific projects of their choice, and researchers from 75% of the most important breeding organizations in Mexico had participated in capacity development activities facilitated by SeeD. These numbers continue to grow and provide evidence of demand for SeeD products that can lead to impact in farmers' fields and consumers' homes. Two PhD and one MSc student shared some of their experiences and learnings from working with the SeeD project during the "Harnessing biodiversity for food security and sustainable development" session of the IAC in New Delhi.

Ankita Suhalia, a doctoral student in botany and crop sciences at Punjab Agricultural University, spoke about her experience as a student within the SeeD initiative:

"My research work aims to dissect drought tolerant and salinity tolerant genotypes from Mexican landraces. This opportunity led to my personal as well as professional growth.

The conference proved to be a memorable and meaningful experience. The conference events were very useful in developing my skills and leadership abilities as a biological scientist. During the "Harnessing biodiversity for food security and sustainable development" session, I had my first time opportunity to speak in front of eminent scientists and subject leaders from national as well as international institutes. The talks by eminent scientists of the field regarding networking were also interesting and the speakers provided good material that I could share with the rest of the students back home about the importance of germplasm in wheat. It was very helpful for me in understanding the importance and quick use of germplasm for wheat improvement. The conference provided rare opportunities for me to get in touch with esteemed scientists and fellow students from other countries. During the sessions, I also had the chance to meet many people who have contributed much to the field and it was inspiring seeing so many like-minded biologists working to make the exploration of germplasm in detailed manner. Furthermore, attending the conference helped me build relationships with other biologists and students for future collaborations.""

Cynthia Ortiz attended the IAC, where she presented a poster and spoke about her experience as a master's degree student with Mexico's Center for Research and Advanced Studies (CINVESTAV) and SeeD:

"My master's research project focused on yellow rust disease, caused by Puccinia striiformis, which is one of the most devastating diseases of wheat, causing significant grain yield losses globally. The aim of my research was to identify genomic regions associated with yellow rust resistance in wheat bi-parental mapping population. The populations were genotyped using the DArTSeq technology, and genomic regions were identified that can be used to improve popular but susceptible wheat varieties using marker assisted breeding. Genomics assisted approaches offer promise for fast-track breeding in wheat to enhance yellow rust resistance and thereby grain yield.

This presentation was part of my continuing education and professional development, as well as an opportunity to share and disseminate the types of research and projects carried out at CIMMYT.""

Laura Bouvet, a postgraduate researcher in crop sciences from the University of Cambridge, UK, spoke about and presented her experience as an intern in capacity development with SeeD:

"Equitable access to genetic diversity and breeding material is at the core of SeeD. This project has been successful in reaching a significant number of scientists at different stages in their professional career. In addition to technical workshops and postgraduate student thesis projects, the initiative is exploring the option of an online training platform in genetic diversity, to reach a higher number of individuals. My role as an intern was to develop the structure of such a training platform and generate educational and promotional material. This included a video explaining the DArTSeq genotyping method used in SeeD, as part of a module on genotypic data, and a video showcasing the benefits of the training platform to different stakeholders.

Working closely with SeeD scientists and across CIMMYT departments, I learnt about the multi-disciplinary and collaborative nature of a large-scale project such as SeeD, two aspects that underlie many crop science projects nowadays. Furthermore, contributing to the capacity building strategy of the project has given me valuable insight into its increasingly important role in agricultural science.""

Part of the SeeD strategy is to invite scientists to apply for visiting scientist opportunities, during which they work with SeeD scientists to apply the tools and products of SeeD to benefit their own programs. A

total of 20 such projects have been initiated. In 2016, SeeD received 11 proposals from Mexican scientists interested to work on such projects, of which five were funded. Topics were characterization of yellow rust resistance of Mexican wheat accessions; genomic characterization of maize accessions in the University of Guadalajara germplasm bank; development and validation of bioinformatics models to select germplasm bank accessions based on allele frequency; genetic resources for forage maize for highland Mexico; and, analysis of pigmented maize landraces. Nine applications were received in 2017, including two on popcorn, one on nutritional quality of maize, one on heterotic patterns for maize, one on Karnal bunt resistance in wheat, and two on diversity and genomics of coffee. The proposals for work on coffee were funded by a special grant from the Mexican Government to the Mexican coffee research institution, which was keen to implement a project similar to SeeD for coffee.

Comments about SeeD from Eminent Scientists and Partners

Several distinguished crop scientists and representatives from international agricultural research funding agencies

Table 2. Comments from participants in the 'Harnessing biodiversity for food security and sustainable development' session of the 1st International Agrobiodiversity Congress, New Delhi, 6-9 November 2016. NB: These comments were recovered from notes taken by the authors during this event, and the authors regret any inaccuracy we may have committed in capturing the intended messages from these participants.

Dr. BS Dhillon, Vice Chancellor, Punjab Agricultural University, Ludhiana	The green revolution had impact due to a number of factors, but the basis was germplasm strength. Germplasm is like a dowry. Elite × elite crosses have strength worldwide, including India, USA and other countries, but considering climate change, change in cropping/farming patterns and other emerging needs, we should look beyond elite germplasm i.e. exotic × elite crosses (pre-breeding).
Dr. RK Singh, Former Country Representative, International Rice Research Institute	Climate change is prominent in Eastern India and this is the reason we need more genetic variation in farmers fields. Broadening of the cultivated wheat gene pool through pre-breeding is urgently required. As an example, "Sahbhagi dhan" rice variety came from a similar approach and has had significant impact in rain-fed regions of Eastern India.
Dr. Steve Visscher, Biotechnology and Biological Sciences Research Council (BBSRC), UK	There is need for partnership with NARS in target ecosystems. It is important for SeeD scientists to convince the donors of how investment will have impact in farmers' fields. Three challenges for the SeeD project are: 1) Convey the work of SeeD in layman words; 2) what are the impacts of SeeD on breeding programs, and 3) what are the real potential and practical impacts of SeeD. Overall, SeeD should channel its investments for impact at farmer field level.
Ms. Lisa Wilson, United States Agency for International Development (USAID), USA	To work with USAID, remember what our mandates are... and SeeD addresses plenty of them. There is need and opportunity to increase partnerships in low andmiddle income countries for a wider impact of the SeeD project; for example, Bangladesh and also in African countries.
Pawan Kumar Agrawal, Indian Council of Agricultural Research (ICAR) representative	Clarity is required about "how to deliver impact at the farmer field level".
Dr. NN Singh, Secretary, Trust for Advancement in Agricultural Sciences	Appreciated the wheat pre breeding efforts ongoing in the SeeD-Wheat project. System change is dynamic and demand is changing. A bottom up approach is required to address this. Collection of the on-farm diversity and close association of farmers with breeders in wheat pre-breeding should be impactful. Large scale involvement of rural youth and women is instrumental in the process.
Dr. Kuldeep Singh, Director, National Bureau of Plant Genetic Resources (NBPGR), India	Breeders too often state that "Biotech is taking money from breeding." On the contrary, biotech is helping the use GeneBank germplasm for wheat prebreeding For example, wheat landrace core formulation by NBPGR as well as Mexican wheat landrace core development in SeeD were done using high density genomics.
Dr. Ravi Singh, Distinguished Scientist, wheat breeder, CIMMYT	"In breeding, when you bring something good, you also bring bad things. Need to understand the genes underlying the traits. Learn the genetics, then mine them carefully to avoid one step forward, two steps back."
Dr. Nagendra Kumar Singh, National Professor, National Research Centre for Plant Biotechnology, India	It is important to break unwanted linkage of gene(s)/QTL(s)/traits with desirable ones prior to prebreeding. As an example in rice, an unholy linkage between drought QTL (qDTY 1.1) and green revolution gene (sd1) has been broken, and semi-dwarf genotypes developed that are being utilized in broadening the genetic base. Similar efforts need to be followed in wheat prebreeding, and genomics approaches can help in this efficiently.
Dr. Sanjaya Rajaram, 2014 World Food Prize laureate	"The program was very well presented, including integration of students. Is there a role for old cytogenetics tools in bringing diversity – translocations?" He suggested to utilize cytogenetics tools and to incorporate "red grain × white grain" crosses in the SeeD wheat prebreeding work to widen the gene pool.
Dr. Gurdev Kush, 1996 World Food Prize laureate	"My only regret is that if I were 30 years younger I would be able to use these technologies." He suggested to utilize all possible technologies in an intelligible fashion for developing prebreeding germplasm. His remarkable contribution in rice i.e. "IR64" was developed through a similar approach.

offered insights, suggestions and comments about the SeeD initiative during the discussion session at the IAC in New Delhi (Table 2). Two scientists mentioned climate change, and the need for agrobiodiversity and pre-breeding to meet the challenges it will present. Four breeders reminded the audience of the large challenges when using exotic (e.g. germplasm bank accessions) material in breeding programs, and of the value of using genetic tools, as in SeeD, to accelerate this process. The 1996 World Food Laureate, Dr. G. Kush lamented that such technologies as used in SeeD were not available to him when he was active as a breeder. Finally, two donor agency representatives reminded us of the need to always show value or return to investments, especially in farmers' fields; and indicated that SeeD addresses many of their priorities, and that there are opportunities to increase partnerships in low and mid-income countries for a wider impact of SeeD.

Conclusion

The SeeD initiative offers an innovative model for enhancing the efficiency and equity of use of genetic resources. This model can be adopted and improved for other crops, as indeed is now happening for coffee in Mexico. Many of the tools and much of the data produced by SeeD can be used for scientific discovery and be applied across many crops. The strategies of the SeeD initiative, outlined herein, are designed to increase not only the use of agrobiodiversity to address challenges to food security, e.g. from climate change, but also to foster equity in the access to and benefits from crop agrobiodiversity. While it is too soon to attempt measuring impacts of SeeD, preliminary outcomes are encouraging, including that large numbers of students and scientists are using the products of SeeD. The observation by a representative of a major international agricultural research funding organization, that there are opportunities to increase impacts of SeeD to benefit low and mid-income countries, raises hopes that resources will be secured to do so.

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Genetic Variability, Heritability and Diversity for Yield Contributing Traits in Reference Varieties of Wheat

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Genetic variability study in 129 wheat varieties (80 bread and 49 durum wheat) revealed high magnitude of variability and high degree of heritability for majority of yield associated traits. Difference between the PCV and GCV was high for harvest index followed by number of grains per spike and grain yield/plant indicating the influence of environment on their expression. High magnitude of genotypic coefficient of variation (≥ 17.0%) was observed for spike length, plant height, grain yield/plant and flag leaf length, which indicates high degree of genetic variability and offers great scope for selection of these characters. High to moderate heritability coupled with high to moderate genetic advance as percentage of mean was exhibited for 7 traits *viz.,* days to flowering, days to maturity, plant height, number of tillers/plant, biomass/plant, flag leaf length and 1000 grain weight, which indicated the predominance of additive gene action in the expression of these characters and very high possibility of improvement through selection for all these characters. Bread wheat varieties *viz.,* CPAN 3004, GW 322, HI 617, HI 1500, DWR 39, DWR 195, GW 89, UP 2113, NP 700 and NP 771 and durum wheat varieties *viz.,* A-28, HI 8381, MPO 215, HD 4530, HD 4672, NIDW 295, Raj 1555 and UAS 415 were found to be superior for grain yield and other traits along with being divergent from other lines, which can be utilized as donors for developing superior genotypes with high grain yield.

Key Words: Wheat, Germplasm, Variability, Diversity, Gene action

Introduction

Wheat is one of the most important staple food grains of human race grown under diverse agro-climatic conditions, occupying nearly 29.65 million ha with a record production of 95.85 million tonnes during 2013-14 in India (AICW&BP Progress report 2013-14). Besides, common wheat, durum wheat (*Triticum durum Desf.*), occupies 5% and *dicoccum* wheat occupies one percent of production in our country. To achieve surplus production with limited land available, genotypes having high productivity and quality traits should be developed through genetic improvement. For a successful breeding programme, the evaluation of germplasm (reference varieties) for assessing genetic variability using phenotypic and genotypic co-efficient of variation, heritability, genetic advance and diversity for yield and yield components is a pre-requisite. Hence, knowledge of nature and magnitude of genetic variability present in germplasm and the extent of heritability of the economic traits is of greater help in identifying the parents with novelty and diversity for planning a suitable breeding strategy.

Grain yield is the complex trait involving many yield components and influenced by genotype, agro-ecological conditions and their interactions. In any selection programme, emphasis on yield and its component characters lies solely on their heritability and genetic advance. High heritability alone is not enough to make efficient selection, unless the information is accompanied by substantial amount of genetic variation (Johnson *et al*., 1955). Success in recombination breeding depends on suitable selection of best parents, exploitation of high heterotic crosses and selection of transgressive segregants. The study was conducted with an objective to find out the extent of variability, heritability, genetic advance and diversity among the available reference varieties of wheat including both bread and durum wheat.

Materials and Methods

The experimental material comprised of 129 wheat varieties (80 bread wheat, 49 *durum* and *dicoccum* wheat), which were evaluated in a randomized block design with two replications; each plot is represented by two rows of 2.5 m long and 23 cm apart under timely

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sown conditions at Directorate of Soybean Research Farm under the supervision of ICAR-Indian Agricultural Research Institute, Regional Station. Recommended package of practices were followed for the genotypes, so as to make them to express their full potential. The data of 13 grain yield and its contributing components were recorded on 5 randomly selected plants from each replication. The mean replicated data on various biometric traits were subjected to analysis of variance as per the standard statistical procedure (Panse and Sukhatme, 1985). Phenotypic and genotypic components of variance were estimated as per the formulae suggested by Lush (1940). Estimates of phenotypic and genotypic coefficients of variation were calculated as per the standard formulae (Burton, 1952). The broad sense heritability was estimated for all the characters as the ratio of genotypic variance to total or phenotypic variance (Lush, 1940). The expected genetic gain or advance under selection for each character was estimated by following the method suggested by Johnson *et al.* (1955).

Results and Discussion

The mean sum of squares based on ANOVA of 129 wheat genotypes (bread, *durum* and *dicoccum* wheat) for 13 characters indicated the presence of high amount of variability among the genotypes. The coefficient of variation (CV) in pooled analysis ranged from 0.5 (days to maturity) to 15.9 (no. of grains/spike). The values of phenotypic coefficient of variation were higher than that of the genotypic coefficient of variation for all the characters (Table 1). The phenotypic coefficient of variation was estimated to be high for grain yield/plant (24.3) followed by number of grains per spike (22.0), biomass/plant (19.6), harvest index (19.5), number of tillers/plant (19.4) and spike length (19.4). These results were in accordance with the findings of Koksal Yagd *et al.* (2007) and Bhoite *et al.* (2008). Genotypic coefficient of variation also showed the similar trend for some of the traits and was observed to be high for spike length (19.1) followed by plant height (18.9), grain yield per plant (18.8) and flag leaf length (17.1) indicating high degree of genetic variability offering great scope for selection of these characters (Yagdi and Sozen, 2009; Riaz-Ud-Din *et al.,* 2010). Difference between the PCV and GCV was high for harvest index followed by number of grains per spike and grain yield/plant indicating the presence of environmental influence on the expression of these traits. Significant variability for characters under study among genotypes represents availability of divergent genetic sources which will be useful in improving grain yield in wheat (Mohammed *et al.,* 2011; Mostafa *et al.,* 2012).

Heritability in broad sense was observed to be high for days to flowering (0.98) followed by days to maturity (0.98), plant height (0.98); and spike length (0.97); while, low for number of grains /spike (0.47) and harvest index (0.40). Genetic advance was found to be highest for plant height (39.5) followed by days to flowering (12.6), 1000 grain weight (11.0) and biomass yield (11.0). Whereas, genetic advance over its mean is high for number of grains per spike (60.4) followed by

Table 1. Estimates of genetic variability for various characters of wheat

Character	Bread wheat			Durum & dicoccum wheat			PCV %	GCV %	h2	GA	GA % over mean
	Mean	Min	Max	Mean	Min	Max					
Days to flowering	75	59	92	78	70	90	8.2	8.1	0.98	12.6	16.6
Days to maturity	114	105	123	115	109	123	3.6	3.4	0.98	8.4	7.3
Plant height (cm)	102	58	145	102	80	139	19.1	18.9	0.98	39.5	38.8
No. of tillers/plant	9.0	6.2	12.9	8.9	6.4	13.3	19.4	16.1	0.69	2.5	27.8
Flag leaf length (cm)	22.2	14.7	31.5	21.4	14.8	28.3	17.6	17.1	0.95	7.6	34.7
Spike length (cm)	8.8	6.3	12.2	6.5	4.4	8.5	19.4	19.1	0.97	3.1	38.8
No. of spikelet/spike	16.0	11.4	20.0	17.3	14.6	20.1	13.4	10.4	0.61	2.7	16.5
No. of grains/spike	39.7	25.1	58.9	41.5	26.3	61.1	22.0	15.1	0.47	8.7	60.4
Hectoliter weight (kg/l)	76.5	64.0	81.5	77.6	66.0	84.5	4.7	4.4	0.88	6.6	8.6
1000 grain weight (g)	42.6	30.9	53.6	49.8	37.9	58.1	13.9	12.8	0.65	11.0	24.2
Harvest index	39.4	27.3	68.0	40.9	27.7	52.0	19.5	12.4	0.40	6.4	16.0
Biomass/plant (g)	41.7	27.1	63.0	39.5	28.1	52.9	19.6	16.0	0.66	11.0	26.9
Grain yield/plant (g)	16.2	10.7	32.6	15.5	10.2	20.3	24.3	18.8	0.60	4.5	28.3

spike length (38.8) and flag leaf length (34.7) (Table 1).These results were in accordance with the findings of Payal-Saxena *et al.* (2007) and Rashidi *et al.* (2011). High to moderate heritability coupled with high to moderate genetic advance as percentage of mean was exhibited for 7 traits *viz.,* days to flowering, days to maturity, plant height, no. of tillers/plant, biomass/plant, flag leaf length and 1000 grain weight, which indicates the predominance of additive gene action in the expression of these characters and consequently greater chance of improving these traits through simple selection. Spike length, no. of spikelets/spike and grain yield per plant showed high to moderate heritability with low genetic advance, which suggested that the presence of non-additive gene action and hence, improvement of these traits could be difficult through direct selection.

On the basis of mean performance of the varieties, three varieties *viz.,* CPAN 3004, GW 322 and HI 617 showed highest grain yield/plant, while HI 1500, GW 322 and NP 846 had showed highest harvest index. Varieties CPAN 3004, HI 617 and DWR 39 showed highest biomass yield/plant, and highest 1000 grain weight was observed in PDW 274 followed by WH 896 and NP 404. Varieties HD 2270, HPW 89 and HS 207 were found to have high flag leaf length, while, K 9644, GW 89 and HPW 89 has high spike length. Varieties NIDW 15, HD 2285 and GW 89 were having highest no. of grains/spike, while, PDW 233, WL 896 and PDW 215 has highest hectoliter weight. Wheat varieties HD 1941, WH 542, WR 544, HD 2285 and NP-4 were observed to be flowered and matured early compared to all other genotypes. Varieties Lal Bahadur followed by HD 1949 and GW 173 were found to be short stature in comparison to other ones under study. Varieties with high number of tillers/plant were NIDW 295, WL 291 and UAS 145, while, high no. of spikelets/spike were seen in CPAN 3004, DDK 1001 and VL 738 (Table 2).

The quantitative assessment of genetic divergence among reference varieties based on grain yield and its contributing traits was carried out using Mahalanobis' D^2statistic. The varieties were grouped into eight clusters each, when bread and durum and *dicoccum* varieties were considered separately and into 12 groups under pooled conditions, indicating high levels of genetic variability existing among them. Plant height followed by days to flowering, no. of grains/spike, grain yield/plant and biomass/plant contributed the most towards divergence among genotypes (Gartan and Mittal 2003, and Sidharthan and Malik, 2006).

Among bread wheat varieties, the average intra-cluster distance (Table 3) varied from 5.1 to 6.8. Cluster VI contained maximum number of genotypes (15) followed by cluster I (12), cluster VII (12), while cluster V contained five genotypes (Table 5). Maximum inter-cluster distance was reported between cluster IV and V (31.6) followed by cluster I & IV (26.5) and cluster III & V (20.4) revealing wide diversity among the varieties included in these clusters. The cluster means showed that varieties with more no. of grains/spike, high harvest index, high biomass yield/plant along with high grain yield/plant were observed in cluster IV, whereas dwarf genotypes with high hectoliter weight were observed in cluster VII. Early flowering genotypes with high 1000 grain weight were present in cluster I. Based on the genetic diversity and per se performance,

Table 2. Superior wheat genotypes among the reference varieties for various characters

Characters	Genotype
Days to flowering	HD 1941, WH 542, WR 544, HS 277 and HD 2278
Days to maturity	WR 544, HD 2285, NP 4, NP 52 and NP 770
Plant height (cm)	LalBahadur , HD 1949, GW 173, HD 1940 and PBW 222
No. of tillers/plant	NIDW 295, WH 291, UAS 415, NP 152 and PBW 222
Flag leaf length (cm)	HD 2270,HPW 89, HS 207, DWL 5023 AND K 9107
Spike length (cm)	K 9644, GW 89, HPW 89, K 9006 and K 68
No. of spikelets/spike	CPAN 3004, DDK 1001, VL 738, Bijaga yellow and PBW 34
No. of grains/spike	NIDW 15, HD 2285, GW 89, CPAN 179 and DWR 1006
Hectolitre weight (kg/l)	PDW 233, WH 896, PDW 215, HD 4672 and HD 2270
1000 grain weight (g)	PDW 274, WH 896, NP 404, DWR 185 and MACS 9
Harvest index	HI 1500, GW 322, NP 846, CPAN 3004 and PDW 215,
Biomass/plant (g)	CPAN 3004, HI 617, DWR 39, HD 1941, and DL 803-2
Grain yield/plant (g)	CPAN 3004, GW 322, HI 617, HI 1500 and DWR 39

Table 3. Average intra and inter-cluster D^2 values between the clusters in bread wheat

Clusters	I	II	III	IV	V	VI	VII	VIII
Bread wheat								
I	5.8							
II	9.1	6.5						
III	11.2	12.6	5.1					
IV	26.5	17.1	12.5	6.8				
V	11.4	15.4	20.4	31.6	5.4			
VI	14.3	15.8	13.0	15.4	16.3	6.4		
VII	13.0	17.8	16.3	31.2	16.5	18.7	6.4	
VIII	10.1	11.4	10.2	14.9	11.5	14.6	15.7	5.6
Durum and dicoccum wheat								
I	6.2							
II	23.3	5.5						
III	19.3	25.9	3.6					
IV	26.3	11.4	26.8	6.4				
V	14.0	11.7	24.6	26.4	4.9			
VI	23.7	11.7	27.0	27.8	16.2	4.9		
VII	14.6	17.1	16.5	23.5	10.6	20.0	4.5	
VIII	26.9	8.1	18.5	17.6	16.5	13.0	9.6	6.0

Table 4. Average intra and inter-cluster D^2 values between the clusters in pooled genotypes

Clusters	I	II	III	IV	V	VI	VII	VIII	IX	X	**XI**	**XII**
I	6.5											
II	14.6	5.1										
III	9.9	15.4	6.2									
IV	39.1	69.8	46.7	0.0								
V	16.5	18.6	10.5	59.4	6.7							
VI	15.5	15.5	16.0	52.3	19.7	5.4						
VII	14.2	16.6	21.5	55.3	13.0	13.4	6.1					
VIII	32.8	27.9	32.8	37.4	38.4	26.8	35.2	8.1				
IX	14.7	10.0	21.2	59.5	10.2	23.1	9.2	36.3	4.7			
X	12.9	9.3	24.0	55.4	21.9	9.5	10.0	26.2	10.4	5.7		
XI	16.0	12.0	11.1	61.2	10.1	20.0	16.0	29.2	11.8	11.3	7.2	
XII	18.3	15.5	11.2	41.8	19.4	18.4	13.7	10.4	19.0	24.5	13.8	6.3

it was observed that bread wheat varieties like CPAN 3004, HI 617, DWR 39, DWR 195, GW 89 of cluster IV; and UP 2113, NP 700 and NP 771 of cluster V can be used as donors for high grain yield along with yield contributing traits.

Among *durum* and *dicoccum* wheat genotypes, the average intra-cluster distance (Table 3) varied from 3.6 to 6.4. Cluster II contained the maximum number of varieties (10) followed by clusters V and VIII (8 each), while cluster IV contained three genotypes (Table 5). Maximum inter-cluster distance was reported between cluster IV and VI (27.8) followed by cluster III & VI (27.0) and cluster I & VIII (26.9) revealing that the genotypes included in these clusters were genetically more divergent. The cluster means showed that early maturing varieties with high 1000 grain weight were observed in cluster V, whereas dwarf varieties with high no. of tillers/plant, no. of grains/spike and high biomass yield/plant were observed in cluster VI. Varieties with high flag leaf length and no. of spikelets/spike along with high grain yield/plant were present in cluster VIII. Based on the genetic diversity and per se performance, it was observed that varieties like A-28, HI 8381 and MPO 215 of cluster IV and HD 4530, HD 4672, NIDW 295, Raj 1555 and UAS 415 of cluster VI can be used as donors for high grain yield along with yield contributing traits.

In pooled analysis (bread, *durum* and *dicoccum* wheat), the average intra-cluster distance (Table 4) varied from 0.0 to 8.1. Cluster VI contained maximum number

Table 5. Grouping of wheat genotypes into different clusters

Cluster	No. of genotypes	Grouped genotypes
Bread wheat		
I	12	AKW 381, HB 208, HD1941, HD 1949, HD 2278, HD 2327, HW 2004, K 8962, K 9465, LOK 1, RIDLEY, WH 542
II	11	AKW 1071, DWR 162, HP 1493, HPW 89 HS 207, HYB 633, K 9006, K9107, K 9644, VL 421, NP 839
III	7	DL 153-2, HD 2428, HI 1500, HP 1731, K 68, K 816, VL 829
IV	7	CPAN 3004, DL 803-2, DWR 39, DWR 195, GW 89, GW 322, HI 617
V	5	NP 4, NP 52, NP 770, NP 771, UP 2113
VI	15	C 306, CPAN 1796, HD 2733, HD 2781, HD 2824, HPW 242, HUW 234, PBW 222, PBW 299, PBW 343, RAJMOLYA RODHAK , SONARA 64, VL 616, VL 738, VL 832
VII	12	DL 784-2, DL 788-2, GW 173, HD 2270, HD 2281, HD 2285, HD 2329, HD 2402, LAL BAHADUR, NP 846, WH 283, WR 544
VIII	11	HD 2236, HI 977, HP 1744, HS 277, HS 295, KALYAN SONA, PBW 373, RAJ 1482, SONALIKA, VL 404, WH 291
Durum and dicoccum wheat		
I	4	BIJAGA RED,GW 1139, HD 4502, MACS 1967,
II	10	MACS 2694, MPO 1106, NIDW 15, PBW 34, PBW 215, PBW 233, PBW 274, PBW 291, RAJ 6560,WH 896
III	4	DDK 1009, DDK 1029, HW 1095, NP 200
IV	3	A-28, HI 8381, MPO 215,
V	8	GW-2, HI 8498, JU-12, MACS-9, MACS 2846, MACS 3125, N-59, NI 5749
VI	5	HD 4530, HD 4672, NIDW 295, RAJ 1555, UAS 415,
VII	7	A-9-30-1, DWR 137, GW-1, HI 7483, JNK-4W-184, NP 404
VIII	8	AKDW 2997-1, BIJAGA YELLOW, DDK 1001, DDK 1025, DWL 5023, DWR 185, RAJ 911, WH 912

Table 6. Grouping of different wheat genotypes (pooled) into various clusters

Cluster	No. of genotypes	Grouped genotypes
I	8	HUW 234, RAJMOLYA, DDK 1001, DDK 1009, DDK 1025, DDK 1029, HW 1095, NP 200
II	7	AKW 1071, BIJAGA YELLOW, HD 4530, HD 4672, NIDW 295, RAJ 1555, UAS 415
III	16	AKW 381, HP 1493, HPW 89, HS 207, K 9006, K 9107, K 9465, NP 771, VL 421, NP 839, VL 829, A-9-30-1, BIJAGA RED, DWR 137, GW 1, HI 7483
IV	1	HI 1500
V	16	DL 784-2, DL 788-2, HB 208, HD 1941, HD 2278, HD 2281, HD 2327, HD 2329, HD 2402, K 8962, LAL BAHADUR, LOK 1, NP 846, RIDLEY, WH 283, WR 544
VI	18	A-28, AKDW 2997, DWL 5032, DWR 185, HI 8381, MACS 2694, MPO 215, MPO 1106, NIDW 15, PBW 34, PDW 215, PDW 233, PDW 274, PDW 291, RAJ 911, RAJ 6560, WH 896, WH 912
VII	13	GW 173, HD 1949, HD 2236, HD 2270, HD 2285, HD 2781, HP 1731, HS 295, HW 2004, K 816, KALYAN SONA, HD 4502, JNK-4W-184
VIII	4	CPAN 3004, DL 153-2, GW 322, HI 617
IX	12	WH 542, GW 2, GW 1139, HI 8498, JU 12, MACS -9, MACS 1967, MACS 2846, MACS 3125, N 59, NI 5749, NP 404
X	14	C 306, CPAN 1796, DWR 162, HD 2824, HPW 42, HYB 633, PBW 222, PBW 299, PBW 343, SONARA 64, VL 616, VL 738, VL 829, DWR 1006
XI	11	HP 1744, HS 277, NP 4, NP 52, NP 770, PBW 373, RAJ 1482, SONALIKA, UP 2113, VL 404, WH 291
XII	09	DL 803-2, DWR 39, DWR 195, GW 89, HD 2428, HD 2733, HI 977, K 68, K 9644

of varieties (18) followed by cluster III (16), cluster V (16), while, cluster IV contains only one variety (Table 6). Maximum inter-cluster distance was reported between cluster II and IV (69.8) followed by cluster IV and XI (61.2), and cluster IV and IX (59.5), while minimum between cluster VII and IX (9.2). Cluster means showed that earliest maturity varieties with dwarf plant types were observed in cluster V, whereas, varieties with high biomass yield/plant and high grain yield/plant were observed in cluster VIII. Varieties with high flag leaf length and high harvest index were present in cluster IV. Based on the genetic diversity and per se performance, it was observed that varieties like AKAW 1071, Bijaga Yellow, HD 4530, HD 4672, NIDW 295, Raj 1555 and UAS 415 of cluster II; WH 291, PBW 373, Raj 1482, NP 770, NP 4, NP 52 and HS 277 of cluster XI; and HI 1500 of cluster IV can be used as donors for high grain yield along with yield contributing traits.

Therefore, as a whole, bread wheat varieties *viz.,* CPAN 3004, GW 322, HI 617, HI 1500, DWR 39, DWR 195, GW 89, UP 2113, NP 700 and NP 771; and durum wheat varieties *viz.,* A-28, HI 8381, MPO 215, HD 4530, HD 4672, NIDW 295, Raj 1555 and UAS 415 can be used as donors to cross among them for developing superior genotypes with high yield in wheat breeding programmes.

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Evaluation of Genetic Diversity in *Saccharum* Species Clones and Commercial Varieties Employing Molecular (SSR) and Physiological Markers

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Genetic diversity of the 38 sugarcane (*Saccharum* spp.) genotypes was evaluated using 50 microsatellite (SSR) markers and seven morphological markers. A complex PCR banding pattern was observed in all the accessions with SSRs markers. The allelic polymorphism information content (PIC) values ranged from 0.105 to 0.790 with an average of 0.37, indicating markers ability to detect high levels of polymorphism. The value of genetic similarity (GS) co-efficient ranged from 0.33 to 0.84, indicated a broad genetic diversity within sugarcane genotypes. Genetic similarity co-efficient indicated low level of genetic diversity among the *S. Officinarum* (0.84 similarity), relatively medium level of genetic diversity in *S. spontaneum* clones (0.78 similarity), and higher degree of genetic diversity in the *S. barberi* clones, and ISH genotypes (0.77 similarity). The SSRs derived from sugarcane were found to be more informative then the transferred SSRs from other related crops. Comparison between morphological and SSRs data revealed a low correlation among two data. These results suggested that the classification based on morphological characters and microsatellite markers will be useful for sugarcane breeders to plan crosses for agronomic traits. Genetically diverse parents could be identified for broadening the genetic base of sugarcane varieties and varietal development in sugarcane.

Key Words: Genetic diversity, *Saccharum* complex, Microsatellite (SSR) markers, Morphological diversity, *Saccharum* species, Sugarcane nobilization

Introduction

Sugarcane alone is responsible for the approximately 70% of row sugar, ~30% bio-ethanol production as-well-as molasses, and paper as byproducts at global level (FAO). As an alcohol crop for biofuel, output to input ratio of sugarcane is higher than the maize (Waclawovsky *et al*., 2010). It produces huge dry-biomass with annual greater yields compared with other major lignocellulosic biofuel plants such as *Miscanthus*, switch grass, and maize (Heaton *et al*., 2008). Energy sugarcane has a potential to produce cellulosic biofuel since it has a high fiber and biomass, and all the fiber, cellulose, and lignin components can be easily converted to energy (bio-ethanol). Hence, it has been established as an important industrial as-well-as farmer's cash crop in many tropical and sub-tropical countries of the world (Singh *et al*., 2011). Though sugarcane have the potential of high biomass and sugar production, breeding programs yet not entirely utilized genetic resource of potential multiple stress resistance and the high yield capacity exists within sugarcane germplasm resources. The detection of the genetic diversity within sugarcane germplasm is crucial, because diversity within a breeding genetic pool is required for making genetic gains in sugarcane (Dillon *et al*., 2007; Singh *et al*., 2012).

The genus *Saccharum* is a group of perennial grasses and belongs to the family *Poaceae*, tribe Andrpogoneae, which includes six species such as *S. officinarum* (noble canes), *S. sinense* (Chinese clones), *S. barberi* (North Indian canes), *S. robustus, S. spontaneum* and *S. edule* (Roach, 1972). *Saccharum* and other related genera i.e. *Erianthus* Michx., *Miscanthus* Anderson, *Narenga* Bor., and *Sclerostachya* make an interbreeding pool of genetic resources termed "*Saccharum* complex" (Daniels and Roach, 1987). The Modern sugarcane varieties derived from introgression between *S. officinarum* and *S. spontaneum* clones (Price, 1963). The F_1 progenies were backcrossed with the *S. officinarum* recurrent parent to expand genes for sucrose biosynthesis and accumulation, and this process is widely known as "nobilization'' (Roach, 1972). Initially, few first hybrids were extensively intercrossed to generate sugarcane

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varieties by nobilization which leads to a narrow genetic base of modern sugarcane hybrid varieties than the other *Poaceae* crops (Creste *et al*., 2010; Singh *et al*., 2013a). Genetically, sugarcane is a highly polyploid and heterozygous crop with highly unstable genetic constitution.

The development of novel sugarcane varieties with high sugar content has proven to be difficult due to the genetic complexity and heterozygous nature (Singh *et al*., 2011). The success for development of elite sugarcane varieties depends on the ability to select parents after efficient evaluation of the genetic diversity among the germplasm. It is the most important to make a new breeding program by using diversified parentage to produce hybrids with advanced agronomic traits and broader genetic base (Santos *et al*., 2012).

Traditionally, morphological traits have been used to identify and characterize *Saccharum* species clones, and the extent of morphological diversity has been used for accurate identification of sugarcane species and commercial varieties. Although, there is a high levels of morphological variability within the genus *Saccharum* which the breeders have used in the past and it provides a large base for selection of agronomic characters. Currently, Molecular (DNA) markers are routinely used for accurate identification, conservation, and management of germplasm stocks of plant species (Karp *et al*., 1997). Moreover, genetic diversity within *Saccharum* germplasm has been analyzed by various molecular markers such as RFLP (Besse *et al*., 1997), RAPD (Selvi *et al*., 2008), SSRs markers (Singh *et al*., 2014a) and AFLP (Aitken *et al*., 2007). Microsatellite (SSRs) markers have preferentially been used due to their simplicity, abundance, variability, co-dominance inheritance, and high reproducibility (Singh *et al*., 2014b).

Sugarcane exhibits a wide range of phenotypic diversity within and between species in different geographical areas and climates. Sugarcane shows a great polymorphism in terms morphological characters such as sucrose content, cane height, girth (thickness), number of internodes, length of internodes, number of leaves, leaf length, leaf width, cane weight etc (Singh *et al*., 2013b). These morphological characters have been used for various purposes including identification of parentage, taxonomical studies, assessment genetic diversity and correlation with characteristics of agronomic importance (CIAT, 1993). Morphological characterization is an important first step towards the assessment of sugarcane diversity (Prakash and He, 1996). Phenotypic characterization in combination with DNA markers based genetic diversity study would be more rewarding for the precise identification and description of closely related *Saccharum* species and commercial varieties.

The objective of the present study was to assess the genetic diversity among the various *Saccharum* species clones and commercial varieties (hybrids) using morphological traits and microsatellite markers.

Material and Methods

Plant Materials

Thirty eight *Saccharum* species clone and commercial varieties of sugarcane were used in assessment of genetic diversity based on morpho-physiological traits and SSRs markers. All the 38 sugarcane genotypes are listed below in Table 1 with their origin and pedigree.

The genotypes include five clones of *S. officinarum* (Badila, Otaheit, Bendjermassimhitam, IJ-76-564, Gunjera), five clones of *S. barberi* (Pathari, Agoule, Lalari, Hemja, Saretha), six clones of *S. spontaneum* (Baheri2, SES135B, WS18, SES515/7, N58, Ramsal), one clone of *S. robustum* (IJ-76-545), fourteen Indian commercial varieties, four Inter-specific hybrids and three non-Indian commercial hybrids (Table 1).

Evaluation of Morphological Traits

The measurements for seven morpho-physiological characters namely Stalk length (SL) in meter (m), Yield/ Clump (cane weight) in kg, No. of Internodes (INTN), Length of Internodes (INTL) in centimetres (cm), No. of Green leaves (NGL), Stalk diameter (Girth) in mm and sucrose content (HR brix %) were recorded on randomly chosen plants. These morpho-physiological characters are measured in most of the sugarcane breeding programmes for the selection of superior genotypes from mapping populations.

Extraction of Genomic DNA

Genomic DNA was extracted from disease free immature fresh leaves of all 38 sugarcane genotypes by the CTAB method (Hoisington *et al*., 1992) with minor modification (Singh *et al*., 2011). The extracted DNA was diluted to a final concentration of ~25ng/µl as determined by agarose-gel electrophoresis using known concentration of uncut λ DNA as standard.

Table 1. *Saccharum* species clones, Indian commercial, Inter specific hybrids and Non-Indian commercial varieties with their respective place of origin and pedigree

S. No.	Name of Genotype/Variety	Place of Origin	Pedigree
A. *Saccharum officinarum* L. (2n=80)			
1	Badila	New Guinea	Natural
2	Otaheit	Java, Indonesia	Natural
3	Bendjermassimhitam	New Guinea	Natural
4	IJ-76-564	Iryan, Java,	Natural
5	Gunjera	Java, Indonesia	Natural
B. *Saccharum barberi* Jesw (2n=81-124)			
6	Pathari	North-Eastern India	Natural
7	Agoule	North-Eastern India	Natural
8	Lalari	North-Eastern India	Natural
9	Hemja	North-Eastern India	Natural
10	Saretha	North-Eastern India	Natural
C. *Saccharum spontaneum* L. (2n=42-128)			
11	Ramsal	India	Natural
12	WS18	WB, India	Natural
13	SES515/7	UP, India	Natural
14	N58	Bihar, India	Natural
15	SES135B	UP, India	Natural
16	Baheri2	UP, India	Natural
D. *Saccharum robustum* (2n= 60-~200)			
17.	IJ-76-545	New Guinea	Natural
D. Indian commercial varieties (hybrids)			
18	CoS91269	Shahjahanpur, India	Bo91×Co1158
19	CoS96268	Shahjahanpur, India	Co1158×Co62198
20	CoS8436	Shahjahanpur, India	MS68/47×Co1148
21	CoS510	Shahjahanpur, India	Co453×Co557
22	CoS767	Shahjahanpur, India	Co419×Co313
23	CoS94527	Shahjahanpur, India	Bo91×Co62198
24	CoS95255	Shahjahanpur, India	Co1158×Co62198
25	UP22	Shahjahanpur, India	Bo91×CoSe40/80
26	UP0097	Shahjahanpur, India	Se1444/91×Se1854/91
27	CoJ64	Jalandhar, India	Co976×CO617
28	CoH70	Haryana, India	Unknown
29	CoJ99192	SBI Coimbatore, India	Unknown
30	CoLk92238	Lucknow, India	Unknown
31	B34-104	Bihar, India	Unknown
E. Inter-specific hybrids			
32	ISH135	Coimbatore, India	Inter specific hybrid
33	ISH168	Coimbatore, India	Inter specific hybrid
34	ISH148	Coimbatore, India	Inter specific hybrid
35	ISH273	Coimbatore, India	Inter specific hybrid
F. Non-Indian commercial hybrids (NICH)			
36	PoJ2878	Java, Indonesia	NICH
37	CP44-43	Canal Point, USA	NICH
38	Q49	Queensland, Australia	NICH

Polymerase Chain Reaction and SSR Analysis

Microsatellite (SSRs) primers were designed from the flanking regions of the simple repeats motifs of ESTs sequences using batchprimer3 online tool. Primers were synthesized by commercial services provider Bangalore GeNei^{TM}, India. Total 4500 EST sequences were retrieved from EST database of National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov). All the collected ESTs were the functional parts of the sugarcane metabolic pathways which many regulatory functions in biosynthetic process.

Table 2. The average and size range of the morpho-physiological characters recorded for phenotypic diversity analysis

S.No.	Genotype Name	HRBrix% (Jan)	Yield/clump (kg)	INTN	INTL (cm)	NGL	SD (mm)	SL (m)
1.	Badila	19.8	3.25	20	10.06	11	23	2.01
2.	Otaheit	22.3	4.35	22	10.64	12	24	2.34
3.	Bendjermasimhitam	21.6	3.5	19	9.74	14	26	1.85
4.	IJ76-564	21.6	6.25	25	13.82	10	27	3.45
5.	Gunjera	17.03	5.1	25	13.2	10	27	3.30
6.	Pathari	12.8	3.4	26	10.22	13	23	2.65
7.	Agoule	18.3	3.2	25	11.4	11	22	2.85
8.	Lalari	19.5	2.15	24	10.62	13	17	2.54
9.	Hemja	20.1	2.5	22	11.24	10	18	2.47
10.	Saretha	14.6	2.5	23	11.74	11	15	2.70
11.	IJ76-545	10.87	6.5	22	14.78	12	30	3.25
12.	Ramsal	4.6	2.0	31	15.68	11	15	4.86
13.	WS18	6.4	2.5	37	11.92	11	18	4.41
14.	SES515/7	10.1	5.0	37	15.34	11	23	5.67
15.	N58	5.8	1.5	24	18.44	10	14	4.42
16.	SES135B	13.0	2.73	26	15.34	11	18	3.98
17.	Baheri-2	3.6	2.4	25	14.98	12	21	3.74
18.	CoS91269	14.77	3.2	24	14.46	12	23	3.47
19.	CoS96268	22.8	5.4	29	15.53	11	22	4.50
20.	CoS8436	20.6	4.1	22	16.1	9	24	3.54
21.	CoS510	21.6	2.6	28	11.74	13	15	3.28
22.	CoS767	20.8	3.8	25	13.32	9	21	3.33
23.	CoS94527	18.2	4.6	24	15.92	11	23	3.82
24.	CoS95255	22.4	4.5	27	15.92	11	24	4.29
25.	UP22	17.2	2.9	29	13.2	11	18	3.82
26.	UP0097	20.01	3.1	26	16.08	6	18	4.36
27.	CoJ64	22.4	2.8	24	15.44	13	24	3.70
28.	CoH70	13.83	5.02	24	17.84	10	26	4.28
29.	CoJ99192	19.17	2.3	21	16.76	12	18	3.51
30.	CoLk92238	19.5	3.6	19	15.7	11	21	2.98
31.	B34-104	18.26	5.3	26	10.98	10	27	2.85
32.	ISH135	17.5	5.5	25	14.94	13	27	3.73
33.	ISH168	9.85	2.4	24	13.32	13	20	3.19
34.	ISH148	17.4	2.4	22	13.62	13	27	3.00
35.	ISH273	13.83	4.5	23	15.14	9	25	3.48
36.	PoJ2878	20.1	3.6	25	12.06	11	26	3.01
37.	CP44-43	17.2	4.1	27	11.46	13	21	3.09
38.	Q49	18.51	2.4	27	13.4	12	16	3.61
	Average	16.52	3.603	25.10	13.73	11.210	21.763	3.45
	Range	3.6-22.8	1.5-6.5	19-37	9.74-18.44	6-14	14-30	1.85-5.67

Identification of unique molecular markers associated with the sugar traits is major objective for maker assisted selection (MAS) in sugarcane energy crop (Singh *et al*., 2012). Accordingly, the newly developed EST-SSRs primers were screened for robust polymorphism by bulk segregation assay (BSA) using a bulk DNA of contrast (high and low sugar) segregating lines of sugarcane mapping population. Total 50 simple sequences repeat (SSRs) or microsatellite polymorphic markers were used to estimate the genetic diversity among 38 sugarcane genotypes. The information regarding the PCR amplification and polymorphism are given in Table 3. SSRs motifs regions were amplified by polymorphic SSR primer pairs in 10 µl reaction volume (Singh *et al*., 2010; 2011).

Diversity Analysis by SSR Markers

To measure the in formative potential of the microsatellite markers, the polymorphism information content (PIC) for each primer pair was calculated according to the formula of Milbourne *et al.* (1997) as follows.

$$\left[PIC = 1 - \sum_{J=1}^{N} P_{IJ}^{2} \right]$$

Where P_{IJ} is the frequency of the *j*th allele for marker *i* and summation extends over *n* alleles. Frequency of the ith allele in the set of 38 genotypes/varieties investigated. DNA bands were scored for the presence (1) or absence (0) in all 38 genotypes and binary data was used to calculate the Jaccard's similarity coefficient using module of free tree. Genetic distance between each pair were estimated as D=1-JS. Clustering was based on a similarity matrix using Unweighted Pair Group Method with Arithmetic average (UPGMA) algorithm; of freeware program Free Tree (Hampl *et al*., 2001). Most universal resampling technique bootstrapping was used to estimate the level of inferred relationships. Tree View, drawing software was used for interactive visualization of the dendrogram (Page, 1996).

Diversity Analysis with Morphological Traits

Cluster analysis was carried out on standardized morphological data based on the Euclidian distance coefficient and Unweighted pair group method with arithmetic means (UPGMA) algorithm using NTSYS-pc version 2.11 (Sokal and Michener, 1958). The dendrogram was generated employing SAHN program of NYTSYS (Rohlf, 2000).

Results

Development of SSR Markers from EST Sequences

Total 189 (4.2%) simple sequence repeat motifs were identified from the non-redundant EST sequences. Among the identified SSRs, tri-nucleotide repeats were found to be most abundant (47.1%) class followed by tetra-nucleotide repeats (16.6%), di-nucleotide repeats (15.8%), penta-nucleotide repeats (14.4%) and hexa-nucleotide repeats (5.5%) (Fig. 1). Eighty seven primer pairs were designed from the flanking regions of the microsatellite repeats (SSRs) and fifty primer pairs were used in the present genetic diversity analysis. These developed EST-SSR markers were able to reveal genetic variability existing within the expressed region of the sugarcane genome which is the more informative then the genetic diversity detected by other genomic SSRs markers (Singh *et al*., 2013c).

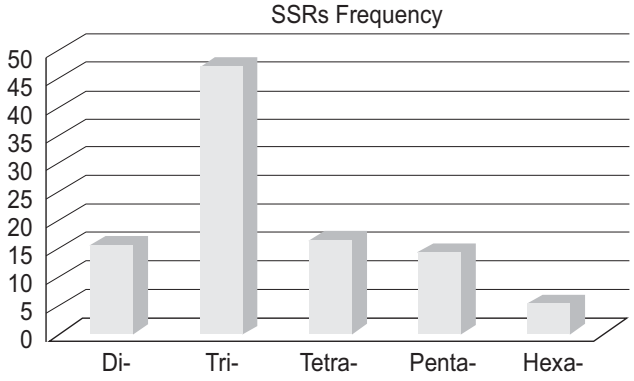

Fig. 1. Frequency distribution of different SSRs & types identified in *Saccharum* species ESTs

The genetic variability prevailing in the functional coding regions of the plants could not be analyzed by many of the molecular markers and most of the molecular markers detects genetic variability in non-coding region of the plant genome. However, these EST based microsatellite markers are less polymorphic then the other genic (derived from genomic sequences) molecular markers, though these exclusively explains the variability in exists in evolutionarily conserved regions of the plants genome (Cordeiro *et al*., 2001; Parida *et al*., 2009; Singh *et al*., 2013c).

Microsatellite Polymorphism and Cluster Analysis

Distinctive PCR banding patterns were found in most of the EST-SSRs markers in all the 38 sugarcane genotypes indicating the variability in expressed regions of the genome. The PCR amplified DNA profiles revealed the potential of microsatellite markers distinguish between inter as-well-as intra-species clones of sugarcane (Fig. 2). Present results corroborate to earlier reports (Singh *et al*., 2013a). Moreover, some previous reports on SSR markers based genetic diversity analysis were in accordance to the present research findings (Selvi *et al*., 2003; Brown *et al*., 2007).

A total of 412 DNA bands were amplified and their size ranged from 50 to 1250 bp with average of 8.29 bands per primer (Table 3). The polymorphism information content (PIC) of markers varied from 0.137 to 0.790 with an average of 0.373 and indicated a good discriminatory power of the functional EST-SSRs.

Morphological Traits' Analysis

The *Saccharum* species clones and cultivated varieties selected for morpho-physiological characterization exhibited high morphological variation in aerial part of the sugarcane. An analysis of variance illustrated that all the characters evaluated were significantly different ($P<0.01$)

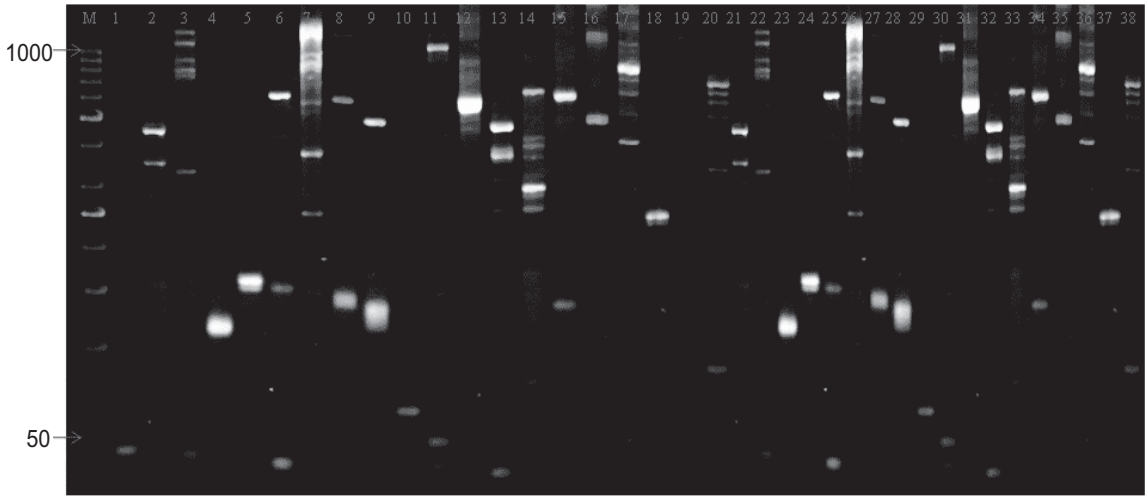

Fig. 2. Amplification profile of EST-SSR markers in 38 sugarcane genotypes belonging to 17 *Saccharum* specie clone, 14 Indian commercial varieties (hybrids), 4 Interspecific hybrids and 3 Non-Indian commercial hybrids. Lane 1-Badila, 2-Otahite, 3-Benjdermassimhitam, 4-Gunjera, 5-IJ76-564, 6-Baheri2, 7-SES135B, 8-WS-18, 9-SES515/7, 10-N58, 11-Ramsal, 12-Pathari, 13-Agoule, 14-Lalari, 15-Hemja, 16-Saretha, 17-CoS91269, 18-CoS96268, 19-CoS94527, 20-UP0097, 21-CoS767, 22-PoJ2878, 23-CP44-43, 24-Q49, 25-B34-104, 26-CoJ64, 27-CoH70, 28-CoJ99192, 29-CoLk92238, 30-IJ76-545, 31-CoS95255, 32-CoS510, 33-UP22, 34-CoS8436, 35-ISH135, 36-ISH273, 37-ISH148, 38-ISH168, L: 50bp DNA ladder (MBI Fermentas, Lithuania). Uncommon DNA banding pattern is showing distinctive nature of genotype by SSR markers.

between the genotypes. The dendrogram generated using phenotypic characters separated genotypes into two major clusters I and II with Euclidian distance ranging from 0 to 140 (Fig. 4). Cluster I contains 37 genotypes and divided into two groups. Group (A) included Baheri2, WS18, Ramsal, and ISH168. Group (B) further divided into two sub groups (a) and (b), Sub-group (a) included CoS91269, Patheri, IJ76-545 and CoJ99192, UP22, CoS91269, CoH70, ISH273, Otaheit, Badila, Bendjermasimhitam, CoS94527, SES135B, Saretha, CP44-43, and Q49. Sub-group (b) includes PoJ2878, ISH135, B34-104, UP0097, Agoule, CoS767, Hemja, CoLk92238, IJ76-564, CoS95255, CoS96268, ISH148, CoS510, and CoJ64. N58 (*S. spontaneum*) formed distinct cluster, which diverged from all the 38 sugarcane genotypes. It was the most diversified wild genotype along with Baheri2, WS18, Ramsal (*S. spontaneum*) and ISH168 genotypes. The genotypes did not form specific groups according to morphological characters in the dendrogram and genotypes related to a common species did not show close similarities in the morphological study. All the recorded physiological parameters with their size range and average are listed in Table 2.

Correlation between Morphological and SSR Data

The mental test showed quite low correlation between morphological and molecular marker based dendrograms (r=-0.03). Both morphological and genetic analysis allowed separation of the sugarcane genotypes into two different clusters. Despite the low correlation between morphological and SSR similarity matrices, there were similar grouping of genotypes in the respective dendrogram.

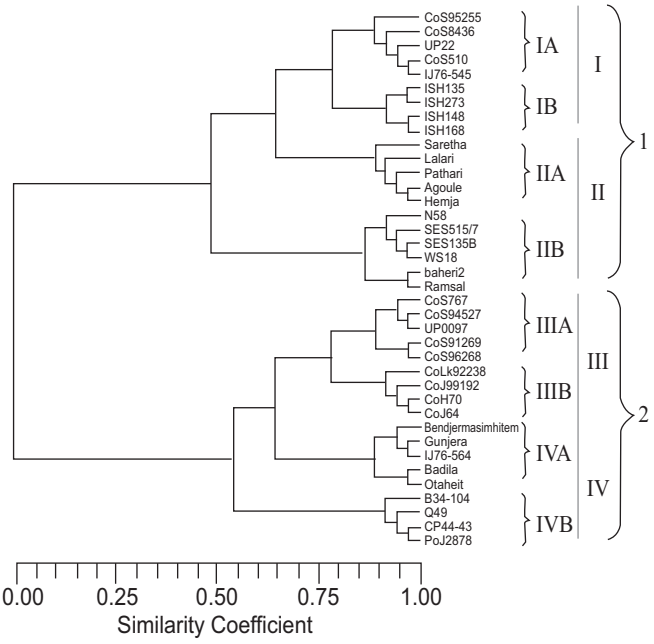

Fig. 3. Dendrogram showing genetic relationship among the 38 sugarcane genotypes based on SSR polymorphic markers. Scale indicates Jaccard's similarity coefficient values

Table 3. Details about the microsatellite (SSRs) marker's name, primer sequences, total no. of DNA bands, size range of bands and polymorphic information content (PIC)

S.No	Marker name	Forward primer (3′- 5′)	Reverse primer (3′- 5′)	Total Band	Band size (bp)	PIC Value
1	SMS1	GGTGTGTTTGAGGTTTAGGT	TGTAATGGCAAGCTCACATA	12	50-900	0.622
2	SMS2	ACCACTTCACAACAGGAGTC	TATTGTATGGGTTCGTTTCC	07	155-1181	0.482
3	SMS3	GGGCAAGAATGTATAGACCA	TTTATCGCCGAATAAGTGAT	04	126-1040	0.598
4	SMS4	TATAAACACACACACGGGAA	TTTGTTCATAGCACCGACTA	06	235-532	0.521
5	SMS5	AAGACCGCACAGTACAAATC	CTGTGTGGTGCTTTGCTT	09	58-629	0.514
6	SMS6	CAGAATACCGCCTATCAGAC	GTTCTGTGCTTTGGTTGG	06	70-640	0.298
7	SMS7	TAGAGGCAACGAAGAAAGAG	CTGGATTAATTGAGCTGGTC	11	92-840	0.185
8	SMS8	TGGGCTAGCTATCCTTACAC	AGCTTCTTACCAGTATGCCA	07	51-529	0.432
9	SMS9	GTGCGAGAGGAACTGTGT	AGCCCTGCCTAACAAGGA	05	59-160	0.210
10	SMS10	GGAGATGTTTGAGAGGGAA	AGAGTAGCATAAAGGAGGCAG	12	59-918	0.416
11	SMS11	ACAATGGAGTTGTATTTGGC	TATTTGCCACGTTGTACTTG	12	57-175	0.414
12	SMS12	ATCTTCACATCCATCATCCAC	ATCTCTCCTTGCTTTGGTTT	10	56-923	0.421
13	SMS13	CCTTGATGTTCAGATAGTTGG	CCGATTCAGCCCTTCGTC	05	57-687	0.521
14	SMS14	AAGAAGAGCCGTAGAAACAAC	ATTGAGCGAGGGATGAAC	10	51-980	0.442
15	SMS15	GTTCTTAGTCCAGCCGTAGTT	ATCGTTGTTGTCGGTGTC	09	60-1250	0.213
16	SMS16	GTTTAAGACAAGATGGTGTAGATG	TACATATTTACATTGTTACTCCGC	04	56-632	0.276
17	SMS17	GCGTCTTCATCATCTGCAAC	TAGAGAGACATGGGGTGCAT	10	57-820	0.613
18	SMS18	GTTGTCGAGATGATACAGAAGTAA	GTACAATATTACACACACAAAGGG	09	55-843	0.152
19	SMS19	CTGCAGTACGGTCCGGAATC	GTACCACCATGGCTCTAGCTTC	07	150-555	0.287
20	SMS20	TCCATCAAGCCGTTCCTC	GCCAAGCAGATAAAGAAGTG	08	60-145	0.511
21	SMS21	ACTCCTCCCGCCTCCACTAC	CTCACCGAAGCAATCAAG	08	66-945	0.539
22	SMS22	AGAGAGGGAGAAGAACAAGAC	ACCAGAAGGACAGAGATGG	09	53-707	0.173
23	SMS23	ATGACAGCAGCACAATGA	CACCCAGTTGAATAAGTGA	05	209-943	0.487
24	SMS24	GAAGCGAATGTGAACTGG	GAGAGCAGCGAGGACAGG	09	146-750	0.219
25	SMS25	CACTTCCCAGAGACCCAG	GACCTTAGCAATCAAGACAGA	06	186-666	0.223
26	SMS26	CTCCCAAAGCAAACCCTT	GTTCTTGACCTTCTTCCTGTC	03	371-954	0.105
27	SMS27	TACTATGGAGGCGGGAGG	TAGAAGAGCACAGAGCAAAC	10	65-925	0.649
28	SMS28	TCAAACCAGGATCTAAGCTCAC	GGTAGTGCCATTGAGGTTGC	08	55-879	0.200
29	SMS29	GCGAGAGAGATAGAGGGAGAGA	AGGTGCCGTTCATGAGGTAGT	11	51-798	0.236
30	SMS30	AATATACTTCTCGATTAATCACCG	CTACTACTACTACCAAGTACGGCG	03	62-431	0.526
31	SMS31	ACTAACTCTCTTCAACTTCCTCTG	AGCTGTTCCTCTTTAGCTAGTTC	04	51-246	0.171
32	SMS32	CACCGCAGCCTGACACAGAACC	AGGAACTCAGCATACTCGTGAC	19	54-1141	0.228
33	SMS33	AATCGGCGCTGACCATGGACTC	AGAACACAACTTTCACCTTGTT	06	51-452	0.428
34	SMS34	AGAAGGTGATCCTCAAGGACAAG	AACTGATCCCTCTTTCATATATTC	04	59-288	0.243
35	SMS35	TAGCAATCTACTCCCTACGTCTAC	GTTGACGTTGATCAGCCCGTTG	08	51-556	0.476
36	SMS36	AAAGACTCCAAGCTCCTGTGTG	CAAGTTTATTAGGGTCTGCAAG	09	54-198	0.169
37	SMS37	TAGAGGAAATAGCAGAACAGG	AGACTGACACCTTTGAGATGA	19	56-465	0.176
38	SMS38	TTTCTTTGGTTATACTGACTTGAC	GGGACAACTAATGTAACTGATTCT	06	51-500	0.137
39	SMS39	GTTAAGCTACTATGGACAACAGG	ACTTAACACTATGTCAGGTCTCAA	05	51-78	0.573
40	SMS40	CGTTATGGAAAGCACGAC	CTTGATGCCGTTGAAGAA	12	57-1029	0.790
41	SMS41	AAGATTCCAAACGCTGAA	AGAGATAGACTCAAAGGGCAA	05	51-95	0.184
42	SMS42	ATGATGACGAGAACGATG	GCAAGGGTGAGCGTGGAA	08	66-830	0.695
43	SMS43	AGGTCATCTCTCTCTTCTCGT	CTCCTTCTCCTCCTTCTTGT	07	60-603	0.263
44	SMS44	GCTCTCCTCCTCCTCTCC	GCCACTTTATCATCCTCAGTT	09	130-1148	0.407
45	SMS45	TTTGTGTCCTCTCTGTTCATT	GCAAGCATCAGTGTTCATC	12	58-1039	0.486
46	SMS46	CAGGACTACAGGGAACAATAA	GAAATACCAGGCTCACTTCA	09	54-990	0.294
47	SMS47	ACGCGTAGGCCGTACCAAAG	GTTAAACCTCAGCCGTGAGT	07	59-199	0.428
48	SMS48	ACGAGTTCAGGGCGCTGATAGAG	ATCACGACGTCATAGTCCGTAAC	08	59-884	0.200
49	SMS49	GCCGAAGCCTCTCCTCTCCTCC	GTCATCAATGACAGAGATGTAGAC	09	59-428	0.413
50	SMS50	GCGTCTCTGCTCTGCACTCTGC	ATTAACATATTCATAGCCCAATTT	11	58-1110	0.411
Total				412	-	-
Mean				8.29	-	0.373

UPGMA dendrogram grouped all the 38 sugarcane genotypes into two major clusters 1 and 2. The cluster 1 divided into two sub-clusters I and II (Fig. 3). Sub-cluster I was further divided into two sub-sub-clusters IA and IB; sub-sub-cluster IA included CoS95255, CoS8436, UP-22, CoS510, IJ76-564 (Indian commercial hybrids, UPCSR), and sub-sub-cluster IB included ISH135, ISH273, ISH148 and ISH168 (Interspecific hybrids). Sub-cluster II also divided into two sub-sub-clusters IIA and IIB; IIA included Saretha, Lalari, Patheri, Agoule and Hemja (*S. barberi*), and IIB included N58, SES515/7, SES135B, WS18 Baheri2 and Ramsal (*S. spontaneum*). Cluster II also divided into two sub-clusters III and IV. Sub-cluster III further divided into two sub-sub-clusters IIIA and IIIB; IIIA included CoS767, CoS94527, UP0097, CoS91269 and CoS96268 (Indian commercial hybrids) and IIB included CoLk92238, CoJ99192, CoH70, and CoJ64 (Indian commercial hybrids, from different parts of India). Sub-cluster IV also divided into two sub-sub-clusters IVA and IVB; IVA included Bendjermassimhitam (BMH), Gunjera, IJ76-564, Badila and Otaheite (*S. officinarum*) and IVB included B34-104, Q49, CP44-43 and PoJ2878 (Foreign commercial hybrids; NICH). Clustering pattern in the present dendrogram is with the accordance to the origin or pedigree, and the genotypes and commercial varieties that shared a common name showed genetic similarities.

Discussion

EST based molecular markers termed EST-SSR were developed from publically available EST database at NCBI website. The publically available EST resources offer an opportunity to develop informative molecular markers for field crops without expenditure. These EST based SSR markers are relatively more informative since they reveals genetic diversity within the SSR streches dispersed in expressed regions of the plant genome (Oliveira *et al*., 2009; Parida *et al*., 2009). The high level of polyploidy and heterozygous nature of sugarcane is responsible for intense PCR amplification pattern (Singh *et al*., 2011). The observed genetic diversity among the 38 sugarcane genotypes was comparatively lower than the earlier study of the five *Saccharum* species and related *Erianthus* (Cordeiro *et al*., 2003). Polymorphic fragment amplified in sugarcane genotypes revealed size variation and the length polymorphism observed between diverse accessions could be due to an accumulation of mutation events during DNA replication and recombination (Parida *et al*., 2006). The genesis of the simple sequence repeats loci is result of the errors in DNA metabolism due to the slippage of DNA polymerase at time of replication (Litt and Lutty 1989; Singh *et al*., 2014a). Thus, SSR markers have been proved more frequently occurring types of markers in sugarcane as well as other crop plants. A better representation of the genetic diversity in sugarcane varieties was obtained with SSR markers based analysis (Brown *et al*., 2007; Singh *et al*., 2012). Similarly, previous study has also shown that the SSR loci give good discrimination between closely related genotypes (Powell *et al*., 1996).

Physiological parameters and SSR markers have been previously employed to the study of genetic diversity in sugarcane varieties and different views regarding genetic base of sugarcane have been predicted. A comparative analysis of the genetic variation in sugarcane is essential for genetic conservation strategies and selection of parents for breeding of desirable economic trait (Singh *et al*., 2013a). Moreover successful conservation of any given

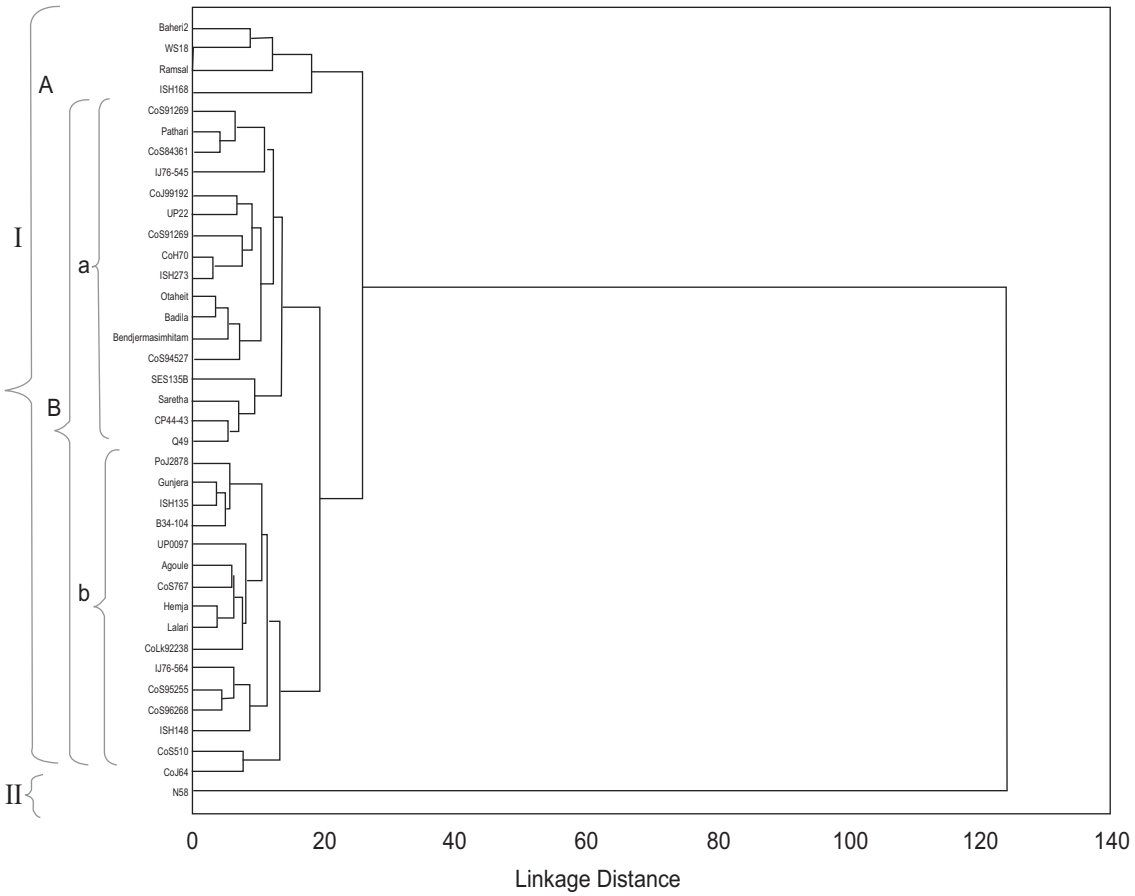

Fig. 4. A dendrogram of 38 *Saccharum* species clones, Indian commercial varieties, Interspecific hybrids and Non Indian commercial hybrids (NICH) based on seven morphological markers

gene pool is largely dependent on understanding the diversity and its distribution in a given region (Zhang *et al*., 1999).

It could be suggested that diverse germplasm sources (other than *S. spontanem* and *S. officinarum*) should be used as parental lines to develop sugarcane varieties. The mental test for association among the matrices derived from SSR and morphological data indicated a poor matrix correlation. It showed that both the methods discriminated very differently among the genotypes. Lesser correlation between morphological and molecular markers has been reported earlier in plants and suggested that it might be due to the independent nature of morphological and molecular variations (Bushehri *et al*., 2005). The poor correlation also could be due to the fact that higher level of genetic variation detected by molecular markers are non adaptive and the selection pressure is influenced by the environment (Vieira *et al*., 2007). Genotypes with the most distinct DNA profiles are likely to carry unique and potentially agronomically useful genes. This makes genomic diversity estimates a potentially valuable predicting source for selecting diverse parent genotypes for favourable heterotic combination that aims to broaden the genetic bases.

Genetic diversity based on morphological traits was higher on an average than SSR markers analysis, which reflects the influence of the environment on the performance of the genotypes. Due to this fact DNA markers and morphological traits could not necessarily gained closely corresponding results (Mart *et al*., 2005). Moreover, mainly two reasons are advocated to explain the limited correlation between DNA markers and morphological studies. First DNA markers cover a larger proportion of the genome than the morphological markers; second DNA markers are less subjected to artificial selection compared to morphological markers (Mart *et al*., 2005).

The development new EST-SSRs will have significant implication for the genetic study and utilization of genetic resources of the sugarcane and related genera. These functional molecular tools will also provide more direct estimate of functional genetic diversity in *Saccharum* species (Oliveira *et al*., 2009).

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Stability Analysis for Fruit Yield and its Component Traits in Indian Bottle Gourd (*Lagenaria siceraria* (Mol.) Standl.) Varieties

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Stability of 18 bottle gourd (*Lagenaria siceraria* (Mol.) Standl.) varieties was studied for fruit yield and yield contributing traits during *Rabi*-summer seasons of 2011-12, 2012-13 and 2013-14. Pooled analysis of variance revealed highly significant differences among the varieties for all the traits except for leaf width, ovary length and fruit weight suggesting enough genetic variability among the genotypes for all these traits. Similarly the mean squares for environments were also significant for all the traits except peduncle length and fruit length indicting that the environments under study are diverse enough. Further, genotype × environment (G × E) interactions were also significant for all the traits suggesting that the traits responded to the environments differently. The environments (linear) also differed significantly for all the traits except leaf width and peduncle length which indicates that the environments selected for testing of genotypes were quite varied in their effect on the performance of genotypes. Considering all the stability parameters, the varieties, Narendra Rashmi and IIHR-19-1 proved to be most stable genotypes exhibiting significantly higher mean fruit yield/ha. For the yield contributing traits viz., fruit number, Narendra Dharidhar, KBGR-12, Pusa Samrudhi and Kalyanpur Long were stable over environments and for fruit weight Kashi Ganga, Punjab Komal and Pant Lauki-3 were stable with highest mean values, unit regression and least deviation which can be exploited for these yield contributing traits in the bottle gourd improvement programs.

Key Words: G×E Interaction, Bottle Gourd, Regression Coefficient, Stability Analysis, Stable Genotype, Vegetable Improvement

Introduction

Bottle gourd [*Lagenaria siceraria* (Mol.) Standl.] commonly known as *Lauki* or *Ghiya* in India is one of the most important member of the family *Cucurbitaceae* and believed to be originated in Africa (Whitaker, 1971). It is commercially grown in all the states of India in both rainy and summer seasons. The immature fruits contain good amount of vitamins and have good medicinal values. In any breeding programme it is necessary to screen and identify phenotypically stable genotype for yield which could perform more or less uniformly under different environmental conditions. It is an established fact that yield is a complex character and largely depends upon its component characters, with an interaction with the environment, resulting into the ultimate product *i.e.* yield. So for breeding a stable variety, it is necessary to get the information on the extent of genotype × environment (GE) interaction for yield and its component characters.

To meet the objective of developing varieties with high yield potential, a wide collection of germplasm must be available so that the evaluation for desirable traits for yield can be exercised and a breeding program for an ideal plant type concept can be made accordingly. A phenotype is the product of interplay of genotype and its environment. A specific genotype does not exhibit the same phenotype under the changing environments and different genotypes respond differently to a specific environment. This variation arising from the lack of correspondence between the genetic and non-genetic effects is known as G × E interaction which is generally considered impediment in plant breeding as it baffles the breeder in judging the real potential of a genotype when grown in different environments. Several workers considered G×E interactions as linear function of environment and proposed regression of yield of a genotype on the mean yield of all genotypes in each environment to evaluate stability of performance

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of genotype (Eberhart and Russell, 1966; Finlay and Wilkinson, 1963; Perkins and Jinks, 1968). The main objective of a breeding programme is to develop varieties that perform well over a broad spectrum of environments. The information about phenotypic stability is useful for the selection of crop varieties as well as for breeding programmes. In bottle gourd, very few studies are available so far on these aspects by Parmar (2000) and Shaikh *et al*. (2012). Hence, the objective of the present study was to explore the effect of G × E on fruit yield and its attributing traits and identifying stable varieties in bottle gourd.

Material and Methods

The experimental material for the present study comprised of 18 bottle gourd reference varieties viz., ABG-1, NDBG-619, NDBG-132, Pusa Sandesh, IIHR-19-1, Pusa Santhusti, Pusa Naveen, Kalyanpur Long, Pant Lauki-3, Narendra Jyothi, Narendra Dharidhar, Arka Bahar, Narendra Rashmi, Punjab Komal, Punjab Long, Pusa Samrudhi, Kashi Ganga and KBGR-12 maintained at the Division of Vegetable Crops under PPV&FRA's DUS project. These 18 bottle gourd varieties were grown in randomized block design (RBD) with three replications at the Vegetable Farm, ICAR-Indian Institute of Horticultural Research, Bengaluru during *Rabi*-summer seasons of 2011-12, 2012-13 and 2013-14. All the recommended cultural and agronomic practices including pest control measures were adopted to raise good crop. To ascertain the comparative behaviour of different genotypes under different environments, observations were recorded on five randomly selected plants from each replication for 15 quantitative parameters such as vine length (m), branch number/vine, node number for first female flower appearance, days taken for first female flower appearance, stem intermodal length (cm), petiole length (cm), leaf length (cm), leaf width (cm), ovary length (cm), peduncle length (cm), fruit length (cm), fruit width (cm), fruit number/vine, fruit weight (g), fruit yield/vine (kg). In all the experiments, plot means (mean of five plants) were used for environment-wise analysis of variance and pooled analysis of variance for the estimation of G×E interaction effects and stability analysis as suggested by Eberhart and Russell (1966).

Results and Discussion

The pooled analysis of variance for different traits revealed highly significant differences among the varieties for most of the traits viz., vine length, branch number/vine, node number for first female flower appearance, days taken for first female flower appearance, stem intermodal length, petiole length, leaf length, peduncle length, fruit length, fruit width, fruit number/vine and fruit yield/vine suggesting enough genetic variability among the genotypes for all these traits. Similar significant differences among the genotypes for all the traits except for days to first picking and fruit weight in bottle gourd were reported by Shaikh *et al* (2012). Thakur *et al*. (1994) also reported significant differences for all the yield and yield contributing traits in bitter gourd. The variances associated with genetic effects were smaller than the variances associated with environmental effects for most of the characters viz., vine length, branch number/vine, node number for first female flower appearance, days taken for first female flower appearance, petiole length, leaf length, leaf width, ovary length, fruit number/vine, fruit weight, fruit yield/vine. This shows that under the present environmental conditions for determination of such characters, the genotypes need to be evaluated in multi-locational trials. Furthermore, the larger variances associated with genetic effects than the variances associated with genotype × environment for most of the traits under the study namely, vine length, node number for first female flower appearance, days taken for first female flower appearance, stem intermodal length, petiole length, leaf length, peduncle length, fruit length, fruit width, fruit number/vine, fruit weight, fruit yield/vine indicates a greater influence and stability of genetic factors relative to the variability associated with the interaction of genotype × environment for these characters in bottle gourd (Shaikh *et al.,* 2012). Similarly the mean squares for environments were also significant for all the traits except peduncle length and fruit length indicating that the environments under study are diverse enough. Further, G × E interactions were also significant for all the traits suggesting that the traits responded to the environments differently. The environments (linear) also differed significantly for all the traits except leaf width and peduncle length which indicates that the environments selected for testing of genotypes were quite varied in their effect on the performance of genotypes. Similar results were earlier reported for yield and yield contributing traits in ridge gourd by Varalakshmi and Subba Reddy (1998).

The G × E linear component was significant for branch number, node for fist female flower appearance, stem intermodal length and leaf length suggesting that

the variation in performance of different genotypes is due to the regression of genotypes on environments and hence the performance is predictable in nature (Krishnaprasad and Singh, 1992; Varalakshmi and Subba Reddy, 1998; Agasimani *et al.*, 2008; Shaikh *et al*., 2012; Vasanthkumar *et al.*, 2012). However, the mean square due for pooled deviation is significant for all the traits except for leaf length suggesting that variation in performance of genotypes is entirely unpredictable.

When stability parameters were studied for 18 bottle gourd reference varieties, it was found that none was ideal variety with stability over environments for all the 15 characters. However, fruit yield/vine is the main trait responsible for overall performance of a variety across environments and in the present study; two varieties, Narendra Rashmi and IIHR-19-1 which recorded significantly higher mean values over population mean (6.89 kg), unit regression coefficient and non-significant deviation from regression were stable over wider environments (Table 1). Similar findings have also been reported by Prasad *et al.* (1987) and Parmar (2000).

The long vine varieties, Pusa Sandesh and Narendra Jyothi were found to be stable for vine length with unit regression and least deviation, similarly NDBG-132 and Kalyanpur Long were stable for branch number (Table 1). The early varieites, Punjab Komal, Pant Lauki-3, Arka Bahar, Pusa Samrudhi, and Pusa Naveen were found stable over different environments for various earliness traits. For node number for first female flower appearance, Punjab Komal and Pant Lauki-3 were stable; Arka Bahar, Pusa Samrudhi, Pusa Naveen for days taken for first female flower appearance; Narendra Rashmi for stem intermodal length and Kalyanpur Long for petiole length were found to have stability over environments having lowest mean value (in the desirable direction) with unit regression and non-significant deviation from regression. Among the other leaf parameters, for leaf length, five varieties viz., Narendra Rashmi, NDBG-619, Pusa Santushti, Pusa Samrudhi and Narendra Jyothi and for leaf width four varieties viz., Arka Bahar, Kalyanpur Long, Punjab Long and Pusa Santushti were stable (Table 1). Several varieties were stable for various fruit traits which are directly correlated with yield; for ovary length, as many as eight varieties, Narendra Jyothi, Pusa Naveen, Punjab Long, Kalyanpur Long, ABG-1, Pant Lauki-3, Narendra Dharidhar and Kashi Ganga were stable with highest mean, unit regression and least deviation (Table 1). For peduncle length, Pusa Sandesh, NDBG-619, Punjab Long had lowest mean (desirable) with unit regression and non significant deviation from regression. IIHR-19-1, KBGR-12, NDBG-619, Pusa Naveen, and Punjab Long were stable for fruit length whereas Narendra Dharidhar, Kalyanpur Long, IIHR-19-1, NDBG-619, Punjab Komal, Narendra Jyothi and Pant Lauki-3 were stable for fruit width. For the yield contributing traits viz., fruit number, Narendra Dharidhar, KBGR-12, Pusa Sanrudhi and Kalyanpur Long were stable over environments and for fruit weight Kashi Ganga, Punjab Komal and Pant Lauki-3 were stable with highest mean values, unit regression and least deviation (Table 1) (Shaikh *et al*., 2012).

Among the varieties studied, the stable and adaptable varieties with high mean performance for fruit yield per vine identified for wider environment and specific (favourable) environment are presented in Table 2. Narendra Rashmi with highest fruit yield/vine followed by IIHR-19-1 would be stable over environments owing to the non-significant deviation from regression value associated with unit regression and high mean performance. Four other varieties viz., Arka Bahar, Pant Lauki-3, KBGR-12 and Narendra Dharidhar had above unit estimates of regression coefficient leading to below average stability hence found to be well adapted to better environment (Shaikh *et al*., 2012). However, Punjab Komal was adaptable to poor environments owing to its negative regression coefficient and least deviation.

Overall, variety, Kalyanpur Long showed stable performance for as many as six traits such as branch number, petiole length, leaf width, ovary length, frit width and fruit number; Narendra Jyothi was stable for four traits viz., vine length, leaf length, ovary length and fruit width. Three other genotypes were stable for more than one important yield contributing trait viz., Punjab Komal for node number, for first female flower appearance, fruit width and fruit weight; Pusa Samrudhi for days taken for first female flower appearance, leaf length and fruit number and KBGR-12 for fruit length, fruit width and fruit number which showed higher mean values than population mean in desirable direction, regression coefficient less than unity and non-significant deviation from regression. These varieties can be cultivated for specific trait(s) of choice under a wide range of agro-climatic conditions or can be used as parents in the hybridization programs in bottle gourd.

Table 1. Mean performance of the stable varieties along with stability parameters for different yield and yield contributing traits in bottle gourd

S.No.	Trait	Stable variety	Mean	bi	S^2di
1	Vine length (m)	Pusa Sandesh	5.01	0.83	-0.01
		Narendra Jyothi	4.95	1.62	0.05
2	Branch number/vine	NDBG-132	18.49	1.05	0.93
		Kalyanpur Long	14.41	1.12	1.85
3	Node number for first female flower appearance	Pant Lauki-3	6.03	0.65	0.23
		Punjab Komal	5.70	1.70	-0.06
4	Days taken for first female flower appearance	Pusa Naveen	40.11	0.99	-4.05
		Arka Bahar	39.34	0.73	0.32
		Pusa Samrudhi	39.85	0.63	6.05
5	Stem intermodal length (cm)	Narendra Rashmi	14.26	0.55	-0.30
6	Petiole length (cm)	Kalyanpur Long	13.14	1.05	-0.44
7	Leaf length (cm)	NDBG-619	18.58	1.16	-0.46
		Pusa Santhusti	18.57	1.04	-0.09
		Narendra Jyothi	17.51	1.83	-0.18
		Narendra Rashmi	19.78	1.97	-0.12
		Pusa Samrudhi	18.32	0.29	-0.11
8	Leaf width (cm)	Pusa Santhusti	22.58	1.09	-1.62
		Kalyanpur Long	23.08	1.16	-0.51
		Arka Bahar	23.56	0.10	8.65
		Punjab Long	22.97	1.13	1.48
9	Ovary length (cm)	ABG-1	4.56	1.15	0.09
		Pusa Naveen	4.66	1.01	-0.02
		Kalyanpur Long	4.59	0.87	-0.07
		Pant Lauki-3	4.54	1.12	0.02
		Narendra Jyothi	4.73	0.97	-0.06
		Narendra Dharidhar	4.42	1.12	0.06
		Punjab Long	4.65	1.17	0.19
		Kashi Ganga	4.32	0.82	0.08
10	Peduncle length (cm)	NDBG-619	9.26	-0.19	0.41
		Pusa Sandesh	8.71	-0.39	-0.15
		Punjab Long	9.87	2.22	0.55
11	Fruit length (cm)	NDBG-619	35.60	1.42	3.56
		IIHR-19-1	45.29	2.18	2.56
		Pusa Naveen	35.52	1.09	0.88
		Punjab Long	34.07	0.60	-1.08
		KBGR-12	37.29	2.82	4.41
12	Fruit width (cm)	NDBG-619	7.08	1.14	-0.09
		IIHR-19-1	6.95	0.08	0.13
		Kalyanpur Long	6.90	0.87	0.46
		Pant Lauki-3	7.50	1.40	0.31
		Narendra Jyothi	7.29	0.61	-0.10
		Narendra Dharidhar	6.54	0.82	0.01
		Punjab Komal	7.25	1.13	-0.01
13	Fruit number/ vine	Kalyanpur Long	5.87	0.79	-0.09
		Narendra Dharidhar	6.27	0.58	0.03
		Pusa Samrudhi	5.93	2.03	0.40
		KBGR-12	6.08	0.94	-0.06
14	Fruit weight (g)	Pant Lauki-3	1.25	1.94	0.02
		Punjab Komal	1.25	1.38	0.03
		Kashi Ganga	1.48	0.90	0.02
15	Fruit yield/ vine (kg)	IIHR-19-1	8.60	0.94	1.43
		Narendra Rashmi	9.37	0.73	1.05

Table 2. Ideal bottle gourd varieties identified for fruit yield per vine in different environmental conditions

Environment	Varieties identified
Wider environment	Narendra Rashmi and IIHR-19-1
Better environment	Arka Bahar, Pant lauki-3, KBGR-12 and Narendra Dharidhar
Poor environment	Punjab Komal

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Genetic Divergence and Gene Source Studies in Durum Wheat Genotypes under Early Sown and Moisture Stress Conditions

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Studies were conducted on genetic divergence for 16 parameters in 102 varieties of durum wheat (*Triticum turgidum var. durum* Desf.). Analysis of variance showed significant differences among varieties for all the traits studied. Based on the magnitude of divergence value, the varieties were grouped into 16 clusters. Intra-cluster distance was maximum for cluster IV (9.6) followed by cluster XIII (7.8). The maximum inter-cluster distance was 104.0 between cluster VII and XIII, indicating high genetic divergence among varieties of these two groups. Maximum contribution towards the divergence was by carotene content (13.2%) followed by sedimentation value (9.4%), grain yield/plant (8.9%) and plant height (8.0%). Gene sources for higher number of tillers/plant (GW 1209); spike length (MPO 1243); number of grains/spike (HI 8627), carotene content (AKDW 4151); sedimentation value (NP 4); low canopy temperature at all the growth conditions (MACS 2846) and grain yield/ plant (HD 4672) were identified for future breeding improvement programme.

Key Words: Cluster, D^2 analysis, Durum Wheat, Gene Source

Introduction

Durum wheat (*Triticum turgidum var. durum* Desf.) is an economically important crop and widely grown in most parts of the world, including India. It is being cultivated in an area of 10 to 11% of the world wheat area, and accounts for about 8% of the total wheat production (Ganeva *et al.,* 2011). Durum wheat is the hardest of all wheat species and consumed mainly in the form of semolina (*suji*) and fast food products (pasta products). The pasta producers require durum wheat which is hard with high yellow pigment and capable of making products with excellent cooking quality. Breeding for varieties with high yield, disease resistance, elevated yellow pigment (YP) concentration, excellent cooking quality and other desirable end-use quality traits are the objectives of durum wheat breeding programs worldwide. Genetic improvement of any species depends primarily on the extent and magnitude of genetic variability present among available germplasm for important traits. Genetic diversity is of paramount importance for heterosis and hybridization between genetically divergent parents and is expected to produce superior hybrids and desirable recombinants. Therefore, it is essential to identify genetically divergent genotypes and utilize them for breeding programme. Precise information on the nature and degree of genetic diversity helps the plant breeder in choosing the diverse parents for hybridization. Mahalanobis D^2 statistics, based on multivariate analysis serves to be a good index of genetic diversity. The present study was aimed to determine the genetic divergence in 102 durum wheat genotypes under moisture stress and early sown conditions, which will help to develop new varieties with desirable characteristics and for identification of gene source for different traits.

Materials and Methods

The field studies were carried out during *rabi* cropping seasons of 2010 to 2013 (October to March) for three years at Indian Agricultural Research Institute, Regional Station, Indore (M.P) India. The experimental material comprising 102 genotypes was sown on 16th October every year under two growing conditions *i.e.,* rainfed and restricted irrigation. Each genotype was planted in two rows of 2.5 m row length and row to row spacing of 30 cm in RBD in three replications. In rainfed condition, no irrigation was applied; whereas, in restricted irrigation condition, only one irrigation was applied at 35-40 days after sowing. Recommended agronomic practices were followed to raise the crop and all care was taken to minimize variations due to environmental and cultural conditions. Sixteen characters

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i.e., days to flowering, days to maturity, plant height, number of tillers per plant, spike length, flag leaf length, number of grains/spike, biomass yield/plant, carotene content, sedimentation value, canopy temperature I, canopy temperature II, canopy temperature III, 1000 grain weight, harvest index and grain yield/plant were recorded. Canopy temperature was measured three times i.e., vegetative stage, flowering stage and grain filling stage. The measurements were made during late morning to early afternoon of cloudless periods (11:00 to 15:00 hours) with infrared thermometer (Model LT-300 sixth sense) focused to 5:1 meter. Multivariate analysis for the estimation of genetic distance among all the varieties was carried out by using D^2 statistic analysis as suggested by Mahalanobis (1936) and clustering of genotypes was done as per the method of Tocher (Rao, 1952).

Result and Discussion

Analysis of variance showed significant differences among varieties for all the traits studied, which indicated existence of genetic variability in the varieties (Table 1). Based on the magnitude of divergence, 102 genotypes were grouped into 16 clusters (Table 2). The maximum number of genotypes were grouped in cluster XII (12) followed by cluster I (11); V (11); IX (11); X (11); IV (8); XVI (7) and one each in cluster VI, III, II, VIII, XIII, VII, XI and XIV. Intra-cluster distance was maximum for cluster IV (9.6) followed by 7.8 for cluster XIII. However, clusters VII, XI and XIV had no intra-cluster distance as they were represented by a single genotype. The maximum inter-cluster distance (104.0) was observed between clusters VII and XIII indicating the presence of highly genetic divergent varieties in these two groups. Minimum inter-cluster distance (9.0) was observed between clusters IX and X showing their close proximity. However, in all cases inter-cluster distances were greater than the intra-cluster distances indicating the presence of genetic diversity among the varieties between different clusters (Table 3). The cluster means indicated substantial variation among 16 clusters (Table 4). Cluster mean values indicated that maximum number of tillers/plant was found in cluster XIII with 3 varieties viz., A 9-30-1, Amrut and CDW 04. Maximum spike length was seen for cluster XIV with one variety (NP 404). Varieties (HI 8627 and CPAN 6236) in cluster VII and VIII showed maximum number of grains/spike. Clusters II, VIII and VII showed high carotene content. Cluster means of XIII, VIII and XII showed maximum sedimentation values. Low mean canopy temperature at all the growth stages was found in clusters XIV, IX and I. Higher mean values of 1000 grain weight was observed among clusters XI and III. Clusters XI, VII, III, XV and VI showed high grain yield/plant.

Genotypes *viz.*, HD 4672, HI 8627, V 21, HD 4676 and HI 8638 showed higher grain yield/plant in cluster XI, VII and III respectively. Maximum contribution towards total divergence was 13.2% by carotene content followed by sedimentation value (9.4%). On the contrary, the other characters have given very little contribution to total divergence for enabling clear discrimination of the genotypes (Table 4). Composition of genotypes among

Table 1. Range, mean, standard error and co-efficient of variance for different characters in durum wheat

Parameters	Mean	Range		SE(±)	CV(%)
		Minimum	Maximum		
Days to flowering	74	58	92	0.3	0.5
Days to maturity	125	111	139	0.3	0.3
Plant height	83	60	109	0.5	0.8
No. of tillers	7.7	5.8	10.3	0.3	4.3
Spike length	7.0	5.2	8.9	0.1	1.0
Flag leaf length	19.0	12.9	23.0	0.2	1.4
No. of grains/spike	41	29	55	0.5	1.4
Biomass/plant (g)	49.3	39.5	60.0	0.1	0.4
Carotene content (ppm)	4.3	2.8	7.8	0.1	1.9
Sedimentation value (ml)	33	20	49	0.4	1.5
Canopy temperature I	20.8	18.2	23.7	0.3	1.9
Canopy temperature II	22.5	20.3	54.0	1.4	7.7
Canopy temperature III	25.2	22.4	27.8	0.3	1.3
1000 grain weight (g)	42.7	31.5	59.4	0.6	1.7
Harvest index (%)	37.6	27.0	54.6	0.6	1.8
Grain yield/plant (g)	15.8	11.2	22.9	0.3	2.5

Table 2. Composition of the clusters of durum wheat genotypes under moisture stress in early sown conditions

Cluster	No. of genotypes	Composition of genotypes
I	11	CDW 04, HI 8498, HI 8691, IWP 5070, MACS 2846, MACS 3125, MPO 1106, MPO 1215, NIDW 70, Raj 1555; Raj 6566
II	4	AKDW 4151, V21/23, WH 896; MPO 1243
III	5	HD 4676, HI 8638, HI 8671, MACS 9; V21
IV	8	GW 1170, HD 4502, HI 7747, HI 8381, HI 8591, MACS 3061, MACS 3063; Raj 6516
V	11	GW 1, GW 1114, GW 1245, HG 110, HI 8722, Jay, Karnataka Local, MACS 1967, N 59, NIDW 15; Sarangpur local
VI	6	Baxi 228-18, Dohad Local, GW 2, HI 8550, IWP 5004-1; Kathia 25
VII	1	HI 8627
VIII	4	CPAN 6236, GS 27, Raj 6562; WH 912
IX	11	B 4446-WA, B 4447-BA, DBP 01-09, GW 1240, HI 8666, IWP 5007, Jairaj, Line 1172, Raj 6069, VD 97-15; Vijay
X	11	Bansi Local, DWL 5023, GW 1209, GW 1225, GW 1244, HI 8645, IWP 5013, Malvi Local, Mandsour Local, NI 5759; NIDW 9
XI	1	HD 4672
XII	12	AKDW 4240, Altar 84, DBP 01-11, GW 1139, HI 8592, HI 8653, HI 8663, MACS 2694, NIDW 295, PDW 215, PDW 233; PDW 245
XIII	3	A 9-30-1, Amrut; CDW 04
XIV	1	NP 404
XV	6	A 206, Bijaga Red, Bijaga Yellow, DWR 137, JU 12; Meghdoot
XVI	7	DBP 02-08, Guji 'S', HD 4709, Motia, MPO 215, Sawer local; Trinakaria

Table 3. Average intra- and inter-cluster D^2 values between the clusters of durum wheat genotypes under moisture stress in early sown condition

Clusters	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI
I	3.6															
II	25.0	7.3														
III	11.6	32.5	6.3													
IV	30.3	21.2	49.0	9.6												
V	10.2	21.2	13.0	30.3	6.3											
VI	22.1	18.5	23.0	22.1	13.7	7.29										
VII	50.4	57.8	34.8	64.0	51.8	57.8	0.0									
VIII	51.8	21.2	59.3	23.0	51.8	22.1	64.0	6.8								
IX	14.4	29.2	28.1	27.0	12.3	29.2	74.0	53.3	5.3							
X	13.0	22.1	24.0	15.2	9.6	10.9	60.8	36.0	9.0	7.3						
XI	31.4	37.2	14.4	51.8	24.0	27.0	47.6	60.1	39.7	26.0	0.0					
XII	10.2	9.6	27.0	13.0	11.6	13.7	56.3	23.0	9.6	14.4	33.6	5.8				
XIII	24.0	34.8	39.7	50.4	15.2	22.1	104.0	60.8	21.2	17.6	46.2	20.3	7.8			
XIV	30.3	42.3	42.3	53.3	16.8	37.2	77.4	81.0	31.4	23.0	46.2	32.5	24.0	0.0		
XV	17.6	32.5	18.5	41.0	13.0	10.9	59.3	50.4	22.1	11.6	20.3	19.4	13.0	19.4	4.4	
XVI	33.6	17.6	37.2	14.4	28.1	116	65.6	16.0	29.2	11.6	29.2	14.4	37.2	44.9	26.0	7.3

the clusters infer that distribution of genotypes from different geographical locations into one cluster may be due to presence of some common genes controlling the most important characters through modifying effect of micro and macro environment affecting genetic diversity and some degree of ancestral relationship among the genotypes (Sangwan *et al*., 2004) . Another feature that genotypes from the same location were placed in separate clusters indicates wide diversity among genotypes from the same location. This showed that the geographical diversity may not necessarily be related to genetic diversity in this species. The present findings are in

Table 4. Mean values of the characters in clusters of durum wheat genotypes under moisture stress in early sown conditions

Characters	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	Contribution (%)
DF	67	78	66	83	66	78	79	89	68	76	71	76	67	79	70	85	6.4
DM	121	129.	122	129	121	126	128	135	121	125	126	126	121	125	123	135	2.5
PH	76	77	83	70	84	99	83	97	76	83	80	76	93	93	98	91	8.0
NT	7.4	7.3	7.3	6.9	7.7	7.4	7.1	7.2	7.9	8.3	9.6	7.6	9.7	9.1	8.3	7.9	5.7
SL	6.7	7.7	6.9	6.9	7.7	7.6	7.5	7.0	5.9	6.7	7.1	6.9	7.7	8.8	7.7	6.6	5.8
FLL	18.5	18.6	19.5	17.1	20.3	19.6	18.9	15.7	18.7	19.7	19.6	18.5	21.1	22.4	21.7	17.9	5.5
NOG	43	41	43	40	42	42	55	54	37	37	42	42	36	35	41	37	6.3
BM	53.2	51.4	56.2	46.5	52	49.5	49.3	42.6	48.1	49.5	51.9	46.8	49.3	54.1	46.7	47.4	5.1
Caro	3.9	7.7	4.5	4.1	4.0	3.9	6.6	7.5	4.2	3.5	5.3	4.8	3.6	3.8	3.5	4.8	13.2
SDS	36	37	36	25	32	35	30	42	32	30	26	41	49	27	33	30	9.4
CT I	18.9	21.2	20.1	22.1	20.2	21.7	20.7	22.7	19.2	21.1	20.2	20.8	20.5	18.7	20.3	21.5	2.9
CT II	20.7	23.2	20.7	25.0	21.6	23.1	22.6	24.2	20.7	22.9	22.0	22.7	22.0	20.7	21.6	23.5	5.4
CT III	23.5	26.1	24.2	26.4	24.7	26.0	25.2	27.1	23.9	25.6	24.5	25.4	24.5	23.4	24.4	26.3	2.3
GW	40.4	42.4	57.1	38.9	42.5	55.7	45.4	44.1	37.0	41.8	59.0	39.0	42.1	35.9	48.5	48.4	7.8
HI	39.0	36.4	41.8	37.5	38.0	35.2	53.6	37.3	37.6	37.6	38.4	37.0	32.1	39.6	36.8	35.9	4.7
GY	15.6	15.1	22.4	14.4	16.1	21.8	22.6	16.2	13.8	15.7	22.7	14.2	13.3	13.9	22.1	16.9	8.9

DF= Days to flowering, DM= Days to maturity, PH= Plant height, NT= No. of tillers, SL= Spike length, FLL= Flag leaf length, NOG= No. of grains/spike, BM = Biomass yield/plant, Caro = Carotene content (ppm), SDS = Sedimentation value (ml), CT I= Canopy temperature at vegetative stage, CT II= Canopy temperature at flowering stage, CT III= Canopy temperature at grain filling stage, GW= 1000 grain weight (gm), HI = Harvest index (%) and GY = Grain yield/plant (g)

Table 5. Gene sources for all characters in durum wheat

Characters	Potential	Varieties
Days to flowering	58, 59, 60, 60, 60	Jay, MACS 9, Vijay, V 21 and NP 4
Days to maturity	111, 117, 118, 118, 118	V 21, HI 8691, Jay, IWP 5070 and Raj 6566
Plant height (cm)	62, 64, 64, 66, 69	IWP 5007, GW 114, GW 1240, HI 7747 and GW 1170
No. of tillers	9.7, 9.6, 9.1, 9.0, 8.9	GW 1209, Amrut, B 4447-BA, NP 4 and GW 1
Spike length (cm)	8.8, 8.6, 8.5, 8.3, 8.2	MPO 1243, NP 404, GW 1245, Karnataka local and Kathia 25
Flag leaf length (cm)	22.7, 22.6, 22.6, 22.4, 22.2	A 206, Bijaga yellow, Dohad local, NP 404 and Karnataka local
No. of grains/spike	54.6, 53.9, 50.8, 49.3, 48.8	HI 8627, CPAN 6236, Altar 84, HI 8550 and HI 8638
Biomass/plant (g)	60.0, 58.6, 58.4, 56.5, 56.4	MACS 9, MPO 1243, HI 8671, GW 1245 and NIDW 15
Carotene content (ppm)	7.7, 7.5, 6.6, 6.5, 6.3	AKDW 4151, WH 896, MPO 1243, V21/23 and WH 912
Sedimentation value (ml)	49.2, 41.8, 40.9, 40.6, 40.6	NP 4, CPAN 6236, IWP 5070, A 206 and Dohad local
Canopy temperature I (^{o}C)	18.7, 18.9, 19.2, 19.2, 19.3	MACS 2846, NP 404, MACS 1967, NP 4 and Jairaj
Canopy temperature II (^{o}C)	20.7, 20.7, 20.7, 20.8, 20.9	Bijaga yellow, HI 8638, Raj 6069, MACS 2846 and HI 8691
Canopy temperature III (^{o}C)	23.4, 23.5, 23.9, 23.9, 24.0	MACS 2846, HI 8638, V 21, MACS 1967 and Line 1172
1000 grain weight (g)	59.0, 57.1, 55.7, 55.6, 53.3	HD 4672, Baxi 228-18, V 21, HI 8550 and Bijaga red
Harvest index (%)	53.6, 44.4, 43.4, 42.9, 41.7	HI 8627, HD 4676, HI 8671, GW 1225 and HI 8638
Grain yield/plant (g)	22.7, 22.6, 22.4, 22.1, 21.8	HD 4672, HI 8627, V 21, HD 4676 and HI 8638

agreement with Deshmukh *et al*., (1999) and Lad *et al.*, (2002) in durum wheat. Thus, the complex composition of clusters indicates that geographical diversity is important but it is not the sole factor determining the genetic diversity (Murthy and Arunachalam, 1966). Based on the analysis, varieties with gene source for all the important parameters have been identified (Table 5). Gene sources for days to flowering (Jay); days to maturity (V 21) and plant height (IWP 5007), whereas GW 1209, MPO 1243, HI 8627 and MACS 9 are gene source for number of tillers, spike length, number of grains/spike and biomass/plant, respectively as these are found superior for the particular traits. MACS 2846 is the gene source for low canopy temperature at all the growth conditions. Since low canopy temperature is the important selection parameter to identify high yielding genotypes, therefore, genotypes showing low canopy temperature values will be utilized for future selection and breeding. HI 8638 with low canopy temperature and high grain yield can be a gene source for grain yield/plant. Keeping these points in view and with the changing climatic conditions, there is a need to select

gene sources in durum wheat genotypes adaptable to early heat stress situations (early sown condition) with minimum irrigation, which will help to improve the heat tolerant character. It will certainly help in increasing its area, production and productivity.

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Molecular Diversity Analysis for Zinc Deficiency Tolerance under Aerobic Rice (*Oryza sativa* L.) Ecosystem

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Rice (*Oryza sativa* L.) is the "Global Grain" because of its use as prime staple food in about 100 countries of the world. Aerobic rice provides for effective use of water as the concept of flooding paddy fields is abandoned in this ecosystem. But due to the transition from flooding of paddy fields to aerobic system of rice cultivation many factors that determine nutrient (Zn) bioavailability changes in addition to water deficit. Achieving higher yields under aerobic conditions requires new varieties of "aerobic rice" combining both the water stress tolerance and nutrient (especially Zn) deficiency tolerance traits. Molecular marker technologies are the effective tools and they are used for the assessment of genetic variability because they are not influenced by the environment. Among the molecular markers, Simple Sequence Repeat (SSR) has proved to be the most powerful tool for variety identification in rice and has much potential in genetic and breeding studies. Among the 16 SSR markers specific to zinc deficiency tolerance used for assessing the genetic diversity, 13 primer pairs (81.25%) were polymorphic. The number of alleles per microsatellite locus ranged from 2 to 5, averaging 2.53 alleles per locus. Polymorphism information content (PIC) values ranged from 0.43 (RM242) to 0.69 (RM1089 and RM3331), with an average of 0.58. The Unweighted Pair Group Method with Arithmetic Mean (UPGMA) dendrogram revealed two major groups with three clusters and the wide range of dissimilarity values (0.68-1.00) which showed a high degree of diversity among the germplasm lines. The results of the genetic diversity will be useful for the selection of the parents for developing zinc deficiency tolerant rice variety under aerobic condition through molecular breeding programme.

Key Words: DNA polymorphism, Genetic Diversity, Polymorphism information content (PIC), Rice, SSR markers.

Introduction

The wonder cereal, rice (*Oryza sativa* L.) is the heart of our culture and the staple food crop consumed by more than 50% of the world's population, which is cultivated over 163 million hectares with the production of 718 million tonnes (FAO, 2013). About 90% of the world's rice is produced in Asia, out of which 20% is produced in India. The area, production and productivity of rice in India is 427.53 lakh hectares, 105.24 million tonnes and 2462 kg/ha, respectively, in 2012-13 (DAC, 2014). India is the second largest producer and consumer of rice in the world, accounting for 22.3% of global production. In addition to food security, rice also plays an important role in socioeconomic and political stability in many countries in Asia, Africa and Latin America. Irrigated rice has very low water-use efficiency as it consumes 3000–5000 litres of water to produce one kg of rice. In rice, among the several production constraints, availability of irrigation water is a major factor as it consumes about 70% of the water available for agriculture (Vibhu Nayar and Ravichandran, 2012). Therefore, ways must be sought to reduce water requirements in rice and increase its productivity. The aerobic condition is maintained by using flush irrigation or sprinklers so that ponding occurs for only short periods of time just after irrigation or rain, if at all. The potential for water savings is large, but aerobic cultivation using conventional lowland rice varieties almost always leads to yield reduction (Atlin *et al*., 2004). Therefore achieving higher yields under aerobic conditions requires new varieties of "aerobic rice" combining both the water stress tolerance and nutrient (especially Zn) deficiency tolerance traits. Thus there is an immediate need to accelerate the genetic improvement of rice for aerobic cultivation, which until recently has received less attention compared to lowland rice. This could be hastened through the identification of molecular markers linked to traits associated with water use efficiency and zinc deficiency tolerance under aerobic conditions in rice and deployment *via* marker assisted breeding. It is difficult to classify the accessions solely

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based on their morphological characters (Gangashetty *et al*., 2013 and Singh *et al*., 2010). The development of reliable methods is necessary to allow for the assessment of genetic variability in germplasm collections or pedigree reconstruction. In various methodologies, DNA based technologies are the most reliable tools allowing for the assessment of genetic variability because they are not influenced by the environment. DNA markers *viz*., RFLP, RAPD, SSR, ISSR etc. can be used to assess the diversity studies (Venkatesan and Bhat, 2015; Kumbhar *et al*., 2013). Among them, SSR markers have great potential in genetic and breeding studies (Matin *et al*., 2012). The present investigation was made to identify the suitable SSR primers for genetic analysis of zinc deficiency tolerance under aerobic rice and to measure the genetic diversity and relatedness among 62 rice germplasm lines using SSR markers specific to zinc deficiency tolerance.

Materials and Methods

The experiments were conducted at Department of Rice, Tamil Nadu Agricultural University, Coimbatore during August/2013-January/2014.

Plant Material

A total of 62 rice germplasm lines (Table 1) comprising of upland and lowland rice cultures were selected and sown directly under aerobic conditions.

Extraction of Genomic DNA

DNA was extracted from the leaf samples of the two parents and the progenies following CTAB method developed by Saghai-Maroof *et al*. (1984) with suitable modifications suggested by Hoisington *et al*. (1994).

DNA Quality and Quantity Check

The nucleic acids were quantified by the methods of Spectrophotometric determination and gel electrophoresis (0.8% agarose gel). DNA concentration for PCR amplification was estimated by comparing the band intensity of a sample with the band intensities of known dilutions that gave good amplifications. The dilutions were carried out by dissolving the genomic DNA in appropriate volume of TE buffer.

PCR Amplification

SSR Markers

The parental survey was performed for 62 rice germplasm lines using 16 SSR markers specific to zinc deficiency tolerance (Bashir *et al*., 2012; Vivek, 2012) for amplifying genomic DNA fragment by polymerase chain reaction. The parental polymorphism survey identified 13 polymorphic markers between germplasm lines. The list of polymorphic markers used for surveying the 62 rice germplasm lines is furnished in Table 2.

SSR Analysis

PCR amplifications were performed in Bio-Rad (MyCycler thermal cycler) and AB PCR machine. About 25 ng of genomic DNA was used as the template. The reaction was carried out in a total reaction volume of 20 µl containing the following components.

Components	Volume (µl)
DNA	2.00
Forward primer	1.00
Reverse primer	1.00
Sterile water	13.6
DNTP's	0.6
Taq buffer	1.5
Taq enzyme	0.3
Total	20.00

The thermal cycler was programmed as follows.

Step 1	Initial denaturing step	94°C for 5 minutes
Step 2	Denaturing	94°C for 1 min.
Step 3	Annealing	55°C for 1 min.
Step 4	Extension	72°C for 2 min.
Step 5	Repeat steps 2 through 4 for a total of 35 cycles (34 times)	
Step 6	Final extension	72°C for 5 min.
Step 7	4°C hold until sample retrieval	
Step 8	End	

PCR products were kept at 4ºC until further use.

Agarose gel electrophoresis was performed at constant power 120 volts for about 3 h to separate amplification products.

Analysis of Genetic Diversity

Genetic diversity among the 62 rice genotypes were evaluated using 13 SSR primers. Each fragment size was treated as a unique characteristic and scored as present (1) or absent (0). Genetic similarity index (UPGMA cluster analysis of the Jaccard's similarity coefficient) was used to construct a dendrogram which illustrated the genetic relationship among the 62 genotypes of

Table 1. Parentage of 62 germplasm lines used in the study

S. No.	Germplasm lines	Parentage	Origin
1	CB-08-702	IR 75417-R-R-R-R-267-3	TNAU, Tamil Nadu, India
2	CB-06-803	PMK3 × Norungan	TNAU, Tamil Nadu, India
3	CB-08-701	IR80013-B-141-4-1	TNAU, Tamil Nadu, India
4	CB-07-701-252	Norungan/TKM9/Norungan	TNAU, Tamil Nadu, India
5	CB-07-701-274	Norungan/TKM9/Norungan	TNAU, Tamil Nadu, India
6	CB-07-701-256	Norungan/TKM9/Norungan	TNAU, Tamil Nadu, India
7	CB-07-701-143	Norungan/TKM9/Norungan	TNAU, Tamil Nadu, India
8	CB-07-701-279	Norungan/TKM9/Norungan	TNAU, Tamil Nadu, India
9	IR84895-B-127-CRA-5-1-1	IR77080-B-34-3 × IRRI123	IRRI, Philippines
10	IR83381-B-B-18-3	IR72022-46-2-3-3-2 × IR77080-B-34-1-1	IRRI, Philippines
11	IR83387-B-B-110-1	IR72022-46-2-3-3-2 × Sambha mahsuri	IRRI, Philippines
12	CB-09-512	OR1797-4/Varapukudanchan	TNAU, Tamil Nadu, India
13	CB-09-516	RR4065-381-245/UPR2893-97-5-27	TNAU, Tamil Nadu, India
14	CB-07-701-12	Norungan/TKM9/Norungan	TNAU, Tamil Nadu, India
15	CO51	ADT43 × RR272-1745	TNAU, Tamil Nadu, India
16	CB-07-701-23	Norungan/TKM9/Norungan	TNAU, Tamil Nadu, India
17	CB-07-701-115	Norungan/TKM9/Norungan	TNAU, Tamil Nadu, India
18	CB-07-701-126	Norungan/TKM9/Norungan	TNAU, Tamil Nadu, India
19	CB-07-701-128	Norungan/TKM9/Norungan	TNAU, Tamil Nadu, India
20	CB-07-701-129	Norungan/TKM9/Norungan	TNAU, Tamil Nadu, India
21	CB-07-701-146	Norungan/TKM9/Norungan	TNAU, Tamil Nadu, India
22	CB-07-701-148	Norungan/TKM9/Norungan	TNAU, Tamil Nadu, India
23	CB-07-701-150	Norungan/TKM9/Norungan	TNAU, Tamil Nadu, India
24	CB-07-701-151	Norungan/TKM9/Norungan	TNAU, Tamil Nadu, India
25	CB-07-701-174	Norungan/TKM9/Norungan	TNAU, Tamil Nadu, India
26	CB-07-701-199	Norungan/TKM9/Norungan	TNAU, Tamil Nadu, India
27	CB-07-701-218	Norungan/TKM9/Norungan	TNAU, Tamil Nadu, India
28	CB-07-701-230	Norungan/TKM9/Norungan	TNAU, Tamil Nadu, India
29	CB-07-701-255	Norungan/TKM9/Norungan	TNAU, Tamil Nadu, India
30	CB-07-701-262	Norungan/TKM9/Norungan	TNAU, Tamil Nadu, India
31	CB-07-701-264	Norungan/TKM9/Norungan	TNAU, Tamil Nadu, India
32	CB-07-701-265	Norungan/TKM9/Norungan	TNAU, Tamil Nadu, India
33	CB-07-701-268	Norungan/TKM9/Norungan	TNAU, Tamil Nadu, India
34	CB-07-701-278	Norungan/TKM9/Norungan	TNAU, Tamil Nadu, India
35	CB-07-701-280	Norungan/TKM9/Norungan	TNAU, Tamil Nadu, India
36	CB-07-701-283	Norungan/TKM9/Norungan	TNAU, Tamil Nadu, India
37	CB-07-701-284	Norungan/TKM9/Norungan	TNAU, Tamil Nadu, India
38	CB-07-701-181	Norungan/TKM9/Norungan	TNAU, Tamil Nadu, India
39	CB-07-701-288	Norungan/TKM9/Norungan	TNAU, Tamil Nadu, India
40	CB-00-11-4	IR50 × Norungan	TNAU, Tamil Nadu, India
41	CB-00-11-7	IR50 × Norungan	TNAU, Tamil Nadu, India
42	CB-00-11-19	IR50 × Norungan	TNAU, Tamil Nadu, India
43	CB-00-11-21	IR50 × Norungan	TNAU, Tamil Nadu, India
44	CB-00-11-22	IR50 × Norungan	TNAU, Tamil Nadu, India
45	CB-00-11-23	IR50 × Norungan	TNAU, Tamil Nadu, India
46	CB-00-12-91	Norungan × IR50	TNAU, Tamil Nadu, India
47	CB-00-12-192	Norungan × IR50	TNAU, Tamil Nadu, India
48	ARB-6	Budda × IR64	UAS, GKVK, Bangalore
49	CB-06-803-2	PMK3 × Norungan	TNAU, Tamil Nadu, India
50	Anna-4	Pantdhan10 × IET9911	TNAU, Tamil Nadu, India
51	CB-00-15-10	IR64 × Norungan	TNAU, Tamil Nadu, India
52	CB-00-15-23	IR64 × Norungan	TNAU, Tamil Nadu, India
53	CB-00-15-44	IR64 × Norungan	TNAU, Tamil Nadu, India
54	CB-00-755-2	IR64/Norungan/IR64	TNAU, Tamil Nadu, India
55	CB-00-15-24	IR64 × Norungan	TNAU, Tamil Nadu, India
56	CB-08-709-2	RR4065-381-245 × UPR2893-99-5-2-1	TNAU, Tamil Nadu, India
57	Apo (IR55423-01)	UPLRI5 × IR12979-24-1	IRRI, Philippines
58	PSBRC-80	IR64 x Moroberekan	Philippine seed board rice, Philippine rice research institute, Philippines.
59	PSBRC-82	IR47761-27-1-3-6 × PSB RC 28	Philippine seed board rice, Philippine rice research institute, Philippines.
60	CB-07-701-22	Norungan/TKM9/Norungan	TNAU, Tamil Nadu, India
61	IR64	IR5857-33-2-1 × IR2061-465-1-5-5	IRRI, Philippines
62	CB-09-123	BPT5204 × CO50	TNAU, Tamil Nadu, India

Table 2. List of polymorphic markers and Polymorphic Information Content (PIC) value

S. No.	Primers	Chromosome No.	Forward sequence	Reverse sequence	No. of alleles	PIC value
1	RM263F	1	CCCAGGCTAGCTCATGAACC	GCTACGTTTGAGCTACCACG	2	0.49
2	RM234F	1	ACAGTATCCAAGGCCCTGG	CACGTGAGACAAAGACGGAG	2	0.49
3	RM21F	2	ACAGTATTCCGTAGGCACGG	GCTCCATGAGGGTGGTAGAG	2	0.64
4	RM212F	2	CCACTTTCAGCTACTACCAG	CACCCATTTGTCTCTCATTATG	2	0.50
5	RM499F	3	TACCAAACACCAACACTGCG	ACCTGCAGTATCCAAGTGTACG	2	0.50
6	RM1089F	5	CAGAAGGATTATCTCGATACC	AATAGGGCTTGAAATAAATTG	2	0.69
7	RM296F	5	CACATGGCACCAACCTCC	GCCAAGTCATTCACTACTCTGG	3	0.49
8	RM164F	5	TCTTGCCCGTCACTGCAGATATCC	GCAGCCCTAATGCTACAATTCTTC	2	0.52
9	RM3331F	7	CCTCCTCCATGAGCTAATGC	AGGAGGAGCGGATTTCTCTC	5	0.69
10	RM6832F	9	GTTGTAAATGCCTGAGTGC	AAAGAGCTAAACCGCTAGG	2	0.61
11	RM26334F	11	GACTCCCTACTAGTGGTTCTGATTCG	CCTTTGACGATTGTGATGCTACG	2	0.62
12	RM242F	11	GGCCAACGTGTGTATGTCTC	TATATGCCAAGACGGATGGG	3	0.43
13	RM87F	12	CCTCTCCGATACACCGTATG	GCGAAGGTACGAAAGGAAAG	3	0.57

rice used in the study. A dendrogram was constructed using similarity index adopting Sequential Hierarchial and Nested (SAHN) using the NTSYS-pc 2.2 (Rohlf, 2005).

Results and Discussion

Molecular markers are a useful complement to morphological and physiological characterization of cultivars because they are plentiful, independent of tissue or environmental effects and allow cultivar identification early in plant development. The molecular markers based on differences in DNA sequences between individuals generally detect more polymorphisms than morphological and protein based markers (Mignouna *et al*., 1998; Tanksley *et al*., 1989). Simple Sequence Repeats (SSR) is used as a primer to amplify regions between the microsatellites. This marker reveals a much larger number of fragments per primer than RAPD analysis (Bajpai *et al*., 2008).

The level of polymorphism among rice germplasm lines was evaluated by calculating allelic number and PIC values for each of the 13 SSR loci specific to zinc deficiency tolerance. A total of 36 alleles were detected at the loci of 13 microsatellite markers across 62 rice germplasm lines. The results revealed that all the primers showed distinct polymorphisms among the germplasm lines studied indicating the robust nature of microsatellites in revealing polymorphism for zinc deficiency tolerance under aerobic condition. Among the polymorphic markers, eight generated two alleles each, four produced three alleles each and only one produced 5 alleles (Table 3). The number of alleles per locus ranged from 2 (RM234, RM3331, RM164, RM212, RM263, RM499, RM296 and RM279) to 5 alleles (RM 1089 and RM3331) with an average of 2.53 alleles across the 13 primers. Similar number of microsatellite markers previously used as subset for genetic diversity analysis of *O. sativa* (Garris *et al*., 2005; Thomson *et al*., 2007; Kanawapee *et al*., 2011; Kumbhar *et al*., 2013; Venkatesan and Bhat, 2015). The Value is comparable to 2-5 allele per SSR locus with an average number of alleles of 2.53 per locus for various classes of microsatellite (Siwach *et al*., 2004; Matin *et al*., 2012). Maximum number of polymorphic alleles (5) was obtained with the marker RM 1089, while the minimum numbers of polymorphic alleles (2) was obtained by using RM234, RM3331, RM164, RM212, RM263, RM499, RM296 and RM279 (Fig. 1).

Polymorphism information content (PIC) value is a reflection of allele diversity and frequency among the germplasm lines. PIC values ranged from 0.43 to 0.69 with an average of 0.58 (Table 3). The highest PIC value 0.69 was obtained for RM1089 and RM3331 followed by respectively RM21 (0.64), RM26334 (0.62) and RM6832 (0.61). PIC value revealed that RM1089 and RM3331 were considered as best markers for 62 rice germplasm lines for zinc deficiency tolerance under aerobic condition. Similar results were reported by Alvarez *et al*., 2007; Matin *et al*., 2012; Kumbhar *et al*., 2013.

Cluster Analysis

The cluster analysis based on unweighted paired group method of arithmetic means (UPGMA) with 13 SSR

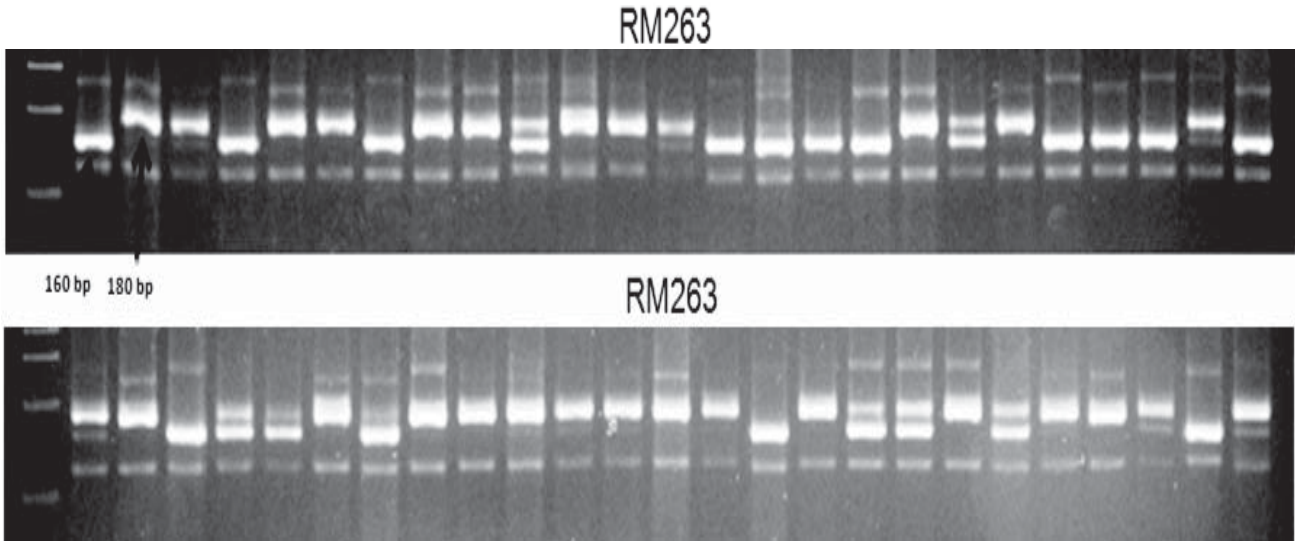

Fig. 1. Representative SSR profile of rice germplasm lines obtained with RM263

Table 3. Distribution of rice germplasm lines into different clusters based on SSR analysis

Cluster	Subcluster	No.of genotypes	Genotypes
I	A	2	CB-08-702 and CB-07-701-12
	B	4	CB-07-701-143,CB-07-701-150, PSBRC-80 and PSBRC-82
II	A	6	CB-06-803, IR83381-B-B-18-3, IR83387-B-B-110-1, CB-07-701-264, CB-00-15-44 and CB-09-123
	B	4	CB-00-11-23, CB-00-755-2, CB-00-15-24 and CB-08-709-2
	C	7	CB-09-512, CB-09-516, CB-07-701-146, CB-00-12-91, Anna-4, CB-00-15-10 and Apo
	D	1	IR84895-B-127-CRA-5-1-1
	E	5	CB-00-12-192, ARB-6, CB-06-803-2, CB-00-11-22 and IR64
	F	4	CB-00-11-4, CB-00-11-7, CB-00-11-19 and CB-00-11-21
III	A	5	CB-08-701, CB-07-701-274, CB-07-701-256, CB-07-701-279 and CB-07-701-218
	B	1	CB-00-15-23
	C	7	CB-07-701-23, CB-07-701-199, CB-07-701-262, CB-07-701-278, CB-07-701-283, CB-07-701-284 and CB-07-701-288
	D	5	CB-07-701-22, CB-07-701-174, CB-07-701-255, CB-07-701-265 and CB-07-701-280
	E	2	CB-07-701-230 and CB-07-701-252
	F	3	CB-07-701-151, CB-07-701-268 and CO51
	G	6	CB-07-701-115, CB-07-701-126, CB-07-701-128, CB-07-701-129, CB-07-701-148 and CB-07-701-181

primers specific to zinc deficiency tolerance allowed the discrimination of cultivars. The UPGMA based clustering grouped 62 rice germplasm lines into three major clusters (Table 3, Fig. 2). Cluster III had maximum number of genotypes (29) followed by cluster II (27), whereas cluster I had six genotypes. Cluster III was the largest and most diverse cluster consisting of 29 genotypes. This cluster was subgrouped into seven sub-clusters (A, B, C, D, E, F and G) at varying degree of similarity. Subgroup B contain highest number of genotypes (7) *viz*., CB-07-701-23, CB-07-701-199, CB-07-701-262, CB-07-701-278, CB-07-701-283, CB-07-701-284 and CB-07-701-288 followed by subgroup G (6) *viz*., CB-07-701-115, CB-07-701-126, CB-07-701-128, CB-07-701-129, CB-07-701-148 and CB-07-701-181, subgroup A (5) *viz*., CB-08-701, CB-07-701-274, CB-07-701-256, CB-07-701-279 and CB-07-701-218, subgroup D (5) *viz*., CB-07-701-22, CB-07-701-174, CB-07-701-255, CB-07-701-265 and CB-07-701-280, subgroup F (3) *viz*., CB-07-701-151, CB-07-701-268 and CO51, subgroup E (2) had CB-07-701-230 and CB-07-701-252 and subgroup B (1) had only one genotype *ie*., CB-00-15-23. Cluster II contained 29 germplasm lines and subclustered into six groups (A, B, C, D, E and F). In this subgroup C contains highest number of genotypes (7) *viz*., CB-09-512, CB-09-516, CB-07-701-146, CB-00-12-91, Anna-4,

CB-00-15-10 and Apo followed by subgroup A (6) *viz*., CB-06-803, IR83381-B-B-18-3, IR83387-B-B-110-1, CB-07-701-264, CB-00-15-44, CB-09-123, subgroup E (5) *viz*., CB-00-12-192, ARB-6, CB-06-803-2, CB-00-11-22 and IR64, subgroup B (4) *viz*., CB-00-11-23, CB-00-755-2, CB-00-15-24 and CB-08-709-2, subgroup F (4) *viz*., CB-00-11-23, CB-00-755-2, CB-00-15-24 and CB-08-709-2, D (1) had only one genotype *ie*., IR84895-B-127-CRA-5-1-1. Cluster I was the smallest group had six genotypes which contains two subclusters (A and B). Subgroup B had 4 genotypes *viz*., CB-07-701-143,CB-07-701-150, PSBRC-80 and PSBRC-82 followed by subgroup A (2) namely CB-08-702 and CB-07-701-12. Similar results were reported by Matin *et al*., 2012 and Kumbhar *et al*., 2013, Venkatesan *et al*., 2011 and Venkatesan and Bhat, 2015. Hence microsatellite marker based molecular fingerprinting could serve as a potential basis in the identification of genetically distance genotypes as well as in sorting of duplication for morphologically close accession. The present study is an attempt at characterizing diversity at the molecular level in this set of germplasm lines for zinc deficiency tolerance under aerobic conditions. Moreover, the cultivars with wide genetic distance can be crossed to widen the genetic base and exploit heterosis. The informative primers would prove useful in marker assisted selection, linkage mapping and gene tagging for zinc deficiency tolerance under aerobic conditions.

The present study revealed a considerable genetic diversity present in the 62 rice genotypes mainly belongs to Tamil Nadu state of India at molecular level and a limited number of SSR markers efficiently grouped these genotypes into 6 clusters on basis of zinc deficiency tolerance. SSR markers such as RM1089 and RM3331 could be useful to breeders for discriminating different rice genotypes for zinc deficiency tolerance under aerobic conditions.

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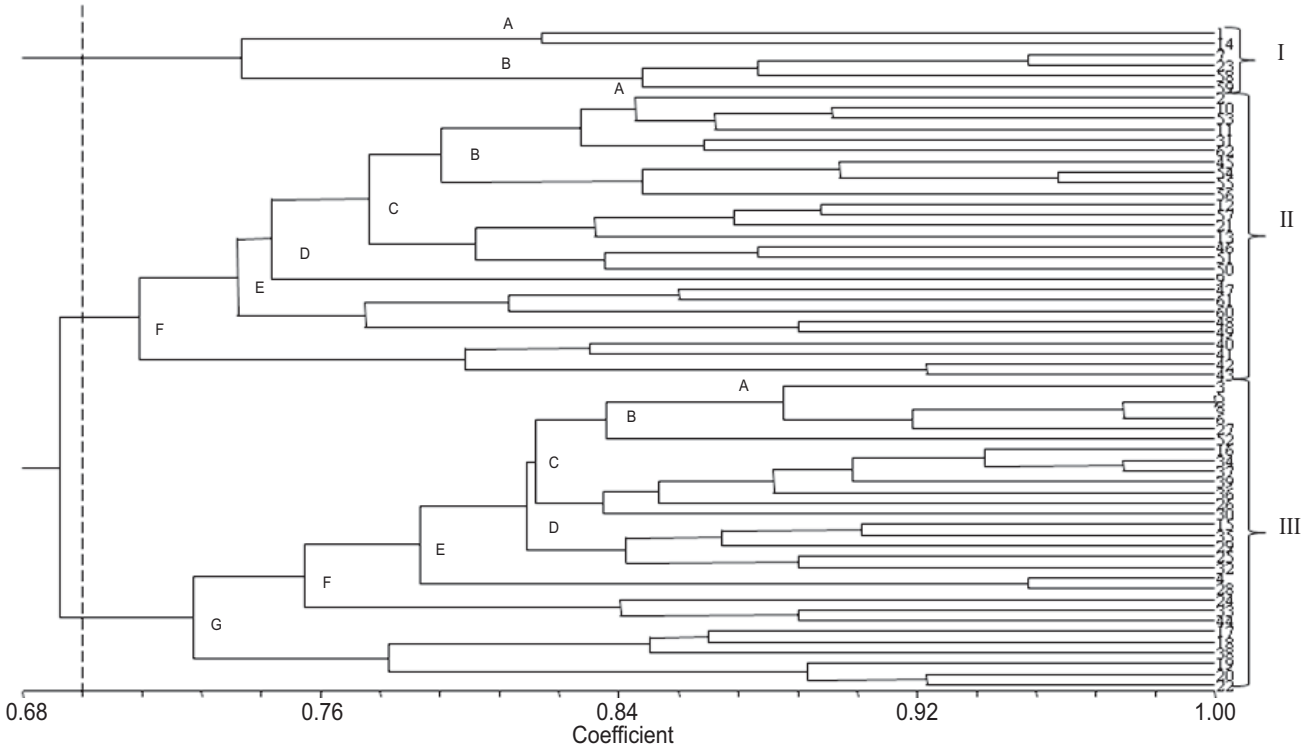

Fig. 2. UPGMA based dendrogram of rice germplasm lines using SSR markers specific to zinc deficiency tolerance

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Genetic Diversity Assessment and Characterization of Indian Mustard (*Brassica juncea* L.) Varieties using Agro-morphological Traits

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Indian mustard (*Brassica juncea* L. Czern & Coss.) is an important source of edible oil in Asian countries. Indian mustard suffers from the lack of distinguishable morphological markers. Effectiveness of a trait in establishing distinctness was determined following a criterion that involved statistical parameter of dispersion measured in terms of range and coefficient of variation. Simultaneously, stability of a trait was estimated as average of coefficient of variation estimates of three year mean values of each genotype. Extent of diversity was estimated and distinctness among cultivated Indian mustard varieties could be established. Varieties were grouped into five different clusters on the basis of multivariate analysis following Euclidean distance and UPGMA method and diverse varieties from different clusters were suggested for hybridization programme. Seed weight, days to maturity, plant height, siliqua angle with main raceme, petal length, siliqua length, plant height/length of main raceme, seeds per siliqua and days to flower initiation were found more effective than leaf length, leaf width and number of leaf lobes. There was no correspondence of grouping between classification based upon mean estimate of traits and clusters based upon multivariate analysis.

Key Words: Distinctness, Diversity, Efficacy, Indian mustard, Stability.

Introduction

Plant Breeders rights have been granted as intellectual property rights in many countries to promote the research in crop improvement. Such rights have been granted in India too under the 'Protection of Plant Varieties and Farmers Rights Act, 2001'. Establishing distinctness on the basis of morphological traits is the pre-requisite to assign the plant breeders rights. Oilseed Brassica crops, the second most important oilseed crop globally after soybean, generally lack distinguishable morphological markers. Indian mustard (*Brassica juncea* L. Czern & Coss.), which is predominantly grown in southern Asia as an important oilseed Brasscica crop, also suffers from the lack of distinguishable morphological markers (Singh et al., 2006). Most of the traits included in descriptor for distinctness, uniformity and stability (UPOV, 1996; PPV&FRA., 2009) are quantitative; hence show continuous variation unlike discrete classes of qualitative traits which are more efficient in establishing distinctness among varieties. Needs to devise more rapid and cost-effective testing procedures to improve the current testing system has been suggested (Cooke, 1999). Different biochemical techniques including comparison of seed oil fatty acid profile by GLC analysis (White and Law, 1991), HPLC analysis of leaf glucosinolates (Adams *et al*., 1999), starch-gel electrophoresis of cotyledon isozymes (Mundges *et al*., 1999) and molecular markers (Tommasini *et al*., 2003) have been reported useful to supplement morphological traits for DUS testing. However, variation within varieties in outbreeding species tends to be high and this lack of uniformity hampers the use of molecular markers for distinguishing between varieties. Preliminary studies have suggested that it might be difficult to identify markers that are sufficiently uniform within varieties and, at the same time, are sufficiently variable between varieties to allow for variety discrimination (Mailer *et al*., 1994; Plaschke *et al*., 1995; Charters, 1996; Robertson *et al*., 1996; Olufowote *et al*., 1997; Donini *et al*., 1998 and Roldan *et al*., 2000). UPOV and Indian test guidelines for DUS testing include only morphological traits. Present investigation aimed to assess the extent of diversity among cultivated Indian mustard varieties and to evaluate the effectiveness of different morphological traits in establishing distinctness using potential morphological traits.

Materials and Methods

Plant material in the present study comprised of 31 released varieties of Indian mustard. The detail of their developing organization and release year has been given

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in Table 1. These varieties were evaluated for three consecutive years in randomised complete block design. Row to row and plant to plant spacing was maintained at 45 and 15 cm, respectively. Recommended practices were followed to raise the good crop. Observations on 15 morphological traits *viz*., number of leaf lobes (LLB), leaf length (cm, LL), leaf width (cm, LW), petal length (cm, PL), petal width (cm, PW), siliqua length (cm, SL), beak length (cm, BL), number of siliquae on main raceme (SMR), siliqua angle with main raceme (0, SA), seeds per siliqua (SS), length of main raceme (cm, LMR), plant height (cm, PH), days to flower initiation (DFI), days to maturity (DM) and 1000-seed weight (g, SW) were recorded. In addition, six variables were computed as ratio between leaf length/leaf width (LL/LW), petal length/petal width (PL/PW), siliqua length/beak length (SL/BL), plant height/length of main raceme (PH/LMR), number of siliquae on main raceme/length of main raceme (SMR/LMR) and days to maturity/days to flower initiation (DM/DFI). Observations were recorded on 60 plants (20 plants from each replication) each year following DUS descriptor (Singh *et al*., 2006).

Mean values of all 21 variables (15 observed and 6 computed) were subjected to statistical analyses. Analysis of variance and multivariate analysis for hierarchical cluster analysis following Euclidean distance and UPGMA method was carried out using linear mixed model and pattern analysis module, respectively, of cropstat 7.2 software (CROPSTAT7.2). Effectiveness of a trait in establishing distinctness was determined following a criterion that involved statistical parameter of dispersion measured in terms of range and coefficient of variation. Simultaneously, stability of a trait in present investigation was estimated as average

Table 1. List of varieties of Indian mustard along with name of developing organization and year of release

Code	Variety name	Year of release	Developing organization	Pedigree
1	Arawali (RN 393)	2001	ARS, RAU Navgaon	Krishna × RS 50
2	CS 52	1998	CSSRI, Karnal	Selection from DIRA 343
3	Geeta (RB 9901)	2003	CCS HAU Bawal	Mutant of RH 30
4	GM 1	1990	SDAU SK Nagar	MR71-3-2 × TM 4
5	GM 2	1997	SDAU SK Nagar	Selection from local germplasm
6	Kanti	2003	CSAUA&T Kanpur	Selection from germplasm collected Kanpur Dehat
7	Kranti	1984	GBPUAT, Pantnagar	Selection from Varuna
8	Krishna	1998	GBPUAT, Pantnagar	Selection from Varuna
9	Laxmi (RH8812)	1997	CCS HAU Hisar	PR15 × RH 30A
10	Maya	2003	CSAUA&T Kanpur	Varuna × KRV 11
11	NDRE-4	2001	NDUAT, Faizabad	TM9 × Seeta
12	PBR 91	1996	PAU, RS Bathinda	(RLM 511× PR 18) × CM1
13	PBR 97	1997	PAU, RS Bathinda	(DIR 202 × PR 34 × V3) × RLM 619 × Varuna)
14	Pusa Agrani (Sej-2)	1998	IARI, New Delhi	Early maturing *B. juncea* × synthetic amphidiploids (*B. campestris* var. toria
15	Pusa Bahar	1991	IARI, New Delhi	(Pusa Rai 28 × Varuna) × (Pusa Rai 30 × T6342)
16	Pusa Bold	1985	IARI, New Delhi	Varuna × BIC 1780
17	Pusa Jai Kisan (Bio-902)	1994	IARI, New Delhi	Somaclone of Vrauna
18	Rajat (PCR 7)	1997	DRMR, Bharatpur	Selection from Katch germplasm line JMG
19	RH 819	1991	CCS HAU Hisar	Prakash × Bulk pollen
20	RH 30	1985	CCS HAU Hisar	Selection from P 26/3-1
21	RH 781	1991	CCS HAU Hisar	(RL 18 × P 26/3-1) × RL 18
22	RL 1359	1988	PAU, Ludhiana	RLM 514 × Varuna
23	RLM 619	1985	PAU, Ludhiana	Gamma ray induced mutant of RL 18
24	Rohini	1986	CSAUA&T Kanpur	Selection from natural population of Varuna
25	Sanjucta Asech	1989	PORS Berhampore	TM 4 × RK 2
26	Saurabh (RH 8113)	1987	CCS HAU Hisar	T 59 × RC 781
27	Swarn Jyoti (RH 9801)	2003	CCS HAU Hisar	Selection from germplasm line RC 1670
28	Urvashi (RK 9501)	2001	CSAUA&T Kanpur	Varuna × Kranti
29	Vardan	1985	CSAUA&T Kanpur	Derived through biparental mating involving Varuna, Keshari, CSU 10 and IB 1775, IB 1786 and IB 1866
30	Varuna (T 59)	1976	CSAUA&T Kanpur	Selection from Varanasi Local
31	Vasundhara (RH 9304)	2003	CCS HAU Hisar	RH 839 × RH 30

of coefficient of variation estimates of three year mean values of each genotype. High estimate indicated low stability and vice versa. A trait having wide range, high estimate of variability (coefficient of variation among genotypes) and low estimate of coefficient of variation among environments (years) was considered effective in establishing distinctness and accordingly relative performance of all studied traits was assessed. Distinctness among Indian mustard varieties was established on the basis of mean estimates for different morphological traits following Indian DUS guidelines for Indian mustard.

Results and Discussion

Combined analysis of variance revealed the existence of significant variability for 19 observations in 31 varieties of Indian mustard. Varieties did not differ for petal width and petal length/petal width (PL/PW). Interactions between variety and year were also significant for all traits except petal width. Ranges were wide for plant height, length of main raceme, siliqua angle, siliquae on main raceme, leaf length, days to flower initiation and days to maturity. Highest variability as depicted by coefficient of variation (Table 2) among varieties was recorded for seed weight (19.4%) followed by siliqua angle (16.9%). Beak length, siliqua length/beak length, leaf length, siliqua length, leaf width, number of leaf lobes, siliquae on main raceme, seeds per siliqua, plant height, plant height/length of main raceme, siliquae on main raceme/length of main raceme, length of main raceme and days to flower initiation expressed moderate variability. Remaining trait had low variability. Days to maturity (CV 3.2%) expressed least variability over years followed by petal length (CV 3.4%) indicating high stability. Leaf length, leaf width, siliqua length/ beak length, beak length and number of leaf lobes were observed as fluctuating over years. Remaining traits plant height, main raceme length, seeds per siliqua, days to flower initiation, days to maturity/days to flower initiation, seed weight, leaf length/leaf width, plant height/length of main raceme, siliquae on main raceme/ length of main raceme and siliquae on main raceme were moderately stable.

On the basis of multivariate analysis, 31 varieties were grouped into 5 clusters (Table 3). RL 1359 and Swarn Jyoti were most resembling varieties followed by Pusa Bold and PBR 97, while, Pusa Bold exhibited

Table 2. Mean, range and coefficient of variation (CV) estimates for 21 traits in Indian mustard varieties

Trait	Mean	Range	Minimum	Maximum	CV among varieties	CV over years
LLB	7.8	2.4	6.4	8.8	7.9	9.6
LL	31.6	13.0	23.3	36.3	9.1	12.8
LW	11.5	4.1	9.3	13.3	8.7	12.3
LL/LW	2.8	0.5	2.5	3.0	4.4	6.5
PL	1.1	0.1	1.0	1.1	2.3	3.4
PW	0.7	0.2	0.6	0.8	4.9	6.6
PL/PW	1.6	0.2	1.4	1.7	3.5	5.9
SL	4.4	2.0	3.7	5.7	8.8	6.2
BL	1.0	0.4	0.9	1.2	9.3	9.8
SL/BL	4.3	1.5	3.8	5.2	9.3	10.4
SMR	49.3	22.5	40.7	63.2	7.8	7.5
SA	28.4	28.1	7.0	35.0	16.9	7.2
SS	15.2	6.8	12.0	18.8	7.8	6.1
LMR	64.7	22.2	55.3	77.5	7.3	6.0
PH	172.6	65.4	128.3	193.7	8.5	5.1
PH/LMR	2.7	1.0	2.1	3.1	8.5	6.9
SMR/LMR	0.8	0.3	0.6	0.9	7.5	7.2
DFI	56.8	16.7	46.8	63.4	6.3	6.2
DM	124.7	17.1	114.4	131.6	3.1	3.2
DM/DFI	2.2	0.5	2.1	2.6	5.2	6.1
SW	4.3	3.0	2.9	5.9	19.4	6.5

(LLB: No. of leaf lobes; LL: leaf length; LW: leaf width; LL/LW: leaf length/leaf width; PL: petal length; PW: petal width; PL/PW: petal length/petal width; SL: siliqua length; BL: beak length; SL/BL: silqua length/beak length; SMR: number of siliquae on main raceme; SA: siliqua angle with main raceme; SS: seeds per siliqua; LMR: length of main raceme; PH: plant height; PH/LMR: plant height/ length of main raceme; SMR/LMR: number of siliquae on main raceme /length of main raceme ; DFI: days to flower initiation; DM: days to maturity ; DM/DFI: days to maturity/days to flower initiation; SW: 1000-seed weight)

Table 3. Grouping of varieties into different clusters on the basis of agro-morphological traits

Cluster No.	No. of varieties	Name of varieties
1	05	CS 52, Geeta (RB 9901), Laxmi (RH8812), RLM 619, Rohini
2	03	Maya, NDRE-4, PBR 91
3	02	Pusa Jai Kisan (Bio-902), Saurabh (RH 8113)
4	07	Arawali (RN 393), GM 1, GM 2, RL 1359, Swarn Jyoti (RH 9801), Urvashi (RK 9501), Vardan
5A	09	Kanti, Kranti, Krishna, Pusa Agrani (Sej-2), Pusa Bahar, RH 819, RH 30, S. Asech, Vasundhara (RH 9304)
5B	05	PBR 97, Pusa Bold, Rajat (PCR 7), RH 781, Varuna (T 59)

maximum distance from Saurabh. Fifth cluster comprising 14 varieties was biggest while cluster 3 comprising two varieties was the smallest. Cluster 5 could be divided into two subclusters; 5A and 5B. Subcluster 5A included Sanjuncta Asech, Pusa Agrani, Pusa Bahar, Krishna, RH 819, Kanti and Kranti while subcluster 5b included RH 781, Varuna, Rajat, PBR 97 and Pusa Bold.

Out of 21 variables studied in present investigation, petal width and petal length/petal width did not have significant variability in present set of varieties. Two parameters; coefficient of variation among varieties (variability parameter) and coefficient of variation over years (stability parameters) were plotted on a two dimensional scatter chart to visualize a biplot (Fig. 1). Trait occupying fourth quarter with high variability estimate and low coefficient of variation over years (stability parameter) were the most effective while traits occupying second quarter with low variability and low stability were unsuitable for establishing distinctness. Traits occupying 3rd quarter may be useful with material having large variability for these traits. Leaf characteristics including leaf length and leaf width displayed low stability; hence, their ratio LL/LW may be more suitable as it had high stability. These findings are in conformity with earlier findings of (Weerakoon and Somaratne, 2010) for length of main raceme and siliqua length in classifying mustard accessions, however, in disagreement for leaf length and leaf width which were recorded at seedling stage while Indian DUS test guidelines prescribes recording of leaf characteristics at bud stage. In general, new varieties

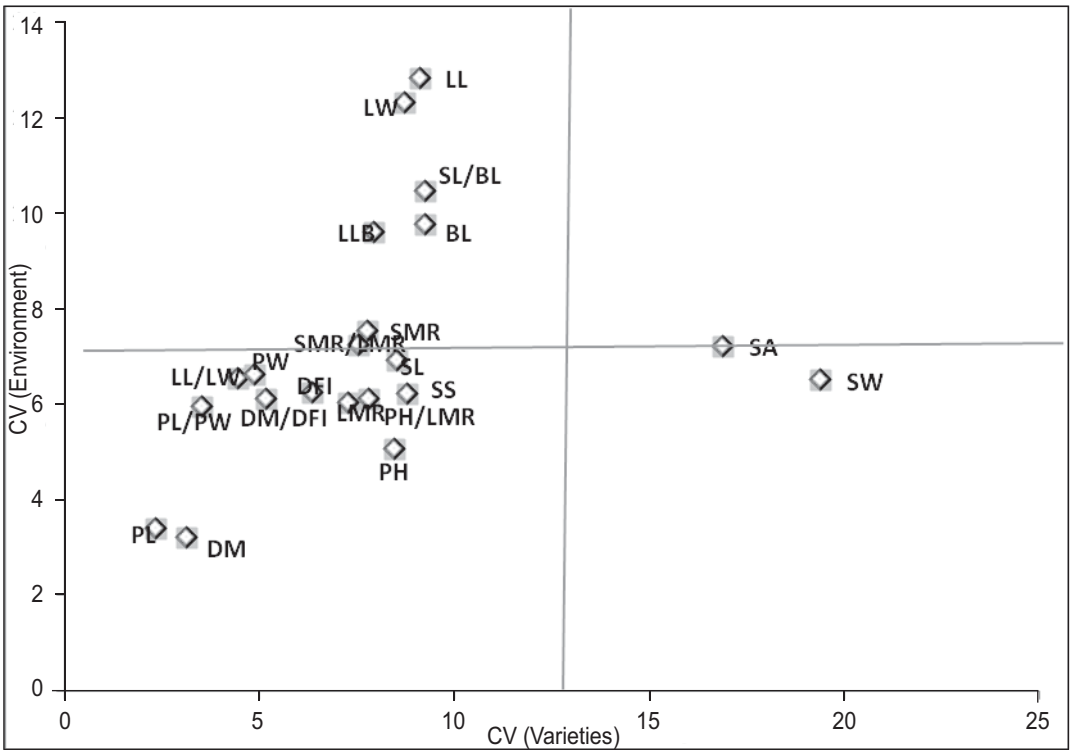

Fig. 1. Biplot of variability and stability parameters for different traits

may be considered "distinct" even in cases where they overlap with other varieties to a limited extent. However, it is necessary to quantify a threshold of the "minimum distance" between the new variety and any other variety from the reference collection, for the new variety to be considered distinct.

An analysis of pedigree of these 31 varieties revealed frequent use of single cultivar Varuna in hybridization programme. Four varieties; Kranti, Krishna, Pusa Jaikisan and Rohini have been derived through selection from natural population of Vrauna, while, seven varieties; Maya, PBR 97, Pusa Bahar, Pusa Bold, RL 1359, Saurabh and Urvashi had Varuna as one of the parentage in their pedigree. Another variety Aravali also had been derived through hybridization between Krishna and RS 50, here Krishna itself is direct selection from Varuna. Hence 12 varieties are the descendant of Varuna. Out of these four; Kranti, Krishna, Pusa Bahar and Pusa Bold have been grouped together in cluster five alongwith Varuna, indicating their resemblance. Reamaining eight varieties have migrated to other clusters which may be due to reconstellation of genes during meiosis and selection during segregating generations.

Distinctness among 31 varieties could be established using 8 morphological traits *viz*., seed weight, siliqua length, days to maturity, length of main raceme, seeds per siliqua, beak length and days to flower initiation (Table 4) proving the worth of these traits in establishing

Table 4. Distinctness based upon eight morphological traits among Indian mustard varieties

sw	sl	dm	ph	lmr	SS	BL	DFI	
small	small	early	Low					Sanjuncta Asech
			medium					Kanti
		medium	medium	medium				CS 52
	medium	early	Low	tall				NDRE-4
			medium	medium				Pusa Agrani
		medium	medium	medium	medium	low		RH 819
						medium	medium	Arawali
							late	Kranti
				small	medium			Vardan
			tall					Krishna
		late	tall					Saurabh
	long	medium	tall					RH 781
		Late	tall					RL 1359
medium	medium	medium	medium					GM 1
			tall					Geeta
		late	medium					PBR 91
			tall	medium	medium	medium	Late	PBR 97
							Medium	Swarn Jyoti
	long	medium	medium	medium	many			Pusa Bahar
			tall					Rohini
		late	tall	medium				Rajat
				tall				RLM 619
bold	medium	medium	medium	small				Urvashi
				medium	medium	medium	Medium	Pusa Jai Kishan
						medium	Late	Maya
					small			Varuna
				long				RH 30
			tall	medium	medium			GM 2
	long	medium	medium	medium	medium			Laxmi
					many			Pusa Bold

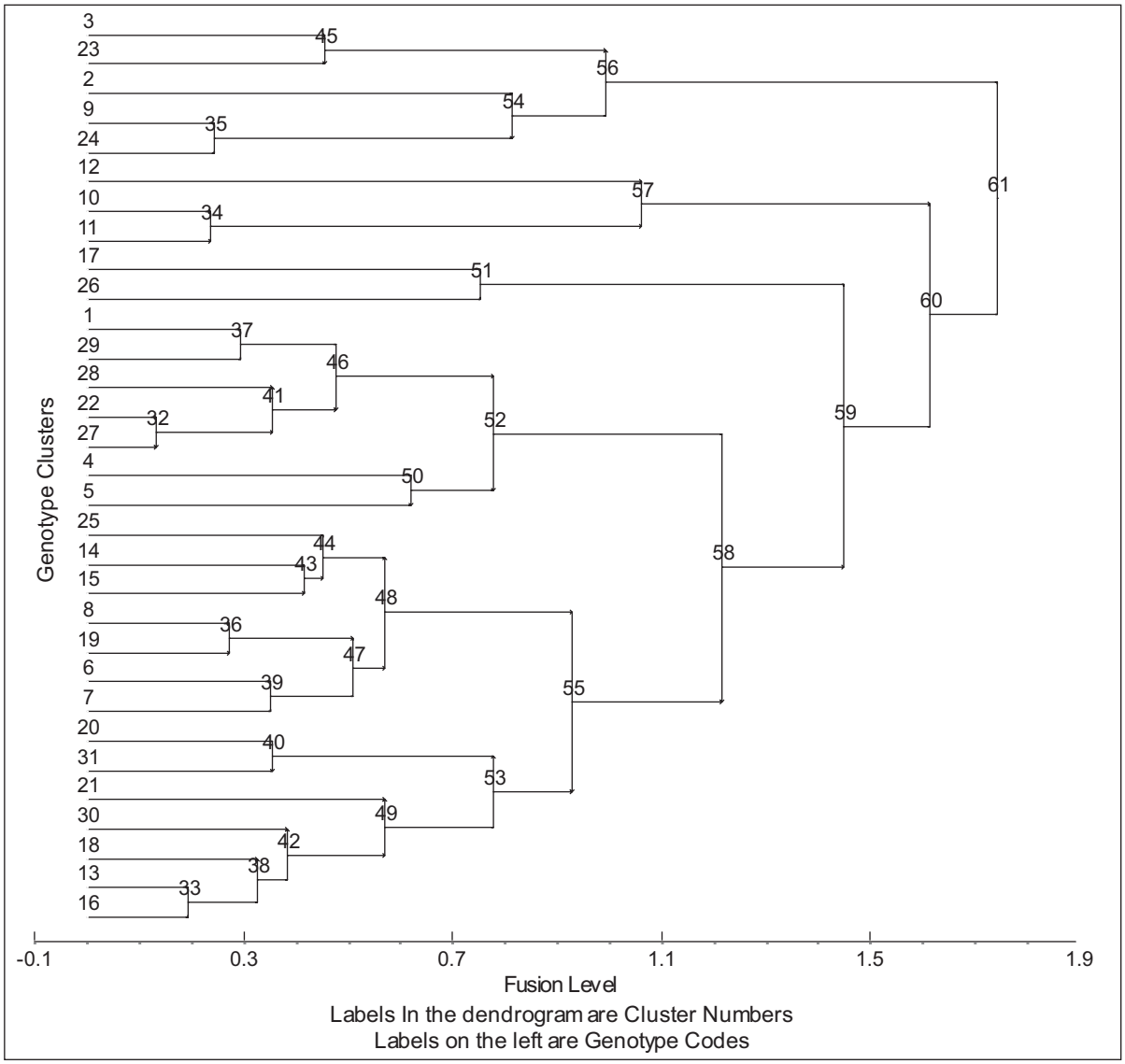

Fig. 2. Dendrogram showing different clustering pattern among 31 Indian mustard varieties on the basis of agro-morphological traits

distinctness. There was no correspondence of grouping between classification based upon mean estimate of traits and clusters based upon multivariate analysis. Though distinctiveness as defined by legislation bears no relationship to genetic distance, however, both approaches established distinctness/variability among Indian mustard varieties. High phenotypic variation in Indian mustard (Singh and Chauhan, 2010; Singh *et al*., 2006; Singh *et al*., 2013 and Singh *et al*., 2014) and canola varieties (Fahmi *et al*., 2012) have earlier been reported. On the basis of above discussion it is reported that seed weight, days to maturity, plant height, siliqua angle with main raceme, petal length, siliqua length, seeds per siliqua and days to flower initiation are more effective than leaf based traits; leaf length, leaf width and number of leaf lobes. Leaf length/leaf width estimates should be preferred over leaf based traits. Distinctness among popular 31 varieties could be established on the basis of 8 morphological traits. Varieties grouped into different clusters (CS 52, Geeta, Laxmi, RLM 619 and Rohini of cluster 1 and RH 819, RH 30, RH 781, Sanjuncta Asech, Varuna and Vasundhara of cluster 5) may be used as parents for hybridization and are likely to through wide spectrum of variability in segregating generations.

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Molecular Marker-based Screening for Bacterial Leaf Blight Resistance Genes in Landraces and Cultivars of Rice in Gujarat

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Bacterial Leaf Blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is the most devastating disease and limiting factor for rice productivity worldwide. Since BLB pathogens are difficult to manage through chemical and other available cultural practices, development of host plant resistance is the most effective means of disease management. So far survey for resistance donor at molecular level in rice is scanty and the landraces of Gujarat state of India have received little attention. Therefore, a molecular screening was conducted to identify the presence of major BLB resistance genes *Xa4*, *xa5* and *Xa21* in landraces and local cultivars of Gujarat. In order to confirm the presence of BLB resistance genes, 25 genotypes were screened using PCR analysis. 10 genotypes (IR20, IR64, IR72, NWGR1095, NWGR2014, IET14726, GR7, GR102, Pankhali 203 and Ratna) were found to be *Xa4* positive. In contrast, only IET18483 genotype was found to be *Xa21* positive. Interestingly, not a single genotype was found to be positive for *xa5* BLB resistance gene. The status of BLB resistance genes in landraces and cultivars of Gujarat will help design BLB-resistance breeding programs.

Key Words: Bacterial Leaf Blight, PCR Screening, Rice Cultivars and Landraces, *Xa* Gene

Introduction

Rice is the oldest domesticated grain (~10,000 years). It is grown on every part of the globe and is the staple food for 2.6 billion people worldwide. The productivity of rice is severely affected by several biotic and abiotic factors. Among biotic factors, bacterial leaf blight (BLB), caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is a devastating disease in the rice-growing countries including India. There are no effective chemical and biological bactericides available for controlling this pathogen. However, for BLB resistance, host plant resistance strategies are the most appropriate to manage the pathogen.

Till date, a total of 42 BLB resistance genes (R genes) have been identified in rice, including *Xa1*, *Xa2*, *Xa3/Xa26*, *Xa4*, *xa5*, *Xa6*, *Xa7*, *xa8*, *xa9*, *Xa10*, *Xa11*, *Xa12*, *xa13*, *Xa14*, *xa15*, *Xa16*, *Xa17*, *Xa18*, *xa19*, *xa20*, *Xa21*, *Xa22(t)*, *Xa23*, *xa24(t)*, *xa25/Xa25(t)*, *Xa25*, *xa26(t)*, *Xa27*, *xa28(t)*, *Xa29(t)*, *Xa30 (t)*, *xa31(t)*, *Xa32(t)*, *Xa33(t)*, *xa34(t)*, *Xa35(t)*, *Xa36(t)*, *Xa37, Xa38, Xa39, Xa40, xa41(t), Xa42.* The recessive resistance genes include *xa5*, *xa8*, *xa9*, *xa13*, *xa15*, *xa19*, *xa20*, *xa24*, *xa25/Xa25(t)*, *xa26(t)*, *xa28(t)*, *xa31(t)*, *xa33(t)*, and *xa34(t)*. Recently, integrated rice science database (available at https://shigen.nig.ac.jp) release the physical map of all the identified BLB resistance genes (Zhang *et al*., 2015). These R genes are known to act in a gene-for-gene interaction manner and are the main sources for genetic improvement of rice for resistance to *Xoo* (McDowell and Woffenden, 2003). Ten of the recessive R genes; *xa5* (Petpisit *et al.,* 1977), *xa8* (Singh *et al*., 2002), *xa13* (Ogawa *et al*., 1987), *xa24* (Kush *et al*., 1999), *xa26, xa28* (Lee *et al*., 2003) and *xa32* (Ruan *et al*., 2008) confer race-specific resistance.

BLB resistance gene *Xa4* is one of the most widely exploited resistance genes in many rice breeding programs and it confers durable resistance in many commercial rice cultivars. However, widespread cultivation of *Xa4* resistance genes containing varieties has led to the development of *Xa4* resistance *Xoo* races in India and most part of south east Asia (Ma *et al*., 1999, Mew *et al*., 1992; Sun *et al*., 2003).Similarly, the *Xa21* gene was identified in the wild species *Oryza longistaminata* and is highly effective against BLB races of South and Southeast Asia (Khush *et al*.,1990). The *xa5* gene, which is naturally found only within the *Aus* subpopulation of rice, provides recessive

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resistance to several *Xoo* races of the Philippines (Garris *et al*., 2003). The information regarding this context is very limited in respect of cultivars and landraces of rice in Gujarat. Therefore, in the current investigation, we performed PCR-based survey for *Xa4*, *xa5* and *Xa21* BLB resistance gene in landraces and cultivars of Gujarat. For the screening of *Xa4* gene, linked marker developed by Ma *et al*. (1999) was used. For *xa5* genes, CAPS markers designed by Iyer *et al*. (2007) were exploited. *Xa21* gene was detected using the STS marker developed by Chunwongse *et al.* (1993).

Materials and Methods

Seeds of the 36 rice genotypes/lines (Table 1) were obtained from Main Rice Research Station, Nawagam, Anand Agricultural University (AAU), Gujarat. The 36 genotypes include six monogenic differentials for BLB resistance genes in the background of IR24, 4 pyramided lines for BLB resistance genes and 5 varieties developed by IRRI. Pyramided lines and IR24 were selected as a positive control and negative control, respectively. Five lines and five advanced breeding lines generated by Main Rice Research Station, Nawagam, along with 8 varieties and 4 landraces released by the station were also incorporated in this study.

Fourteen-day old seedlings grown in petri-plates were used for DNA isolation. Fresh seedlings from ten individuals were bulked together and DNA was extracted

Table 1. List of rice genotypes/lines included in current investigation

S. No.	Genotypes/lines	Descriptions
1	IRBB1	Monogenic differentials in background of IR24 carrying Xa1 gene
2	IRBB4	Monogenic differentials in background of IR24 carrying Xa4 gene
3	IRBB7	Monogenic differentials in background of IR24 carrying Xa7 gene
4	IRBB8	Monogenic differentials in background of IR24 carrying xa8 gene
5	IRBB13	Monogenic differentials in background of IR24 carrying xa13 gene
6	IRBB21	Monogenic differentials in background of IR24 carrying Xa21 gene
7	IRBB57	Pyramided lines carrying Xa4, xa5,xa13 gene (Background IR24)
8	IRBB58	Pyramided lines carrying Xa4, xa13, Xa21 gene (Background IR24)
9	IRBB59	Pyramided lines carrying xa5, xa13, Xa21 gene (Background IR24)
10	IRBB60	Pyramided lines carrying Xa4, xa5,xa13, Xa21 gene
11	IR8	Varieties released by IRRI
12	IR20	
13	IR24	
14	IR64	
15	IR72	
16	NWGR1068	Cross between GR11 X (GR101 X GR3)
17	NWGR1095	Cross between GR11 X IET11763
18	NWGR2014	Cross between Sathi 34-36 X Ratna
19	NWGR2035	Cross between GR11 X Pusa Basmati
20	NWGR99123	Cross between GR4 X IR64
21	IET14726	Advanced breeding lines
22	IET16626	
23	IET17909	
24	IET18483	
25	GR6	Varieties released by Main Rice Research Station, Nawagam
26	GR7	
27	GR9	Upland rice variety released by Nawagam
28	GR11	Varieties released by Main Rice Research Station, Nawagam
29	GR12	
30	GR102	
31	GR104	
32	GAR13	
33	Pankhali203	Aromatic land cultivar
34	Ratna	Released rice variety
35	Sathi34-36	Released upland rice variety of Gujarat
36	SK-20	

using a modified CTAB method as described by Zhang *et al*. (2004). The concentration of extracted genomic DNA was quantified using a spectrophotometer. In order to estimate the quality of isolated DNA, DNA was checked on 0.8% agarose in 0.5x TBE buffer (45mM Tris-borate and 1mM EDTA). For pre-staining, ethidium bromide was added to the gel at a concentration of 10µg/ml before the gel was poured. The samples were run on the gel at 60V until the bromophenol blue dye migrated almost to the end of the gel. Thereafter, electrophoresis gel documentation of stained gels was made using G-BOX from Syngene. Concentrated DNA was diluted to 100ng/ µl using TE (10mM Tris-HCl and 1mM EDTA) buffer and stored at -20ºC till further use.

The genomic DNA was amplified using primers selected on the basis of literature survey. The details of primers, linked genes, sequences and corresponding references are given in Table 2. PCR reactions for SSR/ CAPS were carried out in a reaction volume of 25µl including 2.5 µl of 10 X Taq buffer, 1.0 µl of 50 mM MgCl2 solution, 2.5 µl of 2.5 mM dNTPs mixture, and 0.5 µl of each of the forward and reverse primer at a concentration of 10 pmole/µl, 1.25 µl of 3U/µl Taq DNA polymerase, 3 µl of purified genomic DNA (100 ng/ µl), 1.25µl of glycerol and 13.5 µl of nuclease free water. The PCR components were ordered from Bangalore Genei Pvt. Ldt. The polymerase chain reactions were performed in a thermocycler (Eppendorf) with the following cycle: the initial denaturation at 94°C for 4 min followed by 35 cycles of denaturation at 94°C for 1 min., annealing at 55°C for 45 sec. and extension at 72°C for 2 min. and 15 min. at 72°C for the final extension. In order to determine polymorphism, PCR products were checked on 2.5% agarose in 0.5x TBE buffer (45 mM Tris-borate and 1 mM EDTA). For pre-staining, ethidium bromide was added to the gel at a concentration of 10µg/ml before the gel was poured. The samples were run on the gel at 100V until the bromophenol blue dye migrated almost to the end of the gel. Thereafter, electrophoresis gel documentation of stained gels was made using G-BOX from Syngene.

For Restriction Fragment Length Polymorphism (RFLP) analysis of PCR product, amplified product was digested with different restriction enzymes (Table 3). The PCR reaction were digested by following manufacturer instruction followed by gel electrophoresis on 2% agarose gel contain 10 µg/ml ethidium bromide. Gel documentation was performed as described earlier. The amplified fragments of all the rice genotypes/ lines were observed and compared with positive and

Table 2. List of gene-specific primers used for screening of BLB resistance genes

Gene	Primer Name	Sequence (5'-3')	References
Xa4	RM224	F-TCTCCCTCCTCCTCCTCCTACG	Ma *et al.* (1999)
		R-GATTCAGCACAGCGATTGTTGC	
	MP12	F-ATCGATCGATCTTCACGAGG	
		R-TGGTATAAAAGGCATTCGGG	
xa5	xa5_1F	F-CTCTACCGGAGGTCCACCATT	Iyer *et al.* (2007)
	xa5_2F	F-ACGCTCGACGAGATGGTCTC	
	xa5_4F	F-CTGGAAGAAGCTCTTAATTT	
	xa5_5F	F-CGGATAGCAGCATTTCCAAGAG	
	xa5_6F	F-GATAGCAGCATTTCCAAGAG	
	xa5_1R	R-AGGAACAGCAACATTGCAAC	
	xa5_4R	R-GATTCCTTTAGCAAGGTGTG	
Xa21	PTA248	F-AGACGCGGAAGGGTGGTTCCCGGA	Chunwongse *et al.* (1993)
		R-AGACGCGGTAATCGAAAGATGAAA	

Table 3. Restriction enzymes used in CAPS assay

S. No.	Restriction Enzyme	Reaction composition	Incubation
1	*Bsr*I	5–10 µl PCR product, 2 µl 10X buffer recommended by the manufacturer, and 5 U of enzyme.	65^{o}C
2	*Sml*I	5–10 µl PCR product, 2 µl 10X buffer recommended by the manufacturer, and 5 U of enzyme and 0.2 µl of 100X BSA	55^{o}C
3	*Xho*I and *Hpa*II	10 µl PCR product, 18 µl nuclease free water, 2 µl 10X buffer recommended by the manufacture, 2 µl of restriction enzyme	37^{o}C

negative controls, and for the presence and absence of BLB resistance gene plus and minus sign were assigned respectively.

Results and Discussion

DNA analysis of *Xa4* resistance genes in all the selected rice germplasm exhibited the presence of two different sizes of bands with marker MP12 and three with RM224. With marker MP12 and RM224, the banding pattern of all the genotypes was either similar to the IRBB4, IRBB57, IRBB58, IRBB60 and IR64 (positive control) and IR24, and IR8 (negative control). With marker MP12, the size of the band was 165bp which corresponds to the IRBB4 whereas, the band which corresponds to the IR24 was 144bp in size while with marker RM224 the size of the band was 163 bp corresponding to the IRBB4 and with IR24 was 143 bp. During the gene survey using MP12 marker, out of 36 rice genotypes, 14 genotypes (IRBB4, IRBB57, IRBB58, IRBB60, IR20, IR64, IR72, NWGR1095, NWGR2014, IET14726, GR7, GR102, Pankhali 203 and Ratna) along with a positive control amplified a 165 bp size fragments which indicated the presence of *Xa4* gene. While the remaining 22 genotypes amplified 144bp DNA fragments which showed the absence of *Xa4* gene (Fig. 1a). Ma *et al.* (1999) identified and synthesized MP12 primers based on the sequence of a DNA marker tightly linked to the rice BLB resistance gene *Xa4* for the survey of hybrid rice germplasm. Wang *et al*. (2000) used the same set of primers for the fine mapping of the *Xa4* gene. They analyzed the F2 population of a cross between IR24 and IRBB4 using the same primers and found that *Xa4* is tightly linked to this primer. Arif *et al.* (2008) reported the polymorphism between positive and negative controls with 150bp DNA fragments corresponds to the positive control (IRBB4 and IR64) and 120bp fragments with negative control (IR24) but in present studies the presence of 165bp fragment corresponds to the positive control and 144bp fragments to the negative control were observed. With marker RM224, 14 genotypes amplified

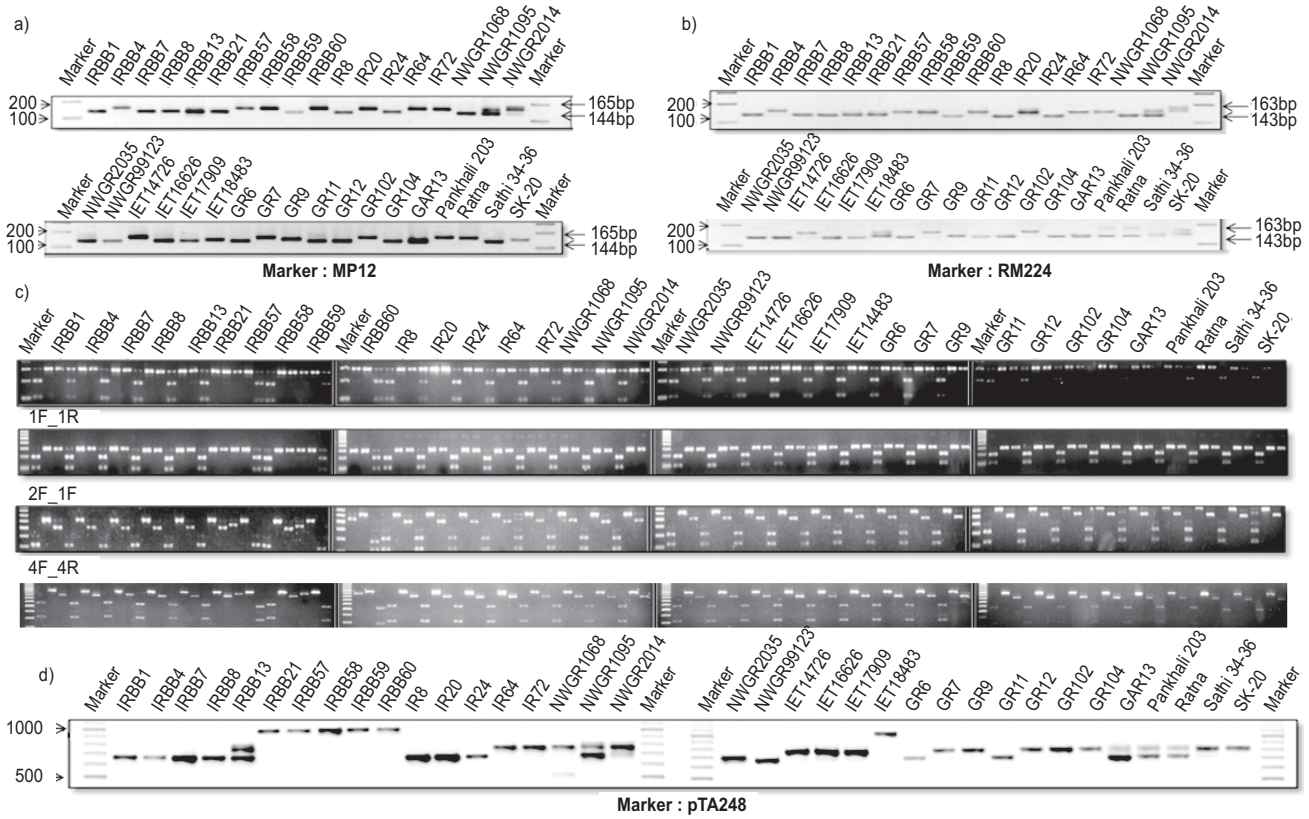

Fig. 1. PCR based genotyping of rice genotypes for BLB resistance gene *Xa4* and *xa5* a) Genotyping of genotypes using *Xa4* linked marker MP12 b) Genotyping of rice genotypes for *Xa4* BLB resistance genes using SSR marker RM224 c) Analysis of rice genotypes for the presence of *xa5* BLB resistance gene using CAPS marker. Each genotype was represented by 3 well, first well contains PCR product digested by *Bsr*I enzyme, second well contain PCR product and third well contain PCR product digested by *Sml*I enzyme d) STS maker pTA248 based genotyping of rice genotypes to screen the presence of *Xa21* BLB resistance gene

Table 4. Primers used for the amplification of CAPS marker

Primers	Distance to FNP	Primer Pair	Product size	Approximate digested product size
xa5_1F	100	1F_1R	299	100, 199
xa5_1R	199			
xa5_2F	61	2F_1R	260	61, 199
xa5_1R	199			
xa5_4F	225	4F_4R	625	225, 400
xa5_4R	400			
xa5_5F	551	5F_4R	951	551,400
xa5_4R	400			
xa5_6F	549	6F_4R	949	549,400
xa5_4R	400			

163bp DNA fragments which indicated the presence of *Xa4* resistance gene whereas 22 genotypes amplified 143bp DNA fragments which showed the absence of *Xa4* resistance gene. Out of 14 resistant genotypes three genotypes namely NWGR2014, Pankhali 203 and Ratna showed the presence of an additional allele of 190bp which was absent in both the positive and negative controls (IRBB4 and IR24 respectively). Sun *et al* (2003) used RM224 to map *Xa4* gene and found polymorphism between the parents of the F2 population. In our study this marker also showed polymorphism and this polymorphism was in synchronization with the polymorphism detected by MP12 except for genotype IET18483, which showed susceptible band with MP12 and heterozygous condition at RM224 loci (Fig. 1b).

The recent identification of the *xa5* gene demonstrated that it encodes the small subunit of transcription factor TFIIAγ. This gene differs in resistant and susceptible cultivars by two nucleotides. *Bsr*I and *Sml*I digest the susceptible and resistant alleles, respectively (Iyer *et al*, 2007). Iyer *et al.* developed seven primers, which were combined into five different sets of primer pairs and was used in the current investigation. Primers xa5_6F and xa5_5F are 96 and 98 base pairs upstream of the first nucleotide of the full-length xa5 cDNA respectively, while xa5_1F, 2F, and 4F are within exon2. xa5_1R and 4R are located in the third intron. Overall, 36 genotypes are screened for *xa5* resistance gene with CAPS markers shown in Table 4. Out of 36 genotypes only 3 genotypes i.e. IRBB57, IRBB59, and IRBB60 (positive control) showed the presence of *xa5* specific bands and rest of the genotypes showed the absence of *xa5* specific bands and banding patterns are identical to the IR24 (negative control) (Fig. 1c). The study conducted by Iyer *et al* (2007) showed a perfect correlation between the CAPS markers genotype and observed phenotype, therefore, the result generated by these markers are reliable to genotyped germplasm for BLB resistance gene *xa5*.

The presence of *Xa21* gene in germplasm was detected by the STS marker pTA248, developed by Chunwongse *et al* (1993). DNA analysis of the genotypes with pTA248 marker exhibited the presence of four alleles of 950bp, 780bp, 650bp and 500bp. Out of these four alleles, 950bp DNA fragments were present in positive controls (IRBB21, IRBB57, IRBB58, IRBB59, and IRBB60) showed resistant nature of the genotype and 650bp DNA fragment is present in negative control (IR24) while the presence of other variants also showed susceptibility of the germplasm. Out of 36 genotypes, six genotypes IRBB21, IRBB57, IRBB58, IRBB59, IRBB60, and IET18483 showed the amplicon of 950bp corresponding to the resistant allele and hence considered as resistant genotypes (further validated at Navagam rice research station, AAU, Nawagam, Data not shown). Eleven genotypes IRBB1, IRBB2, IRBB4, IRBB8, IR8, IR20, IR24, NWGR2035, NWGR99123, GR6, and GR11 showed the presence of 650bp amplicon. Twelve genotypes, IR64, IR72, NWGR2014, IET18483, IET14726, IET16626, IET17909, GR7, GR9, GR12, GR102, GR104, Sathi 34-36 and SK-20 have amplicon of 780bp. Six genotypes showed heterozygosity and out of these five genotypes IRBB13, NWGR1095, GAR13, Pankhali 203 and Ratna were found heterozygous for the allele with two fragments of 780bp and 650bp, and one genotype NWGR1068 was found heterozygous for the allele with two fragments of 780bp and 500bp each (Fig. 1d). Huang *et al.* (1997) reported 3 alleles for pTA248 but in our study, an additional allele of 500bp for pTA248 was found in NWGR1068.The presence of the *Xa21* gene was detected by the STS marker pTA248 located within 1cM of *Xa21* and was originally obtained by sequencing the genomic clone of the RAPD248

fragment using the same primer (Chunwongse *et al*., 1993). Ronald *et al.* (1992) genetically mapped it at 0.1cM distance from *Xa21* gene. pTA248 detected a band of approximately 1Kb in all resistant lines. In current investigation the same polymorphism was also detected between resistant and susceptible lines.

Conclusions

In conclusion, the *xa5* and *Xa21* gene is absent in landraces and cultivars of Gujarat. The use of rice accessions includes IRBB57, IRBB59, and IRBB60 as a donor parents in hybridization program with modern cultivar will accelerate efforts to develop BLB-resistant cultivars through MAS-based pyramiding approaches without compromising yield and grain quality.

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Characterization of Fig (*Ficus carica* L.) Germplasm in Central Kashmir of North Western Himalayan Region

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The present study was focused on collecting, evaluating and characterization of fig germplasm, aiming for the establishment of long term *in-situ* and *ex-situ* conservation. Morphological and chemical characters were evaluated for 13 individual fig accessions grown in two districts of Central Kashmir. The results revealed that fig accessions have a vast diversity in terms of morphological (metric and non-metric) and chemical characters. Intermediate and high plant vigour, open to erect growth habit and dense and intermediate relative degree of branching was observed in the studied accessions. Maximum yield (kg/plant) along with maximum yield efficiency (kg/cm^2) was recorded in ML-G-17 accession closely followed by JN-S-09 (32.82 kg/plant) for yield. Fruit weight varied from 24.40 (B-G-07) to 74.25g (DH-S-06) while width/length ratio ranged between 0.887 (CH-S-03) and 1.150 (YL-G-01). Total soluble solids varied from 9.20 to 20.20 per cent and total sugars from 6.70 to 16.60 per cent in B-G-14 and AH-S-06, respectively. Oblate, oblong and globose fruit shape was recorded. External fruit colour varied from yellowish green to dark purple while internal colour of pulp ranges between light pink and dark red. On the basis of the studied parameters ML-G-17, AH-S-06 and DH-S-04 accessions are found to be promising. These valuable and superior fig accessions have potential for raising the new plants through propagation techniques, deserve further characterization and conserve under *in-situ* conservation for future breeding programmes.

Key Words: Conservation, Fig, Morphological characters, Selection, Survey

Introduction

Ficus carica L., commonly known as Fig is one of the earliest cultivated deciduous fruit tree belonging to the family Moraceae (Watson and Dallwitz, 2004), which is a fruit of warm sub tropical to temperate zone (Westwood, 1993; Murli and Balachandran, 1997). Although, origin of fig is not entirely known, *F. carica* is thought to have originated in Western Asia (Stover *et al*., 2007) to northern India and its local varieties are cultivated in most Mediterranean countries (Zohary and Hopf, 2000). Fig fruit is well known for its attractive taste, nutritive value due to its antioxidant properties and is consumed in fresh or dried form (Solomon *et al*., 2006). It is cultivated for its sweet and fresh fruits and are rich source of amino acids, proteins, carbohydrates, minerals (Wang *et al*., 2003) and vitamins, fibre, potassium, calcium and iron (Chessa, 1997) which are present in higher levels than in other common fruits and are also free of sodium, fat and cholesterol level. Its fruit has also importance in the food industry since it is processed into several products such as preserved fruits, jam, juice, wine and powder. Other parts of the fig tree (leaves, bark, root and latex) are also used traditionally for their medicinal properties.

Total fig production in the world is about 10,31,391 MT and Turkey alone produces about 2,98,914 MT and is also the biggest exporter of fig in the world (FAO, 2014). Fig trees are mostly located on the marginal lands, in mixture with other fruit trees or scattered at the periphery of orchards and in home gardens. In India, the total fig production is estimated to be around 19,000 MT (FAO, 2014) and is largely grown in Pune, Bangalore, Bellary and some isolated patches in Tamil Nadu, Andhra Pradesh. In North India, its cultivation is scattered over some parts of Uttar Pradesh, Uttarakhand, Punjab and Himachal Pradesh (Sharma and Badiyala, 2006). In India, it is considered as an underutilized fruit crop and is a great potential fruit crop to be grown on the wastelands of Indian subcontinent.

Fig tree is an important ethno botanical plant widely spread on kitchen gardens in some pockets of Kashmir valley. Due to perishable nature of fig, they are naturally

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dried under sunlight in Kashmir, which are sweet, soft, chewy with crunchy edible seeds which makes it more tasty. Kashmir figs are high in fiber, providing 20 per cent of the daily value more dietary fiber per serving than any other common dried or fresh fruit. Kashmiri dried figs are good source of calcium, which maintains the bone density and prevents osteoporosis. Moreover they are rich in potassium which regulates the blood pressure and maintain the high blood pressure. Dried Kashmiri figs contain omega 3 and omega 6 fatty acids which prevent heart diseases and are known to heal sore throats due to its high mucilage content. Customers from various regions of India and outside country are placing bulk orders for the dried figs due to their delectable taste and freshness.

Due to an increasing demand of fresh fig production in the country, a number of superior varieties have been cultivated accompanied by advanced handling, attractive packaging and efficient transportation facilities. Therefore, the present study was undertaken as an initiative measure in fig crop which could significantly contribute to the diversification of horticulture in the valley. The inventory study was conducted with the aim to identify promising fig accessions in two districts of Kashmir valley in terms of pomological properties.

Materials and Methods

Experimental Material and Area

Survey and selection studies carried out during 2013 and 2014 in Srinagar and Ganderbal areas of Kashmir valley located in the North Western side of Indian Himalayas aimed to find out fig genotypes which had high productivity capacity and better fruit characteristics. The area under survey had latitude of 34^{o}5'N, longitude of 74^{o}49'N with annual rainfall of 710 mm. The maximum and minimum temperature of the area surveyed was 34^{o}C and -6^{o}C, respectively. Out of a total population of more than two hundred plants, thirteen promising selections (Table 1) were selected and evaluated for various pomological parameters. A total of 41 quantitative and qualitative traits (26 pomology and 15 leaf morphology) were determined according to the fig descriptors prepared by IPGRI and CIHEAM (2003); Aljane and Ferchichi (2009) with some minor modifications that showed high discrimination value depending on our study.

Observations Recorded

Observations were recorded on vegetative, foliage, fruit physical and chemical characters.

Table 1. Full name of the accessions

S.No.	Code	Accession name
1	ML-G-02	Manigam Lar Ganderbal
2	B-G-07	Babawayil Gandebal
3	YL-G-01	Youngura Ganderbal
4	B-G-04	Babawayil Gandebal
5	B-G-14	Babawayil Gandebal
6	JN-S-09	Jawahar Nagar Srinagar
7	ML-G-17	Manigam Lar Ganderbal
8	DH-S-08	Dara Harwan Srinagar
9	DH-S-02	Dara Harwan Srinagar
10	AS-S-06	Arabal Shalimar Srinagar
11	CH-S-03	Chandpura Srinagar
12	DH-S-04	Dara Harwan Srinagar
13	MB-S-05	Meebagh Brain Srinagar

Vegetative and Foliage Characters

Data was recorded on the parameters viz. plant height (m), plant spread (N-S and E-W m), trunk girth (cm), yield (kg/plant). Yield efficiency and trunk cross sectional area was calculated as per Westwood (1993).

$$\text{Yield efficiency (kg/cm}^2\text{)} = \frac{\text{Yield}}{\text{Trunk cross sectional area}}$$

Trunk cross sectional area (TCSA) = r^2 where 'r' is the radius of the tree. Under non-metric traits vigour of the tree (low, intermediate, high), growth habit of the tree (erect, semi-erect, open, spreading, weeping), relative degree of branching (sparse, intermediate, dense) and tendency to form suckers (low= < 3; medium= 3-7; high= > 7) were taken. Maturity of the fruit was recorded when 50 per cent of the fruits were mature and classified them into Breba i.e. first crop bears in the spring on wood from the previous year and Main crop i.e. second crop is borne in the current seasons growth (Stover *et al*., 2007).

For foliage or leaf characters ten leaves of a predominant shape were taken. Number of lobes per leaf, number of leaf per shoot were counted and averaged. Leaf length was measured with the help of scale from the base of the petiole to the tip of the central lobe while leaf width was measured from the left to right of the leaf and both was expressed in centimeter. Leaf area was worked out by length x width (cm^2). Petiole length (mm) was measured with the help of vernier caliper from the base of the stalk attached with the shoot or branch to the tip of the stalk.

Fruit (Physical and Chemical) Characters

For all the fruit physical characters fifteen fresh fruits

Table 2. Vegetative, yield and leaf characters of selected accessions of fig from Central Kashmir

Accession number	Plant height (m)	Plant spread (m)		Trunk girth (cm)	TCSA (cm^2)	Yield (kg/ plant)	Yield efficiency (kg/cm^2)	Number of lobes/ leaf	Petiole length (mm)	Leaf length (cm)	Leaf width (cm)	Leaf area (cm^2)	Number of leaf/ shoot
		N-S	E-W										
ML-G-02	4.74	3.71	3.80	51.0	207.08	25.64	0.124	5.0	4.55	29.45	25.42	748.62	6.0
B-G-07	3.92	3.65	4.21	41.5	137.12	30.32	0.221	4.0	4.04	30.05	26.10	784.31	5.8
YL-G-01	4.91	3.45	3.56	47.0	175.87	28.16	0.160	5.0	6.37	22.57	19.60	374.66	6.3
B-G-04	5.13	3.34	2.98	55.0	240.84	22.40	0.093	5.0	6.50	21.97	19.33	424.68	6.9
B-G-14	4.29	3.97	4.36	46.0	168.47	26.80	0.159	4.0	5.21	21.06	19.23	404.98	5.4
JN-S-09	3.98	3.71	4.49	40.7	131.89	32.82	0.248	6.0	2.33	16.71	16.10	269.03	6.6
ML-G-17	3.92	4.04	4.10	36.8	107.82	34.12	0.316	6.0	3.06	20.08	18.07	362.84	5.7
DH-S-08	4.67	4.37	3.64	40.5	130.59	26.00	0.199	4.0	5.66	20.34	17.53	356.56	6.3
DH-S-02	3.78	4.02	3.95	44.6	158.37	25.50	0.161	4.0	6.30	21.21	19.52	414.02	5.1
AH-S-06	5.21	3.15	2.71	38.1	115.57	30.60	0.265	5.0	5.45	21.36	19.04	406.69	4.9
CH-S-03	4.10	3.84	3.94	45.4	164.10	27.50	0.167	5.0	6.12	20.63	18.86	389.08	5.0
DH-S-04	4.50	3.35	3.66	44.3	156.24	26.00	0.166	4.0	4.94	23.23	20.03	465.30	7.2
MH-S-05	5.00	4.19	4.00	51.2	208.71	25.20	0.121	5.0	5.36	18.20	17.26	314.13	5.6
Mean	4.42	3.71	3.76	44.78	161.74	27.77	0.18	4.77	5.07	22.07	19.70	439.61	5.91
SD	0.52	0.36	0.51	5.37	39.07	3.33	0.06	0.75	1.29	3.82	2.91	153.44	0.73
CoV	11.76	9.70	13.56	11.99	24.16	11.99	33.33	17.40	25.44	17.30	14.77	34.90	12.35

were taken and fruit weight (g) was taken with digital weighing balance, fruit length (mm) and fruit width (mm) was measured with the help of vernier caliper and average was worked out. Neck length (mm) and skin thickness (mm) of the fruit was also measured with the help of vernier caliper. Under non-metric traits external fruit colour, internal pulp colour and fruit shape was taken as per the guidelines of the fig descriptors prepared by IPGRI and CIHEAM (2003). Chemical characters of fruits viz. TSS (%), acidity as per cent citric acid and total sugars (%) were calculated as per the methods given in AOAC (1995). Ascorbic acid (mg/100g) was determined by titration with 2,6 dichloro phenol endophenol blue dye as per the methods given in AOAC (1995).

Statistical Analysis

The average values of measured and calculated parameters were used for analysis and were statistically analyzed as per OPSTAT method.

Results and Discussion

Vegetative Characters

Data on different vegetative characters were presented in the Table 2 which showed wide variability among the studied accessions. Plant height varied from 3.78 m (DH-S-02) to 5.21 m (AH-S-06) closely followed by 5.13 m (B-G-04) and 5.00 m (MH-S-05) with a coefficient of variation of 11.76 per cent. Maximum plant spread in the north-south directions was recorded in DH-S-08 (4.37 m) accession closely followed by MH-S-05 (4.19 m) and ML-G-17 (4.04 m) accessions however maximum plant spread in east-west directions was recorded in JN-S-09 (4.49 m) closely followed by B-G-14 (4.36 m) and B-G-07 (4.21 m). Minimum plant spread in north-south direction (3.15 m) and east-west direction (2.71 m) was recorded in AH-S-06 accession. Accession number B-G-04 recorded maximum trunk girth (55.0 cm) and TCSA (240.84 cm^2) closely followed by MH-S-05 and ML-G-02. Coefficient of variation for trunk girth and TCSA was 11.99 per cent and 24.16 per cent. Maximum yield (34.12 kg per plant) along with maximum yield efficiency (0.316 kg/cm^2) was recorded in ML-G-17 closely followed by JN-S-09 (32.82 kg/plant and 0.248 kg/cm^2, respectively) (Table 2 and Fig 1). Minimum values for both the characters were recorded in accession B-G-14 (22.40 kg per plant and 0.093 kg/cm^2). Similar values for marketable yield were also reported by (Irget *et al*., 2008).

Out of thirteen accessions, eight accessions recorded Intermediate plant vigour while only five accessions have high plant vigour, none of the accessions showed low vigour of plant (Table 3). Among 38 accessions, 19 accessions have intermediate plant vigour, 11 accessions

Table 3. Vegetative and fruit (non-metric) characters of selected accessions of fig from Central Kashmir

Accession number	Vigour	Growth habit	Relative degree of branching	Tendency to form suckers	External (fruit) colour	Internal (pulp) colour	Fruit Maturity		Fruit shape
							Breba Crop	Main Crop	
ML-G-02	Intermediate	Open	Dense	High (>7)	LP	Pink Red	Very late	Late	Oblate
B-G-07	Intermediate	Spreading	Intermediate	High (>7)	LPGB	Pink Red	Very late	Late	Oblate
YL-G-01	High	Semi-erect	Dense	High (>7)	DP	Dark Red	Very late	Mid Season	Oblate
B-G-04	High	Erect	Intermediate	Low (<3)	YG	Pink Red	Late	Early	Globose
B-G-14	High	Spreading	Dense	Medium (3-7)	GYP	Pink Red	Very late	Early	Globose
JN-S-09	High	Spreading	Dense	Medium (3-7)	LP	Pink Red	Very late	Early	Globose
ML-G-17	High	Spreading	Dense	Medium (3-7)	DP	Dark Red	Late	Early	Oblong
DH-S-08	Intermediate	Spreading	Dense	Medium (3-7)	LP	Light Pink	Late	Early	Globose
DH-S-02	Intermediate	Spreading	Dense	High (>7)	PSDG	White Yellow	Late	Early	Oblong
AH-S-06	Intermediate	Erect	Intermediate	Medium (3-7)	LP	Light Pink	Late	Early	Oblong
CH-S-03	Intermediate	Spreading	Intermediate	Medium (3-7)	LP	Pink Red	Late	Early	Oblong
DH-S-04	Intermediate	Semi-erect	Intermediate	Medium (3-7)	PDG	White Yellow	Late	Early	Globose
MH-S-05	Intermediate	Spreading	Intermediate	Medium (3-7)	DP	White Yellow	Late	Early	Globose

Berba crop (Late: 16-30 June, Very Late: > 1 July), Main crop: (Early: 1-10 August, Mid season: 11-31 August, Late: 1-30 Sep),
LP: Light Purple LPGB: Light purple with Green Base DP: Dark purple YG: Yellowish Green
GYP: Greenish Yellow with light purple tinge, PSDG: Purple streaks with dark green base, PDG: Purple with Dark Green Base

have high and 8 accessions have low plant vigour (Podgornik *et al*., 2010). With respect to growth habit among thirteen accessions ML-G-02 showed open, YL-G-01 and DH-S-02 showed semi-erect, BGG-04 and AHS-06 showed erect growth habit while rest of eight observed spreading growth habit. Dense and intermediate type of branching was recorded among the studied accessions. Earlier Podgornik *et al*., 2010 also reported open (16), semi-erect (5), erect (4) and spreading (13) growth habit in 38 accessions while abundant (9), frequent (13) and rare (16) type of branching was noticed. Low i.e. less than three, tendency to form suckers was recorded in BGG-04 accession while ML-G-02, B-G-07, YL-G-01 and DH-S-02 recorded high (> 7) tendency of suckers formation, rest accessions recorded medium (3-7) type of tendency to form suckers. Similar results have also been reported for growth and vegetative characters (Caliskan and Polat, 2012).

Foliage Characters

Number of lobes in leaf ranged between 4.0 (B-G-07, B-G-14, DH-S-08, DH-S-02 and DH-S-03) and 6.0 (JN-S-09 and ML-G-17) while number of leaves per shoot varied from 4.9 (AH-S-06) to 7.2 (DH-S-03) (Table 2). Coefficient of variation for the number of lobes per leaf and number of leaves per shoot was 17.4 per cent and 12.35 per cent, respectively. Petiole length among the thirteen accessions varied from 2.33 (JN-S-09) to 6.50 (B-G-04). Maximum leaf length (30.05 cm), leaf width (26.10 cm) and leaf area (784.31 cm^2) was recorded in accession number B-G-07 closely followed by ML-G-02 (29.45 cm, 25.42 cm and 748.62 cm^2). JN-S-09 recorded minimum values for leaf length (16.71 cm), leaf width (16.10 cm) and leaf area (269.03 cm^2). Coefficient of variation for leaf length, leaf width and leaf area was 17.30 per cent, 14.77 per cent and 34.90 per cent, respectively. Low values as compared to present study was recorded by Podgornik *et al*., 2010 for number of lobes in leaf (3 to 7), leaf length (16 to 23 cm), leaf width (13 to 23 cm) and leaf area (136 to 300 cm^2). However, a marked variation in all the foliage parameters was also reported (Abo-El-Ez *et al*., 2013 and Salimia *et al*., 2013)

Physical Characters

Various physical characters of the fruit were depicted in the Table 4 which shows clear variation for all the studied characters. Fruit weight varied from 24.40 g (B-G-07) to 74.25 g (DH-S-04) followed by 67.11 g (AH-S-06), 63.80 g (MH-S-05) and 62.27 g (CH-S-03) (Table 3 and Fig 1). Accession B-G-07 recorded minimum values for fruit length (35.68 mm) as well as fruit width (40.20 mm) however accession AH-S-06 scored maximum fruit length (60.45 mm) followed by CH-S-03 (56.58 mm), DH-S-04 (53.49 mm) and B-G-14 (53.20 mm). Maximum fruit width was registered in accession DH-S-04 (56.81 mm) followed by MH-S-05 (55.66 mm) and AH-S-06 (54.00 mm). Coefficient of variation for fruit weight, fruit length and fruit width was 38.40 %, 14.39 % and 9.89 %, respectively. Width/length ratio ranged between 0.882 (ML-G-17) to 1.150 (YL-G-01). While working on five cultivars and twenty four selections of

Table 4. Fruit physical and chemical characteristic of selected accessions of fig from Central Kashmir

Accession number	Fruit weight (g)	Fruit length (mm)	Fruit width (mm)	W/L ratio*	Neck length (mm)	Skin thickness (mm)	TSS (%)	Acidity (%)	Total sugars (%)	Vitamin C (mg/100g)
ML-G-02	28.40	40.50	45.20	1.116	6.05	0.95	12.70	0.31	9.00	0.17
B-G-07	24.40	35.68	40.20	1.126	6.40	1.27	15.40	0.22	11.70	0.16
YL-G-01	27.00	40.00	46.00	1.150	3.20	0.88	16.00	0.26	12.80	0.24
B-G-04	43.60	48.41	46.35	0.957	6.12	0.66	13.50	0.28	9.20	0.23
B-G-14	47.25	53.20	51.64	0.970	5.96	0.79	9.20	0.44	6.70	0.21
JN-S-09	37.00	50.00	48.65	0.973	2.08	0.87	11.20	0.40	8.50	0.15
ML-G-17	32.60	50.58	44.66	0.882	8.94	1.03	13.50	0.43	10.60	0.18
DH-S-08	29.27	44.75	46.22	1.032	4.91	1.15	17.60	0.17	14.50	0.22
DH-S-02	41.40	51.98	46.56	0.895	5.12	0.70	13.90	0.16	9.70	0.20
AH-S-06	67.11	60.45	54.00	0.893	3.44	0.85	20.20	0.09	16.60	0.24
CH-S-03	62.27	56.58	50.24	0.887	4.01	0.81	15.50	0.10	12.20	0.20
DH-S-04	74.25	53.49	56.81	1.062	3.87	0.87	19.60	0.07	16.40	0.18
MH-S-05	63.80	52.18	55.66	1.066	3.71	0.79	19.70	0.38	15.40	0.35
Mean	44.49	49.06	48.63	1.00	4.91	0.89	14.88	0.25	11.79	0.21
SD	17.08	7.06	4.81	0.10	1.80	0.17	3.42	0.13	3.20	0.05
CoV	38.40	14.39	9.89	10.00	36.66	19.10	23.00	51.73	27.14	24.44

*W/L ratio = width/length ratio (IPGRI and CIHEAM, 2003)

five year old Caliskan and Polat (2008) reported fruit weight, fruit diameter, fruit length, fruit width/length ratio ranged from 22 g to 52 g, 31.9 mm to 44.2 mm, 30.2 mm to 45.8 mm and 0.84 to 1.30, respectively. Gozlekci (2011), Aljane *et al*. (2012) and Salimia *et al*. (2013) noticed a great variability for fruit weight, fruit length, fruit diameter in different fig accessions.

Among thirteen accessions only three accessions registered oblate (ML-G-02, B-G-07 and YL-G-01) shape, four accessions have oblong (ML-G-17, DH-S-02, AH-S-06 and CH-S-03) shape and fruits are of six accessions are of globose (B-G-04, B-G-14, JN-S-09, DH-S-08, DH-S-04 and MH-S-05) shape (Table 3). Neck length of fruit was varied from 2.08 mm (JN-S-09) to 8.94 mm (ML-G-17) followed by B-G-07 (6.40 mm) and B-G-04 (6.12 mm) while skin thickness of the fruit ranged between 0.66 mm (B-G-04) to 1.27 mm (B-G-07) with a coefficient of variation of 36.66 per cent and 19.10 per cent, respectively (Table 4). Caliskan and Polat (2008) reported among twenty-nine cultivar/selections majority of cultivars have globose shape of fruit and neck length varied from 1.0 mm to 8.9 mm. Polat and Ozkaya (2005), Aljane *et al.* (2012) and Caliskan and Polat (2012) also reported vast variability with respect to fruit shape, neck length and skin thickness.

A great variation was recorded among accessions with respect to non metric traits like external and internal fruit colour, fruit maturity. The external fruit colour among the various accessions ranged from light purple to the dark purple colour (Table 3). The most frequent colour of the skin was light purple in the five accessions (ML-G-02, JN-S-09, DH-S-08, AH-S-06 and CH-S-03) while three accessions (YL-G-01, ML-G-17, MH-S-05) had dark purple colour. Ercisli (2004) and Aljane *et al*. (2012) also reported great variability for skin colour in fig genotypes and cultivars. Internal pulp colour ranges from white yellow (DH-S-02, DH-S-04 and MH-S-05), light pink (DH-S-08 and AH-S-06), pink red (ML-G-02, B-G-07, B-G-04, B-G-14, JN-S-09 and CH-S-03) to dark red (YL-G-01 and ML-G-17). Aljane *et al*. (2012) also reported a great variability for external and internal colour of the fruits while evaluating seventeen fig accessions in Tunisia.

Breba crop of accessions (B-G-04, ML-G-017, DH-S-08, DH-S-02, AH-S-06, CH-S-03, DH-S-04 and MB-S-05) matures late i.e. from 16 to 30 June while accessions (ML-G-02, B-G-07, YL-G-01, B-G-14 and JN-S-09) matures very late i.e. > 1 July (Table 3). Main crop of the accession studied matures late as compared to Breba crop because breba crop develops in the spring on the previous year's shoot growth while main crop develops on the current year's shoot growth and ripens in late summer or fall. With respect to main crop mid season (11-31 August) maturity was noticed in accessions YL-G-01 while accessions ML-G-02 and B-G-07 matures late i.e. from 1st September to 30th September. Rest all the accessions registered early maturity i.e. 1st to 10th August. With five cultivars and twenty four selections

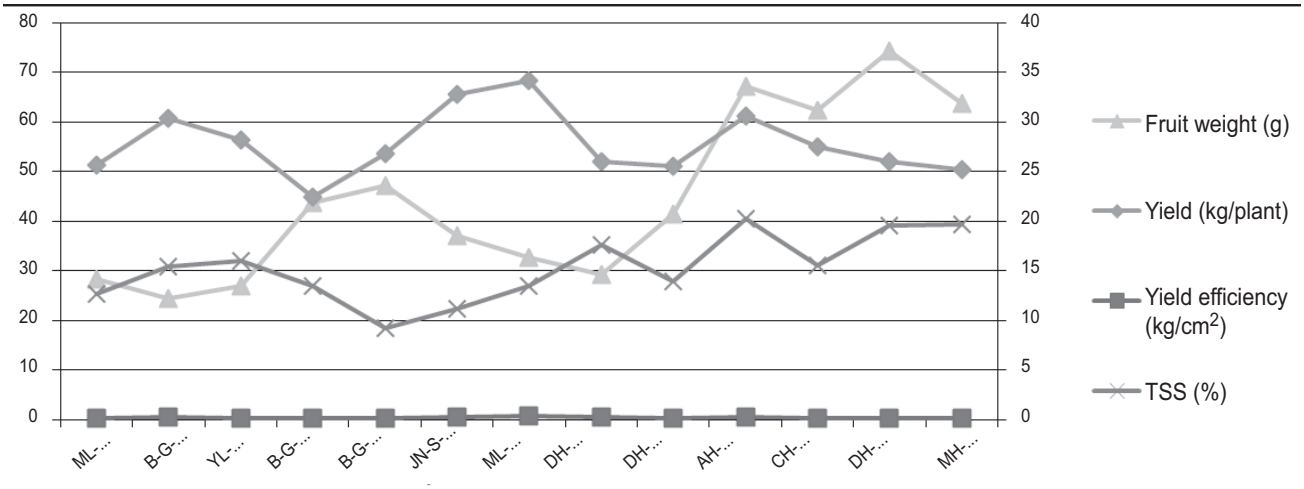

Fig 1. Fruit weight, yield, yield efficiency and TSS of selected accessions of fig from Central Kashmir

having five years of age under Turkey conditions Caliskan and Polat (2008) reported that majority of cultivars and selections matures between 1st to 15th August. Polat and Ozkaya (2005) and Salimia *et al*. (2013) also reported similar timings for the maturity period in twelve fig accessions in Palestine.

Chemical Characters

A great variation was noticed among all the accessions for the studied chemical characters (Table 4). B-G-14 (9.00 %) scored minimum total soluble solids however maximum TSS was recorded in AH-S-06 (20.20 %) followed by MH-S-05 (19.70 %) and DH-S-04 (19.60 %) with a coefficient of variation of 22.19 per cent (Fig 1). Minimum values for acidity was recorded in accession DH-S-04 (0.07 %) closely followed by AH-S-06 (0.09 %) and CH-S-03 (0.10 %) whereas maximum acidity was recorded in B-G-14 (0.44 %). Crisosto *et al*. (2010) reported 15.7 to 19.3 per cent to 0.22 to 0.65 per cent of range for soluble solid contents and acidity, respectively, while Caliskan and Polat (2008) observed a range of 20.1 per cent to 27.4 per cent for soluble solid contents and 0.09 per cent to 0.26 per cent for acidity. Similar variation was noticed by Aljane *et al.* (2012) and Salimia *et al*. (2013) for TSS and acidity among seventeen accessions. Total sugar ranged between 6.70 per cent (B-G-14) and 16.65 per cent (AH-S-06) closely followed by 16.46 per cent (DH-S-04) with a coefficient of variation of 27.14 per cent. Maximum values for ascorbic acid was registered in the accession MB-S-05 (0.35 mg/100 g) and minimum in B-G-14 (0.15 mg/100 g) (Table 4). Similar findings with most of the chemical characters was observed by Polat and Ozkaya (2005), Gozlekci (2011) and Abo-El-Ez *et al*. (2013).

In the present study, high percentage of variation was noticed among the accessions. Maximum genetic diversity was detected in Gutlibagh area of district Ganderbal. Palmer (1981) reported that plant height and number of branches are greatly influenced by location, soil and climatic factors. Caliskan and Polat (2008) reported that random selection from natural populations increases the genetic diversity, whereas cultivars of different geographic origins exhibit high genetic similarity. With a large number of accessions, traits which are dependent on environmental conditions, such as morphological and fruit quality characteristics were not found to be very useful (Papadopoulou *et al.*, 2002; Khadari *et al*., 2003; Baraket *et al*., 2009).

Conclusion

Fig plant is an important ethno botanical plant widely spread on home gardens of the valley. In view of the fact that figs could significantly contribute to the diversification of horticulture in the region, the inventory of fig genetic resources and morphological characterization has been performed with the aim to identify the existing diversity and to plan its conservation strategy. According to above evaluations ML-G-17, AH-S-06 and DH-S-04 accessions of fig were found as the promising type. However, these types need further trials under the same climatic and soil conditions before they can be recommended to growers.

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Evaluation of *Chokuwa* Paddy (Semi glutinous) Landraces of Assam for Nutritive Values and Genetic Diversity Based on Seed Protein Profile by SDS-PAGE

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*Chokuwa*s are a group of unique and indigenous landraces of paddy cultivated in certain pockets of Upper Assam as Kharif paddy. Analysis of nutritive value for this lesser known and little explored paddy group shows that protein content varied from 7.20% to 10.86%, carbohydrate from 68.05% to 81.52% and lipid content from 1.90% to 3.57% with calorific value in the range of 320.87 Kcal/100gm to 376.83 Kcal/100gm. On the other hand dietary fibre varied from 0.92% to 1.40% while total mineral in the form of ash content varied from 0.90% to 1.43%. *Chokuwa*s are rare, endemic with limited number of landraces but molecular analysis based on seed protein profile shows that considerable genetic diversity exists. Dendrogram based on seed protein electrophoretic profile showed three distinct clusters. Notably, five different landraces with same ethnic name "*Soru Chokuwa*" collected from five different localities have been found to be all different from each other in terms of nutritional parameters as well as genetically. Three protein bands with size 20.3, 13.4 and 11.6 Kd were consistently found in all landraces and can be considered as markers.

Key Words: *Chokuwa* rice, Nutritional Components, Seed Protein Profile, SDS-PAGE

Introduction

Re-emphasis on indigenous landraces of crop species, particularly paddy is gaining momentum with the realization that many of them are rich in nutritive and nutraceutical value(Loying *et al.*, 2008, 2010); harbour precious genes for resistance to biotic and abiotic stress (Toledo and Burlingame, 2006) and in view of alarming gene erosion (Swaminathan, 2011). North East India is widely recognized as a secondary centre of origin for paddy. Among them the indigenous paddy landraces of Assam are unique in many ways. The International Rice Research Institute, Manila has a separate and special collection labeled as "Assam Rice Collection" which reflect the importance attached to the paddy landraces of Assam for the valuable traits they possess. Among them a group of landraces called "*Chokuwa*" are unique in many ways and least explored. *Chokuwa*'s are basically kharif paddy and appears to be endemic since their cultivation is restricted to few pockets of Upper Assam. In terms of taste and cooking quality they are intermediate between glutinous rice (*Bora Rice*) and non-glutinous rice. The difference between Bora (glutinous) and *Chokuwa* are in relative proportion of amylose content, while Bora is characterised by trace, *Chokuwa* contain 15-20% amylose (Sarma and Barooah, 2005) However, like glutinous rice they are exclusively used for preparation of various delicacies, rice beer etc and not used for regular consumption. Paddy cultivation in Assam is heavily dependant on kharif paddy (Sali) with 68% area under cultivation accounting for 73% total paddy production (Barthakur,1992) and *Chokuwa*'s have little contribution to overall paddy production and food security. Hence they remain neglected by farmers, policy planners, Govt. Departments and even researchers and hence they are facing the danger of gene erosion. Earlier studies have shown that many indigenous land races are rich in nutritive values, particularly protein, eg. deep water paddy (Loying *et al*., 2010) as well as dietary antioxidants (Loying *et al*., 2008); unique flavor and aroma, eg. Joha (Dutta Roy 2010). The delicacies prepared with *Chokuwa* are highly valued but *Chokuwa*s remain by and large unexplored in terms of genetic diversity, agro-technology, nutritive value etc. The foremost requirement and pre-requisite for conservation programme is study of nutritive values and genetic diversity and other major qualities and traits. The present study attempts to contribute in that direction.

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Materials and Methods

17 indigenous landraces of *Chokuwa* paddy namely *Kajoli Chokuwa*, *Ronga Chokuwa*, *Bor Chokuwa*, *Soru Chokuwa* (5 Nos.) *Bora Chakuwa*, *Nepali Chokuwa*, (3 Nos), *Khesheng Chokuwa*, *Maigum Chokuwa*, *Aam pakhi Chokuwa*, *Gorundra pakhi Chokuwa, and Boga pakhi Chokuwa*. The prefix "Bor" stand for bigger size and "Soru" stand for small size. Five landraces with the same ethnic name *Soru Chokuwa* were collected from five different localities with distinct morphological differences. Similarly three landraces with same ethnic name *Nepali Chokuwa* were collected from different areas. All samples were collected from village area of Lakhimpur, Sonitpur, Dhemaji and Jorhat District of Upper Assam.

The grains were manually dehusked, grounded to fine powder and dried in oven at 60°C till constant weight was recorded for biochemical analysis. Moisture data were recorded and chemical analysis was carried out using dried sample. Crude protein was estimated by working out the total nitrogen by microkjeldahl method (AOAC, 1970). Total Carbohydrate was estimated by anthrone method as outlined by Clegg (1956) with prior digestion of the dry matter using 2.5N HCl for 30 minutes in a hot water bath. For total soluble sugar the sample was extracted with warm 80% ethanol. The mixture was centrifuged to obtain the supernatant and the residue washed twice with warm 80% ethanol. Subsequently ethanol was removed by evaporation and the extract was redissolved with distilled water. From this soluble sugar was estimated by anthrone method. Lipid content was estimated by extracting the sample in Soxhlet apparatus (AOAC, 1970) using petroleum ether (40º-60ºC) and the amount of lipid was determined after removal of petroleum ether by fractional distillation. Dietary fiber was estimated as per the protocol of Sadasivam and Manickam (1996). Total mineral (ash content) was determined by ashing the sample in Muffle furnace at 630ºC for three hours, (AOAC, 1970).Calorific value was computed by using the formula of Sherman (1952)

Seed protein profile were worked out by SDS-PAGE method outlined by Laemmli (1970). Manually dehusked grains (300 mg) were extracted with ice-cold Tris-buffer 0.2 M (pH :7.5). The sample was homogenized and centrifuged at 12000 rpm for 8 minutes at 4ºC. The supernatant was collected and sample weight to extract volume was adjusted to 1:5 ratio. Seed proteins were resolved in two phase acrylamide gel-stacking gel 4%, separating gel 13.5%. Protein bands were visualized by staining with Coomassie Brilliant Blue R-250 stain. Protein molecular weight marker (PMW-M, Bangalore Genei) was co-electrophoresed to determine the molecular weight of the individual protein. Total number of protein bands were counted for each land races and molecular weight of individual band was deduced from standard curve prepared with size marker. Gel scoring was done in terms of presence or absence of particular protein band for a landrace to prepare the frequency distribution table. From this table similarity index (SI matrix) were generated using Nei and Li's co-efficient (1979). The SI matrix was used to generate the dendrogram by unweighted pair group method with arithmetic averages (UPGMA) using the software NTSYS pc.V2.02j.

Results and Discussion

The present work tries to explore the nutritive value of *Chokuwa* rice which has remained little explored despite their potential. Moisture content among the sample varied within the limited range of 2.60% to 4.15% with a mean of 3.35%. It has been found that protein content varied in the range of 7.20% to 10.86% with a mean of 9.12% (Table 1). It is high in carbohydrate as total carbohydrate varied in the range of 68.05% to 81.52% with a mean of 73.65%. Four landraces had total carbohydrate of 80.00% or nearly so. Total soluble sugar was found in the range of 0.81% to 1.78% with a mean of 1.20%. Unlike crude protein and total carbohydrate it is low in lipid content. Total lipid varied from 1.90% to 3.57% with a mean of 2.37%. Compared to other constituents dietary fibre contents were low and occurred in the range of 0.92% to 1.40% with a mean value of 1.17%. Mineral content (Ash content) were very impressive and varied from 0.90% to 1.43% with a mean value of 1.17%. Because of high carbohydrate content calorific value is high and varied from 320.87 Kcal/100gm in *Bor Chokuwa* to 376.83 Kcal/100gm in *Soru Chokuwa-4* with a mean value of 352.48 Kcal/100gm (Table 1).

From nutritional view point the most promising aspect of *Chokuwa* paddy is its high protein content although considerable variation exist among the land races. In general protein content of rice vary from 6% to 14% with an overall mean of 9.5% (Gomez, 1979) or 10.5% (Anon; 1967). The present study is comparable with this as it is 7.20% to 10.86% with the mean of 9.12%. For carbohydrate the mean value in the present

Table 1. Nutritional components of *Chokuwa* (Semi glutinous) paddy landraces of Assam. The values are mean of three replication ± Standard Error of Mean.

S. No.	Name of the cultivar	Moisture (%)	Crude protein(%)	Carbohydrate (%)	Total soluble sugar(%)	Lipid(%)	Dietary fibre (%)	Total mineral (%)	Calorific value (Kcal/100gm)
1	Kajoli	3.20±0.13	10.21±0.58	71.32±0.62	0.82±0.10	3.57±0.14	0.99±0.04	1.15±0.09	358.25±0.66
2	Ronga	3.00±0.13	10.86±0.77	68.39±0.51	1.21±0.16	2.71±0.08	1.16±0.16	0.90±0.04	341.39±0.66
3	Maigum	3.43±0.09	10.47±0.57	69.53±0.55	1.25±0.17	2.95±0.04	1.14±0.07	1.31±0.04	346.55±0.60
4	Bor	4.15±0.10	7.33±0.48	68.05±0.56	0.81±0.12	2.15±0.16	1.34±0.06	1.12±0.08	320.87±0.57
5	Soru-1	2.80±0.13	10.11±0.57	73.39±0.63	1.65±0.16	2.52±0.10	1.21±0.03	1.09±0.05	356.68±0.68
6	Soru-2	3.15±0.06	7.75±0.55	76.12±0.57	0.97±0.05	2.61±0.13	1.33±0.04	1.09±0.05	358.97±0.56
7	Aampakhi	3.63±0.06	8.49±0.66	73.23±0.61	0.96±0.06	2.33±0.10	1.15±0.04	1.26±0.05	347.85±0.61
8	Nepali-1	3.32±0.05	9.63±0.68	70.38±0.61	1.41±0.05	2.33±0.08	1.22±0.06	1.35±0.06	341.01±0.54
9	Soru-3	3.0±0.13	9.16±0.59	71.61±0.64	1.12±0.06	1.90±0.11	1.33±0.08	0.99±0.05	340.18±0.59
10	Bora	3.72±0.06	7.99±0.60	68.36±0.57	1.78±0.06	2.46±0.08	1.32±0.07	1.09±0.08	327.54±0.59
11	Gorundrapakhi	3.12±0.06	7.72±0.58	79.62±0.87	1.11±0.06	2.45±0.09	0.92±0.07	1.43±0.05	371.41±0.63
12	Nepali-2	2.85±0.05	9.77±0.57	79.17±0.72	0.97±0.04	2.32±0.09	1.19±0.07	1.18±0.06	376.64±0.65
13	Khesheng	4.23±0.05	7.55±0.51	77.44±0.59	1.3±0.08	2.03±0.06	1.15±0.06	1.20±0.05	358.23±0.55
14	Soru-4	3.18±0.08	10.81±0.60	79.01±0.47	1.36±0.06	1.95±0.05	1.40±0.04	1.07±0.09	376.83±0.58
15	Soru-5	3.56±0.08	9.55±0.57	75.69±0.40	1.69±0.05	1.93±0.06	0.95±0.08	1.23±0.06	358.33±0.62
16	Nepali-3	2.60±0.05	10.50±0.56	72.57±0.76	1.26±0.05	2.19±0.06	1.17±0.13	1.23±0.07	351.99±0.68
17	Boga pakhi	4.1±0.09	7.20±0.59	81.52±0.43	0.96±0.05	1.99±0.07	0.98±0.05	1.22±0.06	359.39±0.60
Mean		3.35	9.12	73.65	1.21	2.37	1.17	1.17	352.48
CD at 5%		0.33	1.73	1.77	0.31	0.27	0.07	0.19	1.77
CD at 1%		0.48	2.33	2.39	0.42	0.36	0.09	0.25	2.38

study (73.65%) has been found to be comparable to that reported by Baruah *et al.* (2006) which was 73.86%. Both the values are comparable with the range of carbohydrate content for paddy in general (Grist, 1984; Juliano et al., 1964). Ash content, total soluble sugar, lipid and dietary fibre values in the present study have been found to be comparable with other rice in general (Juliano *et al*.,1964).

Seed proteins resolved in polyacrylamide gel revealed a total 14 proteins in the size range of >108 Kd to 11.6 Kd indicating wide variation from very high molecular weight to very low molecular weight protein. Highest number of 10 protein bands were observed in land races *Nepali Chokuwa-2*, *Khesheng* and *Soru Chokuwa-4* with identical profile. Lowest number of 5 protein bands were observed in *Maigum Chokuwa*. The protein bands with molecular weight 20.3, 13.4 and 11.6 Kd were consistently found in all the landraces and appear to be protein molecular marker for *Chokuwa*s. Apart from qualitative difference there were quantitative difference in terms of band thickness and corresponding pixel intensity (Fig. 3). Most prominent band was the one with molecular weight 13.4 Kd. Pixel intensity analysis show that this band is thickest in *Nepali Chokuwa-1* and lowest in *Bora Chokuwa*.

Seed protein profile has been recognized as a molecular tool to characterize a species at interspecific and intraspecific level by International Seed Testing Association (ISTA, 1996). Seed protein profile remain unchanged in seed of different stage of maturity, unaffected by environmental factors, geographical factors etc. (Ladizinsky and Hymowitz 1979, Naik and Kole, 2002) and hence considered to be protein fingerprint. A number of workers have used seed protein profile for molecular characterization as well as genetic diversity study in paddy and many other crops which shows the effectiveness and reliability of this technique. Loying *et al.* (2010) working with deep water paddy rice land races indigenous to Assam found a total 22 protein bands while Dutta Roy *et al.* (2010) working with scented rice (Joha) landraces of Assam recorded a total 16 protein bands. This indicate that depending upon ecotype as well as different paddy type, seed protein profile are highly variable which is reflective of high degree of genetic variability in paddy. In both the studies the marker proteins were three each and in medium to low

molecular weight size range of 26.7 to 15.7 Kd. In the present study a total 14 protein bands were observed and there were three markers in the medium to low molecular weight category. SI matrix and dendrogram analysis showed that there are three major clusters with considerable genetic diversity. Two landraces, *Aampakhi Chokuwa* and *Soru Chokuwa (2)* have been found to be distantly related to the rest as they do not belong to any of the three clusters.

The findings ofthe present study appear to be in conformity with the earlier reports. Among all crop species paddy exhibit highest genetic diversity. Using DNA based markers like RAPD, high degree of genetic diversity was also demonstrated for other group of paddy like glutinous rice (Sarma and Barooah, 2005). Based on seed protein Handique *et al.* (2007) demonstrated significant intraspecific variation among the cultivars of *Vigna umbelleta* (Rice bean) although it is a self pollinated crop. Similar varietal differences together with phylogenetic relationship has been demonstrated for ground nut (Chandran *et al.*, 2002), country bean (Sharma *et al.*, 2010). The most significant aspect of the present study is that the landrace with ethnic name Soru Chokuwa was collected from five different localities. Although same name, they showed some differences in seed morphology. Biochemical analysis revealed that composition of nutritional parameters were different. Seed protein profile showed that phylogenetically they

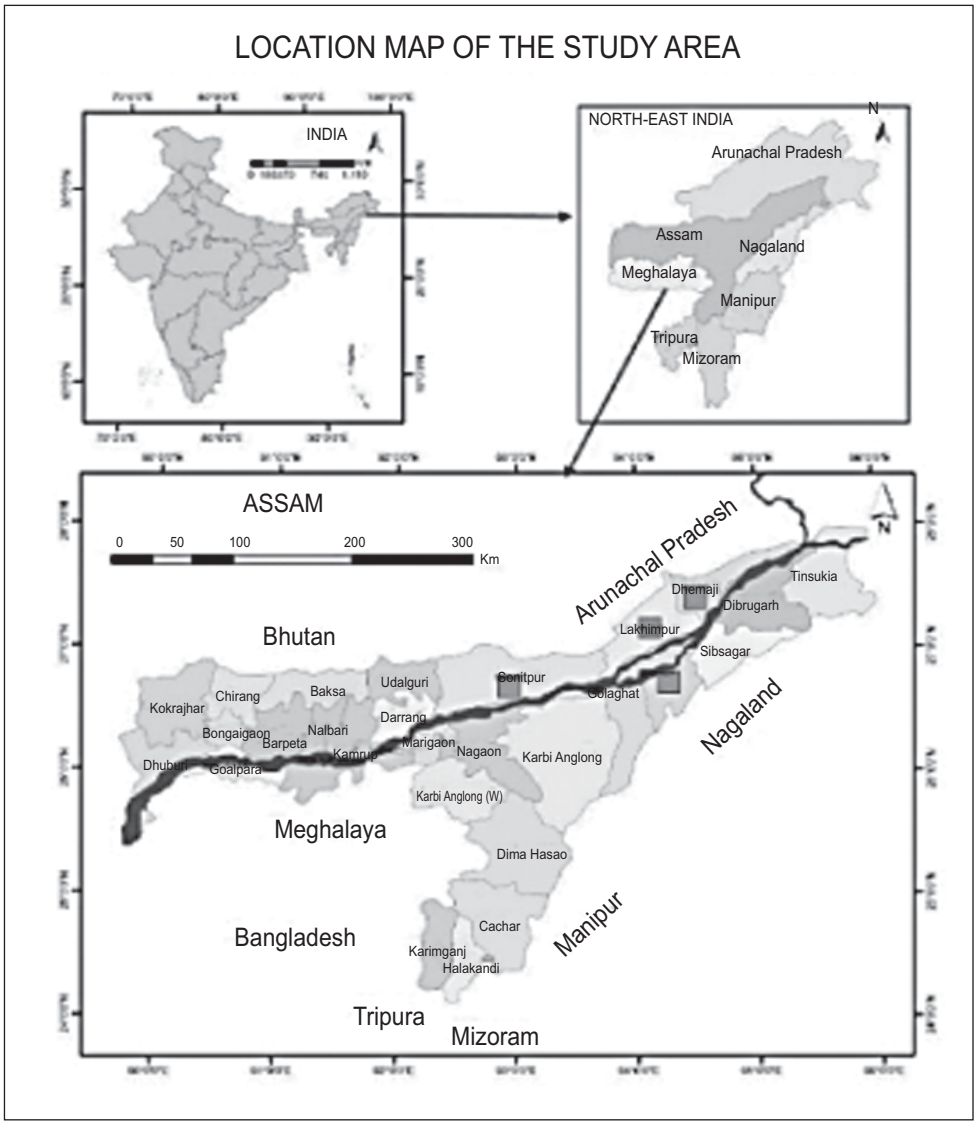

Fig 1. Location Map for sample collection.

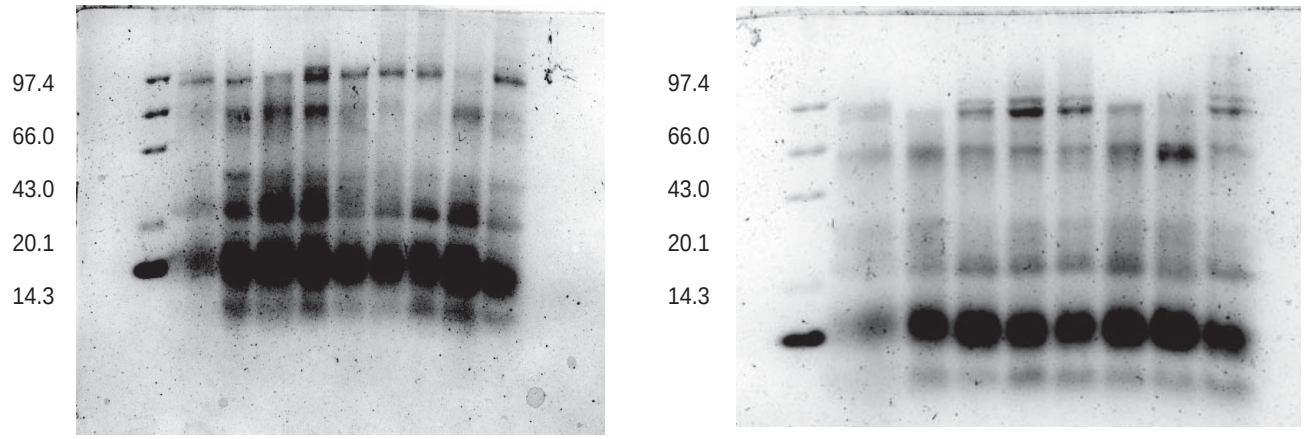

Fig. 2. Seed protein profile of 17 indigenous landraces of Chokuwa paddy (Semi glutinous) resolved in 13.5% polyacrylamide gel. M =Protein molecular weight marker; Lane 1 to 17- Individual landraces of Chokuwa.

Table 2. Frequency distribution of seed protein in *Chokuwa* paddy landraces of Assam

P=Presence of protein band; - Absent.

T-1 Total protein band for a landrace; T-2 Total landrace for a particular protein band.

S.No	Em (cm)	Mol. wt. (Kd)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	T-2
1	0.2	>108.0	-	-	-	P	-	P	P	P	P	-	-	-	-	-	-	-	P	6
2	0.7	>108.0	-	P	-	-	-	P	P	-	P	-	-	-	-	-	-	-	-	4
3	1.0	>108.0	P	P	-	-	-	P	P	-	P	-	-	-	-	-	-	-	P	6
4	1.4	>108.0	-	-	-	-	-	-	-	-	P	-	-	P	P	P	-	-	P	5
5	1.6	108.0	-	-	-	P	P	P	P	-	P	-	-	-	-	-	-	-	P	6
6	1.9	101.5	-	-	P	P	-	P	P	P	P	P	P	P	P	P	P	P	P	14
7	2.5	81.8	-	-	-	-	-	P	P	P	P	P	-	P	P	P	-	P	P	10
8	2.8	66.0	P	P	P	P	P	-	-	P	P	-	-	P	P	P	P	P	P	13
9	3.2	62.0	-	-	-	-	-	-	-	-	-	P	P	P	P	P	P	P	P	8
10	3.5	56.5	P	-	-	-	-	-	-	-	P	-	-	P	P	P	P	-	P	7
11	4.9	34.0	-	P	-	-	P	-	-	P	P	P	P	P	P	P	P	P	P	12
12	6.0	20.3	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	17
13	7.5	13.4	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	17
14	8.5	11.6	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	17
T-1			6	7	5	7	6	9	9	8	13	7	6	10	10	10	8	8	13	

Table 3. Similarity index matrix based on seed protein profile of *Chokuwa* paddy landraces of Assam

0	Cv-1	Cv-2	Cv-3	Cv-4	Cv-5	Cv-6	Cv-7	Cv-8	Cv-9	Cv-10	Cv-11	Cv-12	Cv-13	Cv-14	Cv-15	Cv-16	Cv-17
Cv-1	100	62.5	57.14	44.44	50	36.36	36.36	40	46.15	30	33.33	45.45	45.45	45.45	55.56	40	46.15
Cv-2		100	50	40	62.5	45.45	45.45	50	53.85	40	44.44	41.67	41.67	41.67	50	50	42.86
Cv-3			100	71.43	57.14	40	40	62.5	38.46	50	57.14	50	50	50	62.5	62.5	38.46
Cv-4				100	62.5	60	60	66.67	53.85	40	44.44	41.67	41.67	41.67	50	50	53.85
Cv-5					100	36.36	36.36	55.56	46.15	44.44	50	45.45	45.45	45.45	55.56	55.56	46.15
Cv-6						100	100	54.55	69.23	45.45	36.36	35.71	35.71	35.71	30.77	41.67	57.14
Cv-7							100	54.55	69.23	45.45	36.36	35.71	35.71	35.71	30.77	41.67	57.14
Cv-8								100	61.54	66.67	55.56	63.64	63.64	63.64	60	77.78	61.54
Cv-9									100	42.86	35.71	64.29	64.29	64.29	50	50	85.71
Cv-10										100	85.71	70	70	70	66.67	87.5	53.85
Cv-11											100	60	60	60	75	75	46.15
Cv-12												100	100	100	80	80	76.92
Cv-13													100	100	80	80	76.92
Cv-14														100	80	80	76.92
Cv-15															100	77.78	61.54
Cv-16																100	61.54
Cv-17																	100

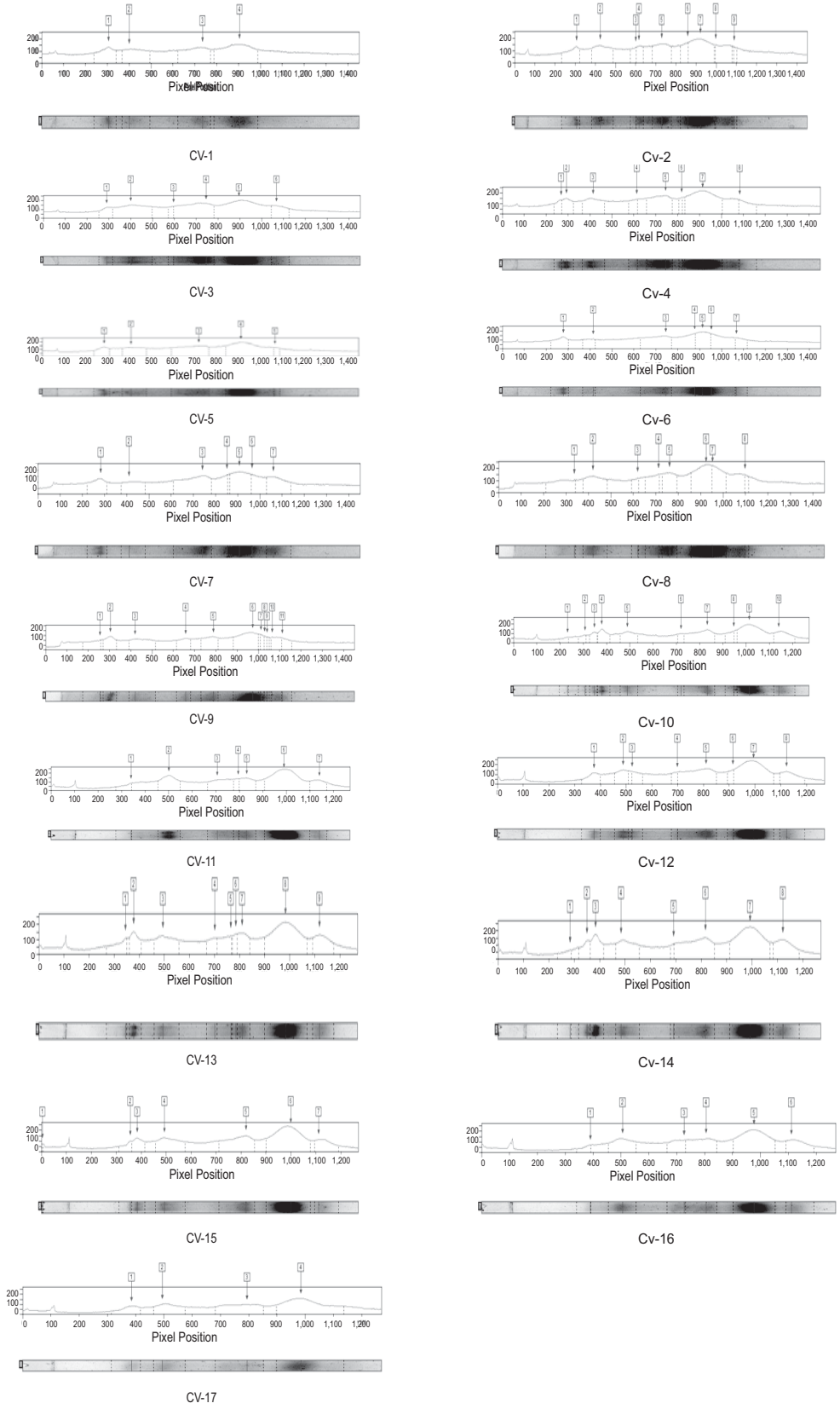

Fig. 3. Pixel intensity analysis for the 17 landraces of *Chokuwa* rice showing the relative proportion of individual proteins and comparision among the landraces.

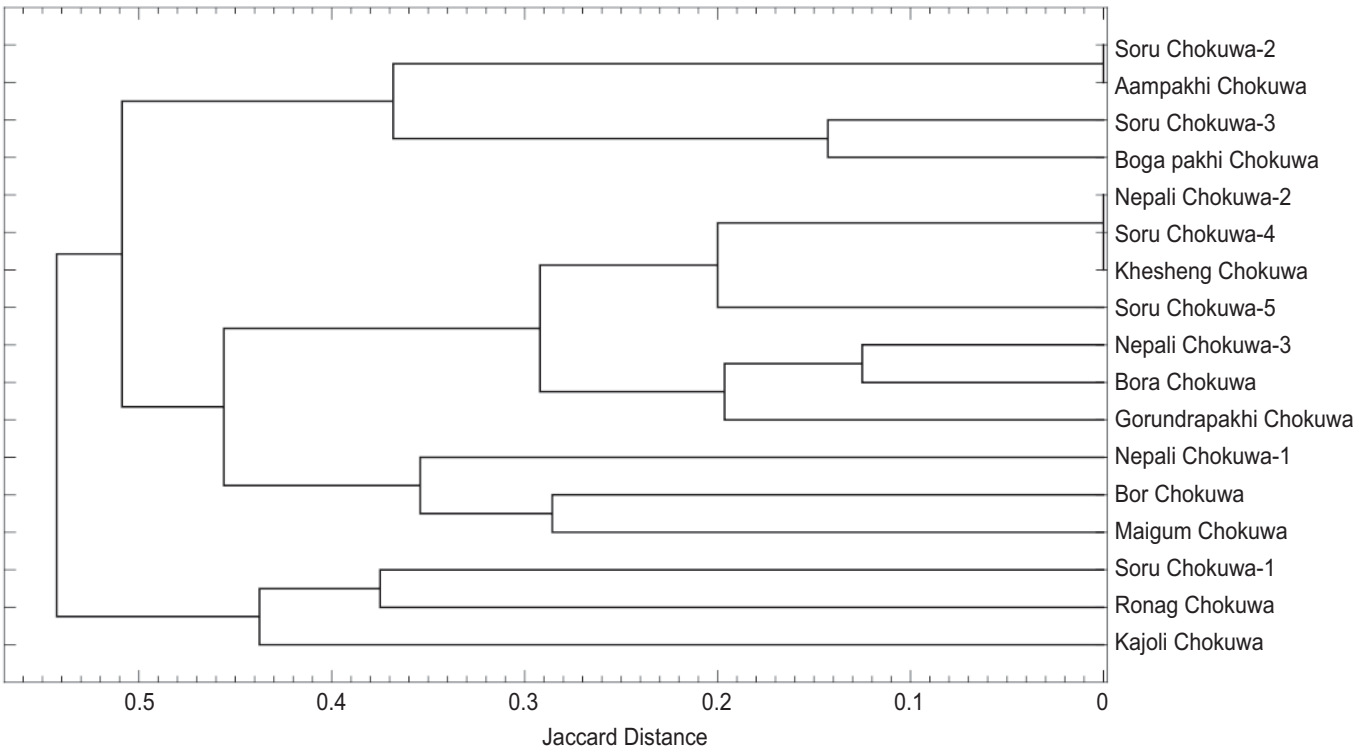

Fig. 4. Dendrogram analysis of *Chokuwa* paddy landraces of Assam based on seed protein profile

were completely different. This shows that these five land races are genetically different although ethnic name is same. Similar observation was made for three land races with the ethnic name *Nepali Chokuwa* collected from three different locations.

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Extended Naturalization Records of Five Non-Native Plant Species to Indian States

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Field survey across various parts of the country coupled with study of literature and examination of herbarium specimens revealed naturalized distribution of five exotic species in various states of India *viz*. *Amaranthus palmeri* S. Wats. (Delhi and Haryana), *Centratherum punctatum* Cass. (Nagaland), *Dysphania pumilio* (R.Br.) Mosyakin & Clemants (Jammu & Kashmir and Rajasthan), *Emex spinosus* (L.) Campd. (Delhi) and *Solanum diphyllum* L. (Andhra Pradesh) which are not earlier reported from these states. The description, phenology, habitat and field notes have been provided in detail.

Key Words: Genetic resource, Indian states, New distribution, Non-native species, Weed

Introduction

Naturalized plants are the plants established as a part of the flora of a particular region other than their place of origin. Maheshwari (1962) reported about 40% of Indian flora comprised foreign elements. These groups of plants predominantly include weeds, exotic crops, especially floricultural plants, crop wild relatives which are deliberately or otherwise got introduced into a region. While undertaking surveys and explorations across different areas, a few species were observed that were not reported in floristic literature pertaining to some Indian states. Such records have been occasionally documented in plant genetic resource studies (Pradheep *et al*., 2011; Sharma, 2012; Pandey *et al*., 2015). Documentation of such species not only helps updating the floristic literature, but also in germplasm collection (in case of crops and related species) as well as in taking prophylactic measures to avoid their fast spread to other areas (in case of weeds). In this paper five such species have been noted as new records to six Indian states. The information on taxonomy, global distribution, phenology and notes on significant findings are provided.

Materials and Methods

The plants were collected at flowering and fruiting stages during explorations undertaken for the past three years (2012-15). Critical observations were made on morphological characters in natural habitats as well as material available in the herbarium. Hand lens (10x) was used for recording observations on micro-morphological characters at field. Herbarium specimens were prepared following the standard procedures (Jain and Rao, 1977) and were studied using Stereoscopic Zoom Microscope (Nikon SMZ1000) and DS Camera Control Unit (DS-L2) at ICAR-NBPGR, New Delhi especially for anthers, stigma, ovary and other minute characters. The botanical information thus obtained was validated with relevant floristic literature, online herbaria at London (BM), Edinburgh (E), Kew (K), Paris (P) and Beijing (PE) including the type specimens. Herbarium specimens were deposited in National Herbarium of Cultivated Plants (NHCP) at ICAR-NBPGR, New Delhi. These species were thoroughly checked against concerned floristic literature (http://efloraindia.nic.in/efloraindia; Kumar, 2001; Pullaiah and Chennaiah, 1997; Naithani, 1990; Shetty and Singh, 1987; Maheshwari, 1963) and were confirmed as new records.

Results and Discussion

1. *Amaranthus palmeri* S.Wats. in Proc. Am. Acad. 12: 274. 1877. Thakur in J. Indian Bot. Soc. 43: 573. 1964. Shetty & Singh, Fl. Rajasthan 2: 728. 1991. Patil in J. Bombay Nat. Hist. Soc. 95(1): 150-151. 1998. [Family Amaranthaceae] Fig. 1 A, B and 2.

Type (lectotype designated by Sauer 1955: 31): United States of America, California, banks of Rio Grande, July 1834, *Berlandier 2407* (GH).

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Fig. 1. New record of plant naturalizations in Indian states/Union Territories – A and B. *Amaranthus palmeri* S. Wats. in Ferozepur Jhirka, Haryana; C. *Centratherum punctatum* Cass. in Mon, Nagaland ; D. *Emex spinosa* (L.) Campd. in Aravalli Biodiversity Park, Delhi; E. and F. *Solanum diphyllum* L. in Malivalasa, Andhra Pradesh (fruiting plant and flowering twig).

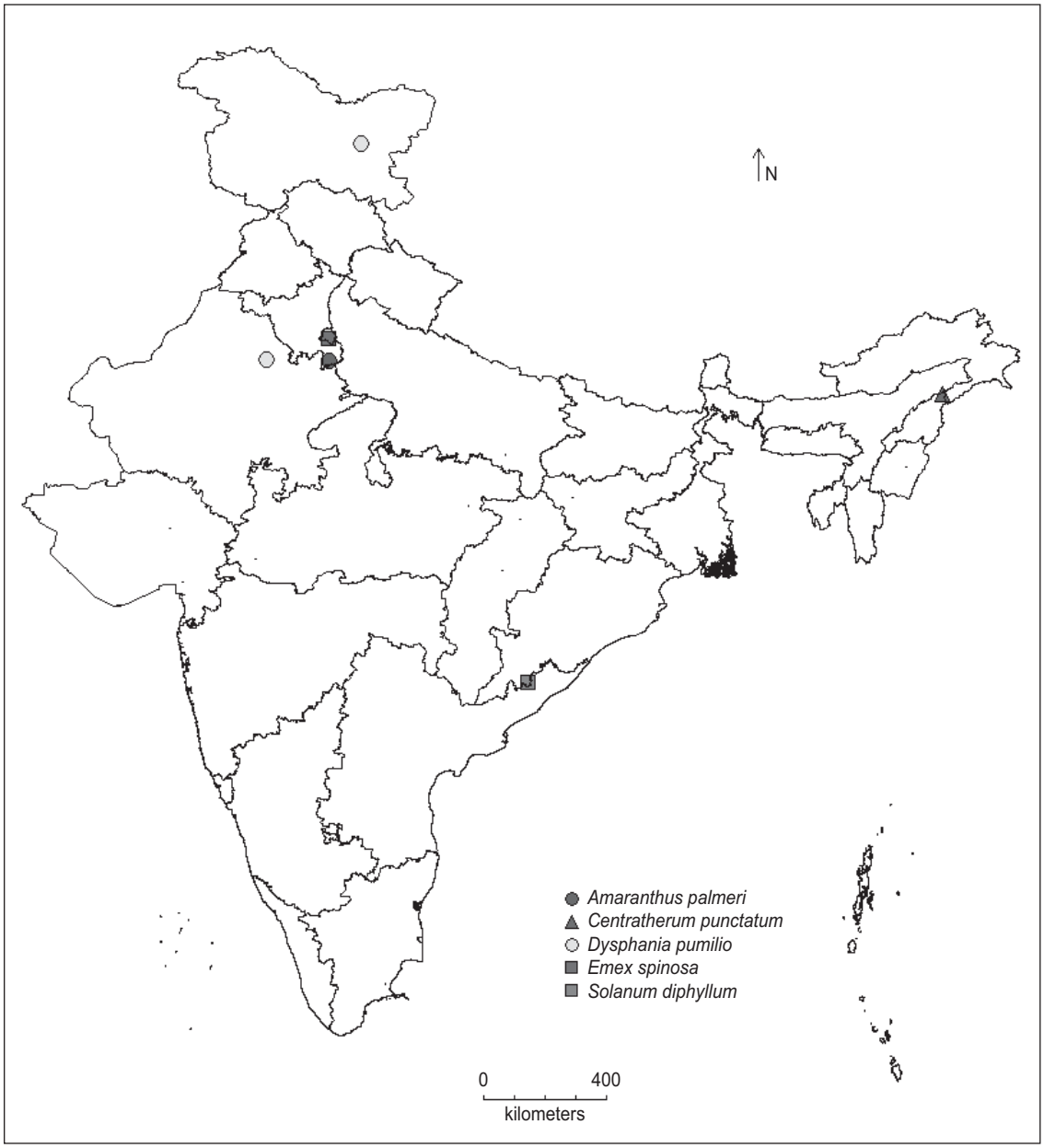

Fig. 2. Map showing new distribution record for *Amaranthus palmeri* S.Wats., *Centratherum punctatum* Cass., *Dysphania pumilio* (R.Br.) Mosyakin & Clemants, *Emex spinosa* (L.) Campd. and *Solanum diphyllum* L.

Herb dioecious, annual, erect, branched, glabrous, up to 1.8 m tall. Stem sulcate-angular, pale green, sometimes pink-tinged. Leaves up to 8 cm long, long-petiolate, petiole often longer than lamina; lamina obovate to lanceolate, obtuse-acute, apex mucronate. Inflorescence terminal, linear-spiked, with occasional few axillary clusters, interrupted in proximal part of rachis. Bract 5-6 mm long, about 2 times longer than tepals, apex acuminate, spine-tipped, particularly of pistillate flowers. Staminate flower tepal 5, unequal, apex acute, stamen 5. Pistillate flower tepal 5, apex acuminate, mucronulate; style branches spreading; stigma 2. Utricle brownish, subglobose, shorter or as long as tepals, rugose. Seed dark brown, about 1.2 mm diam., shiny.

Flowering & Fruiting: Jan.-Dec.

Distribution: Native to Mexico and USA, naturalized in Europe, Asia and Australia. *India*: Bihar, Rajasthan, Maharashtra, Gujarat, Haryana (now), and Delhi (now); along roadsides, wet waste areas and agricultural fields.

Specimens examined: Haryana, Mewat district, Ferozepur Jhirka, 27° 47' 409" N 76° 57' 662" E, 210 m, 23 August, 2015 (flowering twig), *K. Pradheep 2224* [NHCP22212]. Delhi, Central Ridge Reserve Forest, Vandemataram Marg, opp. Buddha Jayanti Park, 28° 36' 396" N 77° 09' 360" E, 234 m, 22 September, 2016 (flowering twig), *E. Roshini Nayar & K. Pradheep/2016-1* [NHCP22379].

Notes: This is the only dioecious *Amaranthus* species (coming under subg. *Acnida*) occurring in India. This aggressive species is spreading very fast on roadsides and crop fields, probably owing to its spiny bracts.

2. *Centratherum punctatum* Cass. in F. Cuvier, Dict. Sci. Nat. ed. 2. 7: 384. 1817. R.R. Rao *et al*., Fl. Ind. Enum.-Asterac. 23. 1988. P.K. Hajra *et al*., Fl. India 13: 394. 1995. [Family Asteraceae] Fig. 1 C and 2.

Type (neotype designated by Kirkman 1981: 15): Brazil, Loreto, *s.d*., *Eiton 4042* (SP).

Spreading herb or undershrub to 50 cm tall. Stem many branched, branches terete, ascending, glabrescent to villous. Leaves 3-5.5 x 1.2-1.5 cm, short petiolate, lamina ovate to elliptic or obovate to spathulate, cuneate to attenuate at base, margin serrate, sparsely pubescent on both sides, teeth with minute mucro. Head terminal, solitary or in cluster of 2-3, peduncle 3-4 cm long. Involucre campanulate, about 1 cm long. Phyllary (involucral bract) imbricate in several series, membranaceus, outer foliaceous, greenish, glabrous or sparsely tomentulose; inner purplish. Corolla purplish or bluish, 5-7 cm long, glandular. Achene not seen.

Flowering & Fruiting: Jan.-Dec.

Distribution: *World*: Mexico, USA, West Indies, Central America and South America. *India*: naturalized in Assam (Talukdar and Deori, 2014) and Nagaland (now); in disturbed places, roadsides.

Specimen examined: Nagaland, Mon district, Mon, 26° 43' 751" N 95° 01' 459" E, 718 m, 24 November, 2013 (flowering twig), *K. Pradheep & Soyimchiten 1601* [NHCP22159].

*Notes***:** Besides Nagaland, the authors observed this neotropical ornamental species as an escape from cultivation in Meghalaya (West Khasi Hills), and Manipur (Tamenglong; http://www.flowersofindia.net/catalog/slides/Brazilian%20Button%20Flower.html accessed 26.05.2016). It was found cultivated as medicinal and ornamental plant in home gardens in the states of Tamil Nadu, Kerala and Maharashtra. An allied infraspecific taxon, subsp. *fruticosum* (S.Vidal) K. Kirkman ex Shih H.Chen, M.J.Wu & S.M.Li, is reported to be naturalized in West Bengal (Chowdhury and Das, 2012), which is distinguished from typical subspecies by large achenes and phyllaries with strong indurate base (Kirkman, 1981).

3. *Dysphania pumilio* (R.Br.) Mosyakin & Clemants, Ukrayins'k. Bot. Zhurn. (Ukr. Bot. J.) 59: 382. 2002. *Chenopodium pumilio* R.Br., Prodr. 1: 407. 1810. Ramayya & Rajagopal in Curr. Sci. 38: 173. 1969. [Family Chenopodiaceae] Fig. 2.

Type: Australia, South Australia, Kangaroo Island, 21 March 1802, *R. Brown 3303* (BM001010202).

Annual herb, prostrate or ascending, aromatic, to 30 cm. Stem branched, glandular, pubescent, green. Leaves alternate, petiolate, petiole about 1 cm long; lamina elliptic-lanceolate or ovate-lanceolate, 2.4-2.7 × 0.75-1.2 cm, glandular-pilose at lower surface, apex obtuse, base cuneate, margin lobed (2-4 each side). Inflorescence in axillary cymes or glomerules, greenish, subglobose, 2.0-3.5 mm in diam. Flower minute; tepals 5, ovate, glandular-pubescent; stamen absent or 1; stigma 2, thread-like. Fruit subglobose. Seed vertical, ovoid, 0.6-0.7 mm, black-brown, testa smooth.

Flowering & Fruiting: Feb.-July & Nov.-Dec.

Distribution: *World*: Native to Australia, and is naturalized in Europe, North America, Japan, New Zealand, Argentina, Iran (Rahiminejad *et al.*, 2004) and China. *India*: Tamil Nadu (Nilgiris), Rajasthan (now) and Jammu & Kashmir (now); in human settlements, occasionally colonizing disturbed areas (roadsides).

Specimens examined: Jammu & Kashmir, Leh district, Leh-Fort Road, 34° 16' 083" N 77° 57' 461" E, 3450 m, 07 September, 2014 (whole plant), *K. Pradheep & Mohar Singh 1717* [NHCP21894]. Rajasthan, Jhunjhunu district, Nawalgarh, 27° 50' 445" N 75° 16' 048" E, 380 m, 24 March, 2013 (a flowering twig), *E. Roshini Nayar 2013* [NHCP21183].

Notes: In Nawalgarh, this weedy species was occurring as scattered rosette along gravelly paths**.** It is characterized by the presence of multicellular glandular hairs. It is distinguished from other species occurring in India – *D. ambrosioides* and *D. botrys* by seeds always vertical (vs usually horizontal), stamen 1 (vs (1-)5), plants slightly aromatic (vs strongly aromatic) (Rahiminejad *et al.*, 2004).

4. *Emex spinosa* (L.) Campd., Monogr. Rumex. 58. 1819. Bhandari, Fl. Ind. Desert 300. 1990. Shetty & Singh, Fl. Rajasthan 2: 745. 1991. *Rumex spinosus* L., Sp. Pl. 33. 1753. [Family Polygonaceae] Fig. 1 D and 2.

Type: Greece, Crete ["Habitat in Creta"], Herb. Linn. 464. 36 (LINN).

Annual herb, glabrous monoecious, upto 30 cm tall. Stem decumbent, branched, often reddish. Leaf alternate, entire; ochrea glabrous, membranous; petiole 2-5 cm long, glabrous; lamina ovate-oblong, 3.5-5.0 × 2.5-3.0 cm, base truncate, apex obtuse. Staminate flowers in axillary fascicles, ovate; tepal 6, greenish, oblong to oblanceolate; stamen 4-6, shorter than tepal. Pistillate flowers in axillary clusters, subsessile, 3-6 per fascicle; tepals 6, connate, arranged in 2 whorls of 3 each, outer ones spinescent; style 3, stigma 3. Nut 3-angled, 4-5 x 3-3.5 mm, enclosed in the spinescent tepals, spine 0.5-1.0 mm long, longitudinally 3-grooved.

Flowering & Fruiting: March-May

Distribution: *World*: Mediterranean region; now naturalized in South America, Eurasia, North Africa and Australia. *India*: Rajasthan, Karnataka, Bihar, Delhi (now); mostly in dry, sandy places and in cultivated fields.

Specimen examined: Delhi, Aravalli Biodiversity Park (near Gurgaon border), 28° 29' 225" N 77° 07' 025" E, 258 m, 19 March, 2015 (fruiting twig), *A. Pandey & K. Pradheep-15/44* [NHCP22091].

Notes: This weed species, though not reported in floristic literature pertaining to the state of Haryana, has been noted as a major weed in wheat and chickpea crops (Punia *et al*., 2009; Chhokar *et al*., 2012). The first author observed occasional occurrence of this species as weed in the experimental fields of Punjab Agricultural University, Ludhiana during March 2015, although unreported from Punjab.

5. *Solanum diphyllum* L., Sp. Pl. 1: 184. 1753. T.K. Paul & M.C. Biswas, *Bull. Bot. Surv. India*, 37: 137-138. 1995. Zhang *et al*., Fl. China 17: 317. 1996. *S. capsicastrum* Link ex Schauer Allg. Gartenzeitung (Otto & Dietrich) 1: 228. 1833. [Family Solanaceae] Fig. 1 E, F and 2.

Type (vide D'Arcy 1974): Herb. Linn. 248. 5 (LINN).

Shrub, glabrous, to 1.5 m tall. Stem erect, terete, dark brown. Leaves unequal-paired, major leaf petiole about 0.5 cm long, winged by leaf bases, lamina entire, elliptic-oblong, 4-6 × 2.5-3.0 cm, base attenuate, apex rounded; minor leaf subsessile, lamina rounded, 1.4-2.0 × 1.2-1.5 cm, entire. Inflorescence leaf opposed, short scorpioid racemes, peduncle short, unbranched. Flowers bisexual, pedicel 5-10 mm long. Calyx constricted at base, c. 2 mm long, 5-lobed. Corolla stellate, whitish, 5-7 mm across, 5-lobed, lobe c. 4 mm long. Stamen 5, equal, epipetalous, anther stout, 1-1.5 cm long; filament short. Ovary glabrous. Fruit a berry, orange, globose, 8-10 mm across, fruiting pedicel erect. Seed yellowish, margin thickened.

Flowering & Fruiting: May-July and June-Sept.

Distribution: Native to Mexico and Central America, naturalized in Taiwan. *India*: Maharashtra, Bihar, Uttar Pradesh, West Bengal, Tamil Nadu and Karnataka, Andhra Pradesh (now); along roadsides.

Specimens examined: Andhra Pradesh, Visakhapatnam district, Araku mandal, Malivalasa, 18°21'370" N 82°53' 404" E, 868 m, 31 October, 2015 (part of fruiting twig), *K. Pradheep & B. Abraham 2083* [NHCP22157].

Notes: In Araku mandal of Andhra Pradesh, this ornamental species appears to be recently naturalised along roadsides and has been spreading fast. Besides, the second author observed it as an escape from cultivation in herbal gardens at Delhi. It is related to a northeast Indian species *S. spirale* Roxb. (Paul and Biswas, 1995).

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Taxonomy, Diversity and Distribution of the Genus *Cucumis* L. in India

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The eco-geographic distribution and taxonomy of 17 taxa of *Cucumis* L. was investigated based on the study of 978 herbarium sheets in seven major Indian herbaria and germplasm collection of 261 accessions. Distribution map was plotted based on authentic identification of herbarium specimens and field observations under natural conditions. Areas of higher concentration/richness have been worked out for individual taxon. Morphological, ecological, phenological features and economic importance are discussed. The ideal time for collection of germplasm is indicated.

Key Words: *Cucumis*, Crop Wild Relatives, Distribution Map, Germplasm Collection, Herbarium Study, Phenology

Introduction

The two major components of agro-biodiversity that offers the broadest range of diversity for breeders are crop wild relatives (CWR) and landraces (LR), but there is currently a gap between their conservation and use and they remain underexploited by the user community (Maxted and Kell, 2012). In this context, studies on diversity, occurrence and distribution of wild relatives of our crop plants assume high significance. In order to meet the needs of future generations, there are five key areas that need to be addressed: climate change mitigation, limited success of traditional characterization to meet breeders' needs, lack of systematic CWR and LR conservation, threats faced by CWR and LR diversity and lack of plant genetic resource informatics cohesion (Maxted and Kell, 2012), out of which we attempt to address in this paper the last two aspects for the genus *Cucumis* L. in India.

The genus *Cucumis* L. in India as per the earlier concept comprised six species viz., *C. melo* L., *C. sativus* L., *C. prophetarum* L., *C. callosus* (Rottl.) Cogn., *C. setosus* Cogn. and *C. hystrix* Chakrav. (Chakravarty, 1982), of which melon and cucumber (*C. melo* L. and *C. sativus* L.) are commonly cultivated for edible fruits used in salads, pickles, curries and juice. *C. melo* subsp. *agrestis* Naud. var. *agrestis* and *C. sativus* var. *hardwickii* (Royle) W. J de Wilde & Duyfjes are found in disturbed habitats and *C. melo* subsp. *agrestis* var. *agrestis* and *C. callosus* (Rottl.) Cogn. are found almost as a weed in man transformed habitats. Among others, *Cucumis indicus* Ghebret. & Thulin, and *C. silentvalleyi* (Manilal, T Sabu & PJ Mathew) Ghebret & Thulin are endemic to Western Ghats and *C. setosus* Cogn. is an endemic of Deccan region. The genus includes 32 species according to Kirkbride's (1993) treatment in the Monograph of the genus *Cucumis*. Molecular studies undertaken by Schaefer (2007) revealed that the genus *Cucumis* is paraphyletic. In order to make it monophyletic, he merged five genera – *Cucumella chior, Dicoelospermum* C.B. Clarke, *Mukia* Arn, *Myrmecosicyos* C. Jeffrey and *Oreosyce* Hookf under *Cucumis* L., and recircumscribed the subgenera and sections. Out of these, *Mukia* is morphologically very distinct with red ripe fruits adapted to bird dispersal. *Cucumella* has the closest morphological resemblance to subgenus *Melo* with yellow flowers, prostrate habit and leaves with shallowly ovate outline.

This paper is dedicated to late Dr. KS Negi who explored Central Himalayan Agro-biodiversity for about three decades and was responsible for conserving many valuable germplasm and also widening our knowledge of Crop Wild Relatives of Central Himalaya.

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Morphologically, *Mukia* and *Dicoelospermum* do not resemble *Cucumis,* but others resemble to a smaller or greater extent. Moreover, crossability studies do not warrant their inclusion in the genus *Cucumis.*

Recently, Dwivedi *et al.* (2010) reported the distribution, collection, extent of variability, associated indigenous traditional knowledge, conservation status of five different *Cucumis* species and prospects of their utilization in crop improvement. Naveen Garg *et al.* (2007) reviewed intra-specific variation in *C. melo*, inter-specific hybridization in *Cucumis* and sources of disease resistance besides phylogenetic affinities among *Cucumis* species.

While there is unanimous opinion about origin and domestication of cucumber in India, there are two view points on the origin of melon, majority for an African origin, but recent reports support for its Indian origin (Sebastian *et al*., 2010; Joseph John *et al.*, 2013). Identifying gaps in existing *ex situ* germplasm collection is increasingly important as the large scale coarse-grid collecting in the past is to be complemented with a more targeted fine-grid approach. To make the most efficient use of limited resources, germplasm collectors must have a clearly defined set of target taxa and should know where to find species within their target region. A concerted attempt was made to locate, collect, identify and conserve landraces and wild species of *Cucumis* throughout the country and the results are summarized in this paper.

Materials and Methods

As a part of the eco-geographic survey of the genus, the authors visited major herbaria in India during 2008-2011. All the *Cucumis* sheets lodged at Botanical Survey of India, Western Regional Centre, Pune (BSI), Botanical Survey of India, Eastern Regional Centre, Shillong (ASSAM), Central National Herbarium, Howrah (CAL), Calicut University Herbarium, Kozhikode (CALI), The Madras Herbarium, Coimbatore (MH), National Herbarium of Cultivated Plants, New Delhi (NHCP) and ICAR-National Bureau of Plant Genetic Resources Regional Station, Thrissur, Kerala [(NBPGR (T)] were examined. Online herbarium images from Royal Botanic Garden, Edinburgh, Scotland (E), South China Botanical Garden Herbarium, Guangzhou, China (IBSC), Royal Botanic Garden Kew, UK (K), Museum National d' Histoire, Paris, France (P), Chinese National Herbarium, Beijing, China (PE) and North West Agriculture and Forestry University, Shaanxi, China (WUK) were also studied. Based on herbarium data, the entire stretch of Western Ghats was explored extensively, besides specific sites in Deccan Plateau, Central India, Konkan region of Maharashtra, Andaman Islands, Lakshadweep and North East India for collection of germplasm and ecological data. We have observed all the taxa in the field (wild) and augmented several collections through field trips for conservation, characterization and herbarium curation. The seed germplasm is being maintained in the Medium Term Storage (MTS) at ICAR-National Bureau of Plant Genetic Resources Regional Station, Thrissur, Kerala.

Passport data of 261 accessions collected were incorporated into an Exploration Database developed by Indian Council of Agricultural Research-National Bureau of Plant Genetic Resources, India. The GIS software (DIVA-GIS, ver. 5.2 developed by IPGRI) was used to plot individual taxon distribution, areas of greater concentration and for identifying gaps/inadequacies in the existing collections. The eco-geographic and phenological data recorded while collecting germplasm from the original habitat were supplemented with the herbarium observations to find out the natural range, habitat preference, flowering and fruiting periods, etc.

Results and Discussion

Herbarium Representation

Several reports of eco-geographic studies in the wild genepool (Gemeinholzer *et al.,* 2010; Guarino *et al.,* 2002; Kameswara Rao and Bramel, 2000; Maxted *et al.,* 2008) indicate the usefulness of this technique in germplasm collection and conservation. Out of 978 herbarium sheets, the NHCP had the highest number (327) followed by CAL (212), BSI (202), MH (128), NBPGR (T) (98), CALI (7) and ASSAM (4). A total of 83 sheets were found to be labelled under *C. pubescens* and *C. trigonus* of which 56 sheets should be placed under *C. melo* subsp. *agrestis. C. pubescens* is synonym of *C. melo* subsp. *agrestis* and *C. trigonus* is a synonym of *C. callosus. C. melo* has the highest representation among various *Cucumis* species. Herbarium wise representation is given in Table 1.

Herbarium survey indicates prevalence of *Cucumis setosus* in certain pockets of Maharashtra being cultivated for the fruits popularly known as 'meika' and wide distribution of wild and feral forms of *C. melo* subsp. *agrestis* var. *agrestis* and *C. sativus* var. *hardwickii*

Table 1. Representation of *Cucumis* taxa specimens in some major Indian herbaria

S.No.	Taxa	BSI	ASSAM	CAL	CALI	MH	NHCP	NBPGR (T)	Total
1	*C. africanus* L.f.						1		1
2	*C. anguria* L.						3		3
3	*C. callosus* (Rottl.) Cogn.	24		24		28	20	8	104
4	*C. dipsaceus* Ehrenb. ex Spach						2	1	3
5	*C. hystrix* Chakrav.			4			10	2	15
6	*C. indicus* Ghebreti. & Thulin	1		2			7	1	10
7	*C. melo* L. – cultivated	53	2	50		13	76	16	210
8	*C. melo* subsp. *agrestis* (Naud.) Pangalo	31		65		32	35	14	177
9	*C. melo* L. subsp. *agrestis* (Naud.) Pangalo var. *momordica* (Roxb.) Duthie & Fuller						64		64
10	*C. melo* L. subsp. *agrestis* (Naud.) Pangalo var. *utilissimus* (Roxb.) Duthie & Fuller						19		19
11	*C. metuliferus* E. Meyer ex Naudin						2	1	2
12	*C. muriculatus* Chakrav.						2	1	3
13	*C. prophetarum* L.	11		8	1		5	5	30
14	*C. sativus* L. - cultivated	46	2	15		14	27	9	113
15	*C. sativus* L. var. *hardwickii* (Royle) Alef.	16		27	2	36	38	33	152
16	*C. setosus* Cogn.	20		17			9	1	46
17	*C. silentvalleyi* (Manilal, T. Sabu & P. J. Mathew) Ghebret. & Thulin				4	5	2	9	20
18	*Cucumis* spp. (unidentified)						5		5
	Total	202	4	212	7	128	327	98	978

NBPGR (T) = ICAR-NBPGR Regional Station, Thrissur, Kerala

throughout the country with overlapping distribution. *C. hystrix* is under-represented, even though it was spotted from all over Mizoram, parts of Arunachal Pradesh, Nagaland, Manipur and Assam with possible extended distribution in adjoining North East Hill states.

As per the information from herbarium labels and passport data, taxa under the genus *Cucumis* in India are adapted to soil types such as gravelly (Rajasthan), black cotton (Deccan Plateau), sandy loam (Uttar Pradesh), river alluvium and lateritic loam (Kerala), calcareous coral sand (Lakshadweep) and volcanic (Andaman Islands). The low frequency of herbarium collections from the states of Arunachal Pradesh, Nagaland, Tripura, Sikkim and elsewhere in North East (NE) India is rather an indicator of poor botanical exploration undertaken in these states as evidenced from sample survey (nevertheless we made significant cucurbit collections in NE India for the past five years). Very less representation of *Cucumis indicus* and *C. silentvalleyi* in herbaria signifies the niche-specificity of both the species, restricted to a few localities in Western Ghats. Species-specific targeted approach is essential for conservation (*ex situ*) of niche specific/RET taxa.

Species Distribution and Areas of Greater Concentration

Cucumis melo and *C. sativus* are widespread under cultivation throughout the country. Both have wild forms distributed across a long stretch of geographical area in the country. *C. melo* subsp. *agrestis* var. *agrestis* grows in wild habitats throughout the country including Lakshadweep and Andaman and Nicobar Islands where non-bitter types are found. However, it is conspicuously absent in Kerala characterized by high rainfall and acidic soils, except in Attappadi (an area with black cotton soil and less rainfall). The tiny state of Goa offers good scope for collection of variability in snap melon (*C. melo* subsp. *agrestis* var. *momordica*) as original Portuguese introductions still persist in home gardens. Similarly, Kerala state has many selections of oriental pickling melon (*C. melo* subsp. *agrestis* var. *conomon)* by local farmers suited to their ethnic and cultural needs. We have come across three specific named named types; 'Kallanvellari' (IC613488) restricted to Thiruvananthapuram and Kanyakumari districts, 'Kanivellari' – across Kerala, cultivated as a summer vegetable in paddy fallows and 'Ponavellari', now nearly

extinct but a component once prevalent in slash and burn hill agriculture in northern Malabar (Kasaragod/Kannur). *C. silentvalleyi* and *C. indicus* are niche specific; former occurs in specific pockets of southern Western Ghats as ephemeral. It is restricted to Kerala state (Thrissur, Palakkad, Wayanad and Idukki districts) and bordering Tamil Nadu (Coimbatore district). *C. indicus* grows in northern Western Ghats and *C. setosus* is an endemic species restricted to central India including Maharashtra state and border districts of adjoining states *viz*., Dang district in Gujarat; Hoshangabad and Umaria districts in Madhya Pradesh and Belgaum district in Karnataka.

Cucumis hystrix is a wild species indigenous to northeastern India, Myanmar, Thailand and Yunnan province of China. In India, it is found in areas bordering Myanmar and Bangladesh with good population density in Mizoram. It has been classified as a species in the subgenus *Cucumis* based on its morphology (Kirkbride, 1993). It was rediscovered in natural and wild conditions (Chen *et al.,* 1998) and subjected to subsequent genetic characterization. This is the first Asian *Cucumis* species described with chromosome number 2n=24 (Chen *et al*., 1997a). This finding challenges the basic chromosome numbers theory that African *Cucumis* have x=12 and that Asian *Cucumis* have x=7, which has governed the understanding of systematics and phylogenetics in *Cucumis* for decades. Although, *C. hystrix* possess the same number of chromosomes as *C. melo*, yet its fruits have a typical cucumber taste and flavour and has been found to be closer to *C. sativus* (than to *C. melo)* on the basis of isozyme patterns (Chen *et al.,* 1997b), SSR and RAPD marker analysis (Zhuang *et al.,* 2004).

Cucumis muriculatus is a new species record for India (Joseph John *et al*., in preparation). Even though considered synonym with *C. hystrix* (De Wilde and Duyfjes, 2007), it is different from *C. hystrix* for many vegetative, floral and fruit characters. It has been collected from two sites in Mizoram and one site from Nagaland and often with overlapping distribution with *C. hystrix*.

Cucumis prophetarum is localized in Gujarat and Rajasthan and specific regions of Maharashtra and Delhi. *C. callosus* has been found to occur naturally in the wild state in the whole of Deccan Plateau and Indo-Gangetic plains comprising the states of Tamil Nadu, Karnataka (barring coastal and Western Ghats area), Maharashtra, Madhya Pradesh, Uttar Pradesh, Jharkhand, Chhattisgarh, Odisha, West Bengal, Punjab, Haryana, Assam and Himachal Pradesh (Joseph John *et al.,* 2013). *C. sativus* var. *hardwickii* is widely distributed in Khasi hills (Meghalaya), throughout Western Ghats (parts of Maharashtra, Goa, Karnataka, Kerala and Tamil Nadu), Himalayan foothills (parts of Uttarakhand and Himachal Pradesh), Eastern Ghats (parts of Odisha and Chhattisgarh), Chota-Nagpur Plateau in Jharkhand, Central Plateau region (parts of Madhya Pradesh, Chhattisgarh and Maharashtra) besides sporadically in parts of Rajasthan (Aravalli Hills). However, its maximum diversity and distribution is in Western Ghats. The Konkan collections are much like cultivated cucumber but bitter with both truly wild and feral forms. It possesses a multiple fruiting and branching habit not present in the cultivated cucumber (Horst and Lower, 1978). It possesses useful characters such as prolific fruit bearing and high number of laterals (Bisht *et al.,* 2004). Polymorphism in *C. sativus* var. *hardwickii* is higher than in *C. sativus. C. melo* subsp. *agrestis* and *C. callosus* occur as sympatric populations in the Deccan Plateau. Overlapping distribution of *C. sativus* var. *hardwickii* with *C. silentvalleyi* in southern Western Ghats and with *C. indicus* in northern Western Ghats was observed. Species distribution maps based on geographic coordinates generated from herbarium locality information are given in Fig. 1.

Cucumis melo subsp. *agrestis* has wide distribution having many ecotypes (including some cultivated types) adapted to various ecological conditions prevailing in the country. The major distribution areas are Indo-Gangetic plains and Deccan Plateau and rain-shadow areas in the leeward side of Western Ghats. Highly saline tolerant forms growing in calcareous coral sands of Lakshadweep are unique entities. 'Kachuri', a semi-domesticate form of this with fruity flavour is common in arid Central India.

The two exotic introductions, cultivated *C. metuliferus* E. Mey. *ex* Naudin and naturalized *C. dipsaceus* Ehrenb. *ex* Spach have also been collected from peninsular India (Tamil Nadu and Karnataka), the former from farmer's field and the latter as an escape from cultivation. *C. metuliferus* fruits are used as dessert/ substitute for slicing cucumber in tribal area in Sikkim and Darjeeling. Distribution of wild species of *Cucumis* in India is given in Table 2.

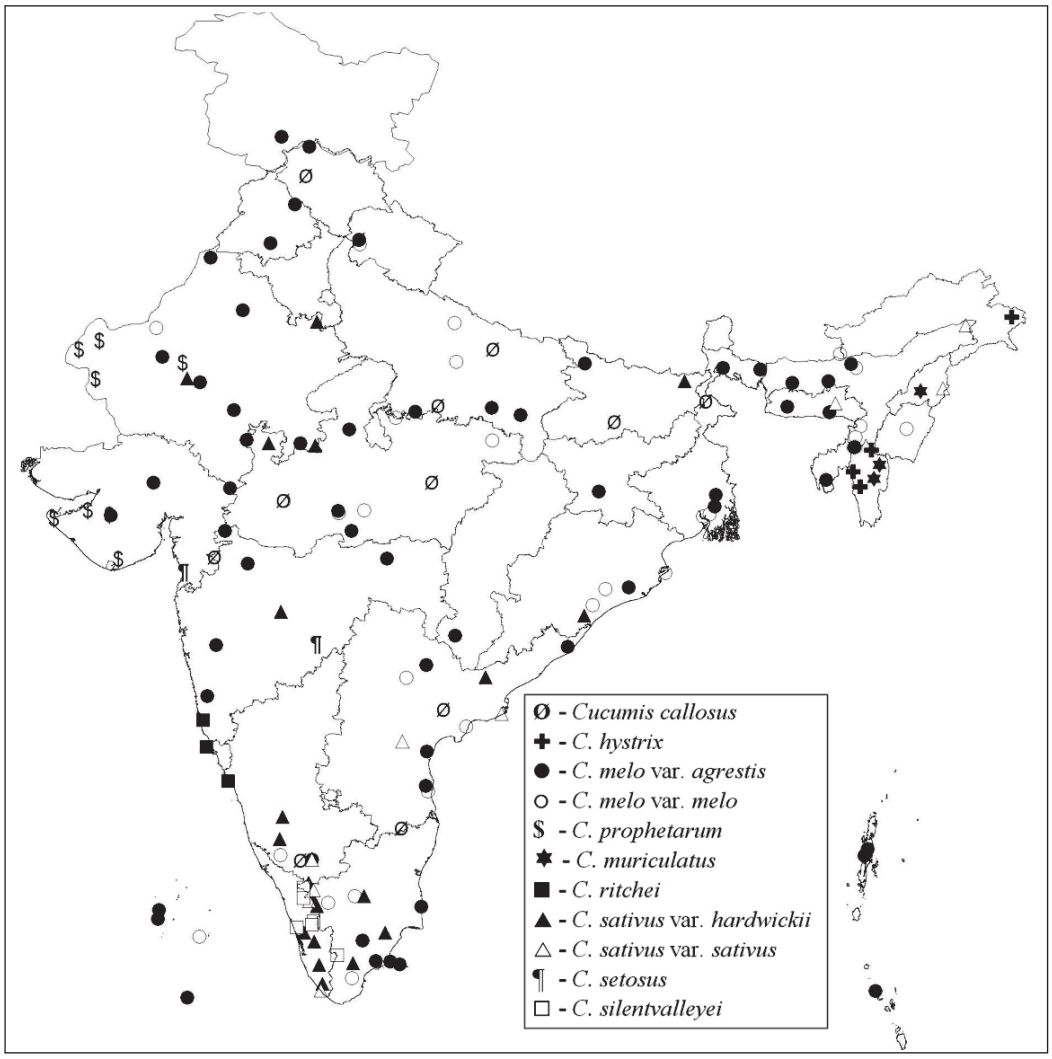

Fig 1. Distributional map of different *Cucumis* species of India based on herbarium records

Morphological Variability

This study has brought out areas of higher concentration of inter and intra-specific diversity in the genus (Table 2). It is difficult to distinguish between cultivated and wild types in case of *Cucumis melo* and *C. sativus* unless live plants are studied. Over-dependence on herbarium data and arriving at conclusions without studying live specimens should be avoided where crop wild relative gene exchange and overlapping distribution/occurrence are common. The difference may be insignificant such as variation in size, shape and colour of the fruit, size of the flower, etc. Smaller size of the fruits and seeds could be observed in the wild taxa of *C. melo* and *C. sativus*. Many workers consider *C. callosus* as the progenitor of melon (Sebastian *et al.,* 2010; Parthasarathy and Sambandam, 1989; Joseph John *et al.,* 2013) and *C. sativus* var. *hardwickii* as the progenitor of *C. sativus* (Nesom, 2011). Morphological variation in wild melon analogous to that in cultivated melon could be located from Eastern Ghats and Deccan Plateau. Some specimens from Lakshadweep and Andaman Islands were non-bitter ones which were with slender branches and profuse bearing. The number of fruits per plant varies from 300-400 in Nicobar collections of *C. melo* subsp. *agrestis*. IC539841, from Nicobar was characterized with miniature fruits that were non-bitter. The plants were highly branched and heavy bearing and adapted to coastal areas with high rainfall and low sunshine hours. Morphologically distinct and unique collection of this wild melon is consumable directly and offer scope for

genetic enhancement. Drought and salinity tolerance could be expected in collections from Lakshadweep which grow prolifically in coastal calcareous soils on beach. Both were found to be easily crossable with all types of cultivated melons and to produce viable fertile progeny (Bhat *et al*., 2014). These materials will be valuable for melon improvement in the context of climate change related abiotic stresses.

Cucumis melo types show considerable variation in leaf lobing, branching habit, size of flower and fruits, etc. and also adapted to various niches/region. Chakravarty (1982) states that leaf characteristics are important in classification of *Cucumis* taxa. Leaf-lobing varies with different entities of *Cucumis viz.* round unlobed (*C. melo* var. *melo*) slightly lobed (subsp. *agrestis*), deeply lobed (*C. callosus* and *C. prophetarum*) and variously angled (*C. sativus*, *C. indicus, C. setosus, C. hystrix* and *C. muriculatus*). Tuberous tap root is a modification for adaptation to extreme temperature stress in *C. callosus,* specimens of the same from Rajasthan state showed extreme drought tolerance, prolific flowering and fruiting under extreme water stress and were resistant to fruit fly. The ovary of *C. sativus*, *C. hystrix, C. muriculatus* and *C. prophetarum* are tubercled or muriculate while that of *C. melo*, *C. indicus*, *C. silentvalleyi* and *C. setosus* are variously pubescent. However, the nature of tubercles varies among the four species in the former case.

Accessions of *Cucumis sativus* from Andaman Islands and North East region were found to produce large number of fruits and showed tolerance to downy mildew. Long fruits ranging from 20-30 cm were observed in accessions IC 613458 and IC 595510 from Mizoram and Tripura respectively. Cucumber with yellow and orange flesh having high carotenoid content was collected from Tripura, Mizoram and Manipur. Ivory white salad cucumber is unique to Hassan district of Karnataka. Large, cooking type cucumber *C. melo* var. *conomon* landraces with high shelf life, namely 'Mulsouthe' and Doppasouthe' were located in coastal Karnataka midlands and also in Vindhyachal part of Madhya Pradesh. The wild taxa such as *C. indicus*, *C. silentvalleyi, C. setosus* and *C. hystrix* bear bitterless fruits and thus have a prospect for direct domestication; they might contribute genes for crop improvement programmes. High rainfall and low sunshine hour tolerance of *C. silentvalleyi* may be a useful trait for incorporation in melon cultivars, especially oriental pickling melon. Distribution and specific traits of melon and cucumber cultivars are given in Table 4.

Phenology

A perusal of phenological data compiled based on herbarium study, revealed a specified period for flowering and fruiting. The actual field study showed slight variation in various localities based on factors such as date of onset of monsoon, soil moisture content, day length, soil and topographic factors. Germination in all the wild species coincide with pre-monsoon rains which is normally in May-July in most part of the country except in Karnataka plains and parts of Deccan Plateau in Tamil Nadu, Andhra Pradesh and Telangana where North East monsoon in October is the normal time for seedling emergence. Seedling morphology is of importance in identifying and locating species for rehabilitation in simulated *in situ* habitats. However, there are no illustrated seedling keys to identify various *Cucumis* species in the juvenile stage. The phenological data is useful to *ex situ* conservationists to plan collection trips in different regions when maximum seed quantities are available. Phenological observations are given in the Table 3. No seed dispersal agents were found. Ripe fruits fall to the ground and disintegrate leaving a soil seed bank for next growth season.

Current Status of Taxonomy

Correct botanical identification of the studied germplasm is paramount to linking it to related information for utilization purpose. The authors consider *Mukia* and *Dicoelospermum* as separate genera and do not favour their merger within *Cucumis* barring *Cucumella*. *Cucumis* L. *sensu stricto* in India comprises only six species (Chakravarty, 1982). A morphological key for field identification of various *Cucumis* species coming under the general pool of cucumber and melon are given below.

Key Diagnostic Characters of Cucumis Species in India

- Prostrate habit, leaves broadly round, fruits smooth or pubescent, ovary softly hairy, not tuberculate, cultivated – *C. melo*
- Climbing, ovary hispidulous, young fruits tuberculate, leaves angular, cultivated – *C. sativus*
- Fruits setose, hairy, wild – *C. setosus*
- Fruits echinate/muriculate, prostrate, leaves round, fruits round, longitudinally white banded and with recurved aculei, wild – *C. prophetarum*

Table 2. Distribution of wild species of *Cucumis* in India

S. No.	Species	Indian distribution and areas of greater concentration
1	*C. callosus* (syn. *C. trigonus* Roxb.)	Andhra Pradesh, Telangana, Tamil Nadu, Karnataka (Deccan areas), Maharashtra, Rajasthan, Gujarat, Odisha, West Bengal, Jharkhand, Chhattisgarh, Uttarakhand, Uttar Pradesh and Madhya Pradesh. Areas of greater concentration: Coimbatore, Salem, Tirunelveli, Ramanathapuram, Chengalpet, Sivagangai and Rajapalayam in Tamil Nadu; Prakasam, Krishna, Chittoor and Nellore in Andhra Pradesh; Rangareddy, Medak and Hyderabad in Telangana; Bellary in Karnataka; Dhule, Thane and Pune in Maharashtra; Ganjam in Odisha; Ranigunj, Jalsuka and Sunderbans in West Bengal; Indore, Jabalpur and Umaria in Madhya Pradesh; Raipur in Chhattisgarh; Dehradun in Uttarakhand; Banda, Gonda, Agra, Bahraich and Saharanpur in Uttar Pradesh; Jodhpur in Rajasthan; Rajgir and Sahibganj in Bihar; Sutlej river bank in Punjab and Palamau and Ranchi in Jharkhand (Joseph John *et al*., 2013).
2	*C. melo* subsp. *agrestis* var. *agrestis*	Occasional as a weed of cultivated fields throughout the country except high rainfall areas of Western Ghats and North-eastern India. Areas of greater concentration: Minicoy and Kavaratti in Lakshadweep; North, South and middle Andaman in Andaman & Nicobar Islands; rain shadow areas of Attappadi in Kerala; Coimbatore, Ramanathapuram and Madurai in Tamil Nadu; Nellore, Prakasam, Krishna and Srikakulam in Andhra Pradesh; Warangal in Telangana; Mysuru in Karnataka; Dhule, Nagpur and Pune in Maharashtra; Churu, Chittaurgarh, Jodhpur, Jaisalmer, Sri Ganganagar and Bhilwara in Rajasthan; Hoshiarpur in Punjab; Doda in Jammu & Kashmir; Hamirpur in Himachal Pradesh; Bastar region in Chhattisgarh; Rewa in Madhya Pradesh; Allahabad, Lakhimpur-Kheri and Mirzapur in Uttar Pradesh; Jalpaiguri, Somra and Kolkata in West Bengal; Goalpara, Kamrup and Darrang in Assam; Dehradun in Uttarakhand and Lohit district in Arunachal Pradesh.
3	*C. melo* subsp. *melo* (cultivated - including varieties)	Cultivated throughout the country as vegetable and as table fruit. Infraspecific variability in cultivated melon is region specific: *C. melo* var. *melo*, *C. melo* var. *conomon*, *C. melo* var. *cantalupensis*, *C. melo* var. *momordica* and *C. melo* var. *utilissimus* are cultivated.
4	*C. sativus* var. *hardwickii*	Common in Western Ghats and occasional in Western Himalayas and rare in Deccan Plateau, Eastern Ghats and Khasi Hills. Areas of greater concentration: Hassan and Chikmagaluru in Karnataka; Alwar and Jhalawar in Rajasthan; Ernakulam, Idukki, Wayanad, Pathanamthitta and Palakkad in Kerala; Nilgiri, Coimbatore, Namakkal and Karaikudi in Tamil Nadu; East Godavari in Andhra Pradesh, Shahdol in Madhya Pradesh and terai region in Uttarakhand.
5	*C. setosus*	Maharashtra state and adjoining districts of the neighbouring states. Areas of greater concentration: Pune, Satara, Nasik, Kolhapur and Khandala in Maharashtra; Dang in Gujarat; Hoshangabad and Umaria in Madhya Pradesh and Belgaum in Karnataka.
6	*C. prophetarum*	Occasional in Rajasthan, Maharashtra, Gujarat, Delhi and bordering areas (as extended distribution from Africa through Arabia and West Asia and Sindh region of Pakistan). Areas of greater concentration: Jodhpur in Rajasthan.
7	*C. hystrix*	Rare in Brahmaputra Valley, occasional in Naga hills (Nagaland); Mishmi hills (Arunachal Pradesh) and Tura mountains (Meghalaya); Lohit district (Arunachal Pradesh); Dampa Wild Life Sanctuary, Champhai, Kolazib and Aizwal districts of Mizoram; Changlang in Arunachal Pradesh and Churachandpur and Jessami (Ukhrul) in Manipur with possible occurrence elsewhere in North-Eastern Hill states.
8	*C. muriculatus*	Very rare in Champhai, Mamit and Kolasib districts of Mizoram and Tuensang district of Nagaland.
9	*C. silentvalleyi*	Endemic to southern Western Ghats of Kerala and Tamil Nadu (Sholayar, Silent Valley, Nelliyampathy, Valparai) on moist rocky crevices. As ephemeral in rainy season, grows on humus rich rocky crevices, near dripping water source. Areas of greater concentration: Kollathirumedu, Anakkayam and Malakkappara Ranges in Sholayar forest, grasslands near Sholayar Dam site (Coimbatore District, Tamil Nadu), Periyar Tiger Reserve in Idukki, Vellarimala, Malabar Wildlife Sanctuary, Wayanad Wildlife Sanctuary, Moozhiyar and Gavi in Pathanamthitta and grasslands in Silent Valley National Park.
10	*C. indicus*	Amboli Ghat (type locality), at two kilometre stretch in Sindhudurg district of Maharashtra and also in Dandeli Wildlife Sanctuary at 250 m near Karwar, Jog Falls and Devarayii in Belgaum district, Karnataka.

- Fruits ovate-oblong with mamillate protuberances, calyx not recurved, climbing, leaves angular, stem scabrid, wild – *C. hystrix*
- Fruits turbinate with hyaline appendages, calyx recurved, climbing, leaves angular, stems soft hairy (brown), wild – *C. muriculatus*
- Retrorse hairs on ovary and fruit; ephemerals – *C. silentvalleyii*
- Antrorse hairs on ovary and fruit; fruit snout like at both ends, ephemerals – *C. indicus*

Further, taxonomy of various cultivar groups of *melo* has been found confusing for which also based on analysis of Indian specimens a key is provided. Cultivated melon is an exceptionally and highly diverse group with at least five cultivar subgroups in India, each with specific morphological characters, typical use and geographic localization. The cultivated melons fall under two subspecies, *C. melo* subsp. *melo* and *C. melo* subsp. *agrestis*. The subsp. *agrestis* comprises var. *conomon* (oriental pickling melon) and var. *momordica* (snap melon) and the subsp. *melo* comprises var. *flexuosus* (snake melon), var. *reticulatus* (netted melon) and var. *maltensis* (Sarda melon).

Morphological Key to the Subspecies of Cucumis melo

- Ovary pilose to villose, hairs long – subsp. *melo*
- Ovary with retrorse or antrorse appressed short hairs – subsp. *agrestis*

Key to the Cultivated Varieties of Cucumis melo subsp. *melo*

- 1a Fruits elongated (snake like), non-sweet, slender, curved, eaten raw (salad) – *flexuosus*
- 1b Fruits globose or oblong-elliptic – 2
- 2a Netted or with longitudinal bands, flesh mildly (melting) sweet, soft, aromatic – *reticulatus*
- 2b Fruits of monocolour, flesh firm, white, mildly sweet – *maltensis*

Key to the Varieties of Cucumis melo subsp. *agrestis*

- 1a Wild or feral, small fruits, inedible or edible – var. *agrestis*
- 1b Cultivated, sweet or non-sweet, non-bitter, edible – 2
- 2a Fruits 20-30×10-15 cm, cylindrical-oval, skin cracks on ripening, flesh granular, mealy, sweet,

Table 3. Phenological traits of various *Cucumis* species helpful in planning exploration

S. No.	Species	Phenology	Best time for collection/seed maturity and locations
1	*C. melo* subsp. *agrestis*	Seedlings emerge with pre-monsoon showers and come to flowering and fruiting within 30 days.	October-December Namakkal (Tamil Nadu), Malacca (Nicobar), Vindhya hills (Madhya Pradesh). May-June Eastern Ghats, Telangana, Andhra Pradesh
2	*C. callosus*	Seedlings emerge with pre-monsoon showers and come to flowering and fruiting within 60-90 days.	September-October (Tamil Nadu plains) October-November (central and western India)
3	*C. melo* subsp. *melo* (cultivated- including varieties)	Germination with monsoon rains.	September-October April-May (var. *conomon* in Kerala) October (Brahmaputra Valley) (*C. melo* var. *acidulus*) April (Andhra Pradesh, Telangana) Snap melon (Goa-Karnataka) - harvested in October. Snake melon (Tamil Nadu) - harvested in March.
4	*C. sativus* var. *hardwickii*	Germination with pre monsoon showers. Flowering in August-September and senescence by December-January.	October-December (Western Ghats) October (Uttarakhand) November (Meghalaya)
5	*C. setosus*	Germination with monsoon rains. Flowering and fruiting in September-October.	October-November (Maharashtra and Madhya Pradesh)
6	*C. prophetarum*	Germination with monsoon rains. Flowering and fruiting in September-October.	September-October (Jodhpur)
7	*C. hystrix*	Germination with pre monsoon rains. Flowering in July-August.	Late October-November (Mizoram), November-December (Manipur, Arunachal Pradesh)
8	*C. muriculatus*	Germination with pre monsoon rains. Flowering in July-August.	November-December (Mizoram)
9	*C. silentvalleyi*	Germination with pre-monsoon showers and flowering and fruiting in July-September (rarely extending to October).	August-October (southern Western Ghats)
10	*C. indicus*	Germination with pre-monsoon showers and flowering and fruiting in July-September (rarely extending to October).	Mid October-November (northern Western Ghats, Konkan)

Table 4. Diversity of cultivated taxa of C*ucumis* in India

S.No.	Common name; Taxa/ Botanical identity	No. of accessions studied	Useful traits	Distribution	Remarks
1	Oriental pickling melon; *C. melo* subsp. *agrestis* var. *conomon*	12	Long fruits, long shelf life	West coast, Kerala; Kanyakumari (Tamil Nadu); Dakshina Kannada (Karnataka); Andhra Pradesh; Dibrugarh (Assam); Lohit (Arunachal Pradesh)	Cooking types 'Dosakka' (Andhra Pradesh) 'Kanivellari' (Kerala) 'Kallanvellari' (South Kerala) 'Ponavellari' (North Malabar)
2	Snap melon; *C. melo* subsp. *agrestis* var. *momordica*	5	Dessert/ summer fruit, refreshing juice, mild sweet taste, carotenoid rich forms, skin cracking during fruit maturity is a typical phenomenon	Goa, Tamil Nadu, Mizoram, Madhya Pradesh, Punjab, Kerala, Tripura, Odisha, West Bengal	'Pottuvellari' (Kerala) 'Phoot' (North India)
3	Musk melon; *C. melo* var. *reticulatus*	5	Dessert, sweet taste, refreshing juice. Thick netted appearance on the fruit and long shelf life.	Uttarakhand, Punjab, Indo-Gangetic plains	'Karbhuj'
4	Winter melon; *C. melo* var. *maltensis*	1	Firm flesh, white, high shelf life, non-cracking skin	North West plains	Zarda
5	Snake melon; *C. melo* var. *flexuosus*	2	Salad, tender fruit for thirst quenching	Rajasthan, Indo-Gangetic plains, Tamil Nadu	Upto 90 cm long fruit
6	'Kachur'/ 'Kachri'; *C. melo* subsp. *agrestis* var. *agrestis* (semi-domesticated)	1	Fresh fruit aromatic	Rajasthan	Bitter and non-bitter (ripe stage) forms
7	'Kheera'/ 'Kakri'/ cucumber; *C. sativus* var. *sativus*	35	Fresh tender fruits used as salad, mature ripe fruits used as vegetable. Long shelf life, fruit weight 1- 6 kg	Andaman, Karnataka, Goa	Carotenoid rich forms collected from Manipur, Tripura and Mizoram, ivory coloured forms from Hassan, Karnataka (Hassan Local) and mildly sweet, good table types from West Bengal

aromatic, soft and melting on ripening, highly perishable – var. *momordica*

- 2b Fruits 15-20×10-12 cm, oblong-ovate, non-sweet, firm white flesh and intact skin, extra long shelf life – var. *conomon*

Adaptability and Habitat Preference

Cucumis silentvalleyii is highly adapted to specific habitats where humus accumulates in shallow soils combined with dripping water source areas during South West monsoon season (Joseph John *et al.,* 2015). *C. sativus* var. *hardwickii* exhibits a clear cut preference for high rainfall and high humidity regions with big fruited forms in eastern Madhya Pradesh and Khasi Hills and small fruited forms in Himachal Pradesh and Uttarakhand. However, all wild cucumbers are bitter and inedible. On the contrary, *C. melo* subsp. *agrestis* var. *agrestis* occurs more in field boundaries, also as weed in field, does not prefer high rainfall prevailing in Western Ghats and thus are mutually exclusive in distribution.

Conclusion

Lack of information on a taxon's precise distribution in different ecosystems is a major constraint to collection and conservation (Arora, 1998). The mega-diversity hotspots in the Indian region – Western Ghats and Eastern Himalayas – represent two mutually exclusive pockets of diversity for these taxa. The findings of the eco-geographical analysis give a clear cut picture of areas of distribution, diversity richness, infraspecific variability and phenology. The distribution maps give a holistic picture of the distribution of component taxa, areas of overlapping distribution and higher concentration that need to be targeted for maximum assemblage of genetic diversity using which a prospective collector can have access to the exact site (location). Comparing the gene bank passport data with distribution map helps to ascertain

area-wise gap in germplasm collection sites. Analysis of species distribution maps based on herbarium survey and locality data of collections currently maintained in Medium Term Storage (MTS) reveal the need for more intensive exploration in diversity rich pockets. Tribal areas in the whole of North East India and Uttara Kannada district for cooking type *C. sativus*; Tamil Nadu and Uttar Pradesh plains for *C. melo* var. *flexuosus* (snake melon); Mizoram, Nagaland and Manipur for *C. hystrix* and *C. muriculatus* are some suggested areas for exploration in the near future. A good representation of diversity in cultivated melon and cucumber has been assembled from Mizoram, Tripura, Nagaland, Manipur, Sikkim-Darjeeling, Meghalaya, Kerala, Karnataka, Goa, Andaman and Lakshadweep. However, other species and areas need extensive coverage. *C. setosus, C. hystrix, C. muriculatus, C. silentvalleyii* and *C. indicus* which are niche specific and endemic, needs to be specially targeted for *ex situ* conservation. *C. silentvalleyii* germplasm is difficult to gather in sufficient quantity due to small clutch size, predation and quick seed dispersal on ripening. Same is the case with *C. indicus,* due to its fragile habit and niche specificity. With the information generated here on rarity, crossability relationship and actual genebank status, prioritization of species for conservation can be planned. A perusal of the long-term storage status of *Cucumis* in the National Genebank indicates that out of the total 1,881 accessions, cultivated melon (1,024) and cucumber (422) together accounts for 76.87% of the holding. Out of the remaining 435 wild accessions (23.13%), 212 are of *C. callosus* (11.27%), 156 are of *C. sativus* var. *harwickii* (8.29%) and 21 are of *C. prophetarum* (1.12%). Endemic and rare taxa like *C. hystrix* (2), *C. muriculatus* (1), *C. setosus* (1) and *C. silentvalleyii* (1) are only meagrely represented. Exotic species like *C. metuliferus* (1) and *C. dipsaceus* (1) and the species falling under tertiary gene pool such as *Mukia javanicus* (2), *M. leiosperma* (2) and *M. maderaspatana* (8) are also under-represented (Source: PGR Portal). Being orthodox and without any apparent issues on cold storage and retrieval of viable plants, *ex situ* conservation of all species is not a problem once sufficient seeds are harvested either directly at source from wild habitats or regenerated *ex situ* in simulated conditions.

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Developing Mini Core of Rice Germplasm for Submergence Tolerance

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We report here development of a mini-core subset of submergence tolerant rice genotypes containing 21 accessions representing the genetic diversity derived from 5716 accessions collected from flood-prone rice growing areas. The mini-core was developed using PowerCore software on the basis of five quantitative and one qualitative trait. The resultant mini-core had 12.52% of mean difference (MD%), 68.13% of variance difference (VD%), 168.38% of variable rate (VR%), and 98.21% of coincidence rate (CR%) with the core collection, which brought about full coverage of 6 traits. The diversity of mini-core and entire set estimated by Nei index was 0.676 and 0.536 respectively. Cluster analysis was done using UPGMA method. These 21 accessions were classified into four clusters containing 12, 5, 3 and 1 accession in clusters 1, 2, 3 and 4, respectively. In nutshell, the mini-core developed in this study is a representative subset of the rice germplasm collected from different parts of rainfed lowlands of India.

Key Words: Mini-core subset, Power Core, Rice (*Oryza sativa* L.), SAS, Submergence tolerance

Introduction

Genetic resources enable plant breeders to create novel gene combinations and select crop varieties that are more suited to the needs of diverse agricultural systems. Geographically, rice is being grown in lands as far as 50 ^{0}N (Aiwei, China) to 30 ^{0}S (New South Wales, Australia). It is grown at altitudes ranging from below sea level (Kerala, India) to 2761 m above the sea level (Jumulla valley, Nepal), thus making its presence in all the environments and all continents of the world (Chang, 2000). The adaptation to extremely variable conditions in rice offers a hope to combat the current challenges imposed by variable abiotic stresses, as well as means to cope with the adverse effects of climate change, to secure food and livelihood. This is because genes associated with tolerance to various abiotic stresses are probably available within the cultivated gene pool, offering considerable opportunities for genetic improvement. The approach involving identification of tolerant germplasm and associated QTLs /genes has paid rich dividends in developing stress-tolerant high-yielding cultivars for commercial utilization (Xu *et al*., 2006; Sarkar *et al*., 2009; Singh *et al*., 2009; Septiningsih *et al*., 2009). Since the introduction over fifty years ago, the adoption of high-yielding semi-dwarf rice cultivars in unfavourable lowlands and coastal areas has not been successful. Apparently, adoption of these improved rice varieties in favourable irrigated areas could make significant impact on yield improvement, but with limited success in unfavourable ecosystems, as in coastal belt of water logging and submergence prone areas. Developing rice varieties with wider adaptation and broader tolerance to prevailing stresses would be more viable for these areas, where abiotic stresses are variable and complex, and growing conditions are too risky to persuade farmers for investment in inputs (Singh *et al*., 2010). Insertion of genes from alien species could also improve the tolerance to stresses, however, identification of specific genotypes within the cultivated gene pool and their utilization in breeding through conventional or biotechnological approaches still remain the most trusted and successful approaches to date.

A wealth of plant germplasm is accessible worldwide, with about 6 million accessions held in over 1400 gene banks. Yet, these collections are barely tapped (<1%) by breeders, owing to the scarcity of information on their characterization, other than the taxonomic status and geographical origin (Glaszmann *et al*., 2010). Together more than one lakh germplasm lines of rice are available in the National and International rice gene banks, of which more than 30,000 lines are available at the ICAR-National Rice Research Institute, Cuttack. The vast germplasm collections are accessible, but their use in crop improvement programme is limited, efficiently accessing genetic diversity is still a challenge. The complete and high-quality sequence of the rice

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genome has thrown light on population dynamics and the impact of selection during domestication. Germplasm characterization has now gained analytical power for resolving the genetic basis of trait variation, diversity patterns, and their adaptations in a definite or variable environment. Although many rice germplasm accessions have been collected and conserved in the Indian gene bank, the utilization, characterization and management of these resources have not kept pace due to the recurring problem of duplication among accessions, which results in a high rate of genetic redundancy. To overcome this problem, Frankel (1984) suggested sampling of the collection to a manageable sample or 'core collection'. A core collection contains a subset of accessions from large collections that captures most of available diversity of species (Brown, 1989a; 1989b). Core sets are derived from the wide spectrum of genetic diversity of the whole collection, most of this diversity is expected to be retained. Extensive evaluation of the core set leads us to guide more efficient utilization of the entire collection (Brown, 1989b). Core sets have been made for different crops including rice. However, to make a core set taking germplasm based on their sensitivity of a specific abiotic stress is hitherto scanty. Therefore, the objective of this study was to develop a core set of rice germplasm accessions sensitive to submergence using data on both qualitative and quantitative traits.

Materials and Methods

Collection of Rice Germplasm

In almost all the countries of the South and Southeast Asia, rice productivity in rainfed lowlands is generally low compared to the favourable ecosystems (Sarkar *et al*., 2009). Farmers predominantly grow local tall, photoperiod sensitive rice genotypes in medium-deep and deepwater sub-ecosystems. High yielding semi-dwarf cultivars are rarely grown in these areas.

Multiple surveys and germplasm collection activities were undertaken at different years along the coastal and rainfed areas of the India that are prone to salt stress and flooding. The areas such as West Bengal, Bihar, Odisha and Assam are frequently encountered by excess water stress from the mighty rivers, the Ganges, Koshi and the Bramhaputra respectively. Thus in the present study, all these old collections conserved in the rice gene bank were used for screening experiment. Besides, some fixed breeding materials developed by International Rice Research Institute (IRRI), Philippines and the SAUs located in eastern India were also included.

Screening for Submergence

As we know that quality of floodwater influences the survival of plants, hence to screen the cultivars we focused on the susceptible cultivars and depending on the quality of floodwater, an assumption was made so that mortality of the susceptible check scores nearer to 100%. When the floodwater was clear, 8 days after complete submergence, we checked the susceptible cultivars. Extreme yellowing of leaves and softening of base was a harbinger of plant death and on that basis we took decision about the total days of submergence. Under clear water, in general, we gave submergence stress for 10-15 days depending upon the condition of susceptible check.

Plants were established using direct seeding in a field tank (40 m × 8 m × 0.8 m) in lines that were 20 cm apart and with 15 cm between hills. Chemical fertilizers were added as N:P:K at 0:30:30 kg/ha, respectively. Twenty-one-day old seedlings were completely submerged under 70-80 cm of water for 10-15 days. Plant height was taken before and after submergence to determine the elongation ability to identify which accession is suitable for flash flood versus stagnant water conditions. Finally, the number of survivors was counted after 10 days of de-submergence and percentage survival was determined as:

Survival (%) = (Number of hills after 10 days of de-submergence / number of hills before submergence) × 100 (Sarkar and Bhattacharjee, 2011).

Data Collection and Trait Evaluation

Data of six phenotypic traits were collected for all the 5716 rice genotypes. Out of these, five were quantitative and one was qualitative trait. The phenotypic traits include plant height before submergence, plant height after submergence, elongation, elongation %, survival % and submergence-type. The plant height before submergence and after submergence was recorded maintaining twenty-one-day old seedlings which were completely submerged under 70-80 cm of water for 10-15 days. Elongation was calculated as the difference between plant height after submergence and plant height before submergence. Submergence-type was divided into four categories: tolerant, medium tolerant, avoiding and susceptible. Survival due to complete submergence

depends on either quiescence or escape strategies (Sarkar and Bhattacharjee, 2011). Therefore, the plant which avoids the complete submergence for survival through greater elongation was termed as 'avoiding type' whereas the tolerant type restricts the elongation, remains inside the water and still survives under such situation. Survival percentage of 80 and above was considered as 'tolerant type' whereas above 50% to below 80% was considered as 'moderately tolerant'. Elongation and elongation % were calculated as follows:

Elongation = Plant height after submergence - Plant height before submergence

Elongation% = (Elongation / Plant height before submergence) × 100 (Sarkar and Bhattacharjee, 2011).

Sampling Strategy and Data Analysis

Sampling the core collection was performed by the PowerCore software described by Kim *et al*. (2007). These variables were automatically classified into different categories or classes by the PowerCore programme based on Sturges' rule = 1 + Log2(*n*), where *n* is the number of observed accessions (Kim *et al*., 2007). Five genotypes namely FR 13A, Sabita, IR 42, Swarna and Swarna-Sub1 were preferentially included in the mini-core without validation using PowerCore because these are the check varieties.

The frequency distribution between entire set and core set for all the six traits were evaluated by the χ^2 test. The resulting mini-core was compared with the original core collection to assess its homogeneity. Nei genetic diversity index (Nei, 1972) was estimated for both the core and mini-core collections. Homogeneity was evaluated for the six phenotypic traits using the Newman-Keuls test for means, the Levene test (Levene, 1960) for variances, and the mean difference (MD%), variance difference (VD%), coincidence rate of range (CR%), and variable rate of coefficient of variance (VR%) according to Hu *et al*. (2000).

$$MD(\%) = \frac{1}{m}\sum_{j=1}^{m}\frac{|M_e - M_c|}{M_c} \times 100$$

where M_e is the mean of entire collection and M_c is the mean of core collection.

$$VD(\%) = \frac{1}{m}\sum_{j=1}^{m}\frac{|V_e - V_c|}{V_c} \times 100$$

where V_e is the variance of entire collection and V_c is the variance of core collection.

$$CR(\%) = \frac{1}{m}\sum_{j=1}^{m}\frac{R_c}{R_e} \times 100$$

where R_e is the range of entire collection and R_c is the range of core collection.

$$VR(\%) = \frac{1}{m}\sum_{j=1}^{m}\frac{CV_c}{CV_e} \times 100$$

where CV_e is the coefficient of variation of entire collection and CV_c is the coefficient of variation of core collection.

Coverage of all the phenotypic traits in the original core collection was estimated in the mini-core as proposed by Kim *et al*. (2007):

$$Coverage(\%) = \frac{1}{m}\sum_{j=1}^{m}\frac{D_c}{D_e} \times 100$$

where *D*c is the number of classes occupied in the minicore subset, *D*e is the number of classes occupied in the original core collection for each trait, and *m* is the number of traits, which is 6 in this case.

The cluster analysis was performed over the submergence traits using the un-weighted pair group method of arithmetic means (UPGMA) method.

Results

A total of 5716 accessions of rice germplasm were tested for submergence tolerance. Based on 6 traits, the heuristic search identifies 21 accessions as core set (Table 1).

Frequency Distribution of Submergence Traits

The frequency distribution for all the submergence traits between entire set and core set were tested using χ^2 tests. The χ^2 probability for distribution were non significant for PH(BS), PH(AS), elongation, elongation (%), and survival (%) but significant for submergence-type (Table 2) between entire set and core set. This indicates representative similarity between entire set and core set except for submergence type trait. The graphical distribution analyses of entire set for five quantitative traits were shown in Figure 1 (a-e).

Table 1. The minicore subset of 21 accessions along with their name and six traits developed using PowerCore software

Name	Source	PH (BS)	PH (AS)	Elongation	E (%)	Survival (%)	Submergence type
FR 13A	Odisha	49	65	16	32	93	Tolerant
Sabita	West Bengal	45	106.2	61.2	136	81	Avoiding
IR 42	IRRI, Philippines	38	72	34	89	0	Susceptible
SWARNA	Maruteru, Andhra Pradesh	33	68	35	106	20	Susceptible
SWARNA-SUB1	IRRI, Philippines	30	43	13	39	70	Medium Tolerant
AC-42238 (Kalam)	West Bengal	14	56	42	300	62	Medium Tolerant
AC-1786	Odisha	16	95	79	494	84	Avoiding
AC-42107 (Mugakatia); IC 575291	Odisha	25	84	59	236	16	Susceptible
Baikoili	Odisha	65	117	52	80	95	Avoiding
AC-975	Odisha	19	102	83	437	41	Susceptible
IR 84645-2-11-71-B	IRRI, Philippines	56	56	0	0	50	Susceptible
AC-42108 (Kankada Bichha); IC 575292	Odisha	19	87	68	358	34	Susceptible
Ranjei	Odisha	67	122	55	83	80	Avoiding
IRGC - 45059	IRRI, Philippines	47	75	28	60	100	Tolerant
RAU - 1415-12-7-6-4-3-8	Bihar	5	58	53	1060	7	Susceptible
IR64-SUB1	IRRI, Philippines	25	33	9	36	78	Medium Tolerant
AC-42268 (Bajal), IC 57544, JRS-215	West Bengal	34	126	92	271	65	Avoiding
AC-42103 (Gitanjali),IC 575287	West Bengal	37	109	72	195	118	Avoiding
AC-653	Odisha	12	82	70	583	53	Susceptible
AC-37983, Badshabhog	Chhattisgarh	32	0	0	0	25	Susceptible
AC-1303B	Odisha	76	101	25	33	65	Avoiding

PH - Plant Height, BS - Before Submergence, AS - After Submergence, E - Elongation,
IRRI - International Rice Research Institute

Table 2. Comparison of frequency distribution for 6 traits between entire set and core set of rice

Traits	Df	$\chi 2$ value	Probability value
PH(BS)	1	0.6350	0.4255
PH(AS)	1	0.0636	0.8009
Elongation	1	0.1303	0.7181
Elongation (%)	1	0.9666	0.3255
Survival (%)	1	3.4994	0.0614
Submergence type	1	4.1188	0.0424

Df - Degrees of freedom

Comparison of Mean and Variance

Comparative values for the ranges, means, and variances of 6 phenotypic traits among the entire set and mini-core subset are presented in Table 3 and demonstrate that the mini-core subset covers the range of variation for each trait. The equality of mean and homogeneity of variance between entire set and mini-core was analyzed using SAS 9.2 software. The Newman-Keuls test results indicate the presence of homogeneity of means between the core collection and mini-core subset for 2 (33%) traits (PHAS and elongation) out of the 6 traits analyzed. Two (33%) traits (survival % and submergence-type) had homogeneous variances between the two collections as revealed by the Levene's test.

The MD%, the VD%, the CR%, and the VR% are designed to comparably evaluate the property of core collection with its initial collection. Over the entire 6 phenotypic traits, the MD% was 12.52%, far less than the significance level of 20% (Hu *et al*., 2000) between the entire set of 5716 and the mini-core set of 21 accessions selected by the PowerCore search. The VD% was 68.13%, far more than the significance level of 20% between the two collections, as four out of six traits are significant variance between entire set and the mini-core set (Table 3). The VR% compares the coefficient of variation values of the 6 phenotypic traits measured in the core collection with the mini-core subset in general and determines how well the variance is being represented in the mini-core. More than 100% of VR% is required for a core collection to be representative of its original collection (Hu *et al*., 2000). The mini-core had 168.38 VR% over its originating core, which is much more than the required VR%, indicating good

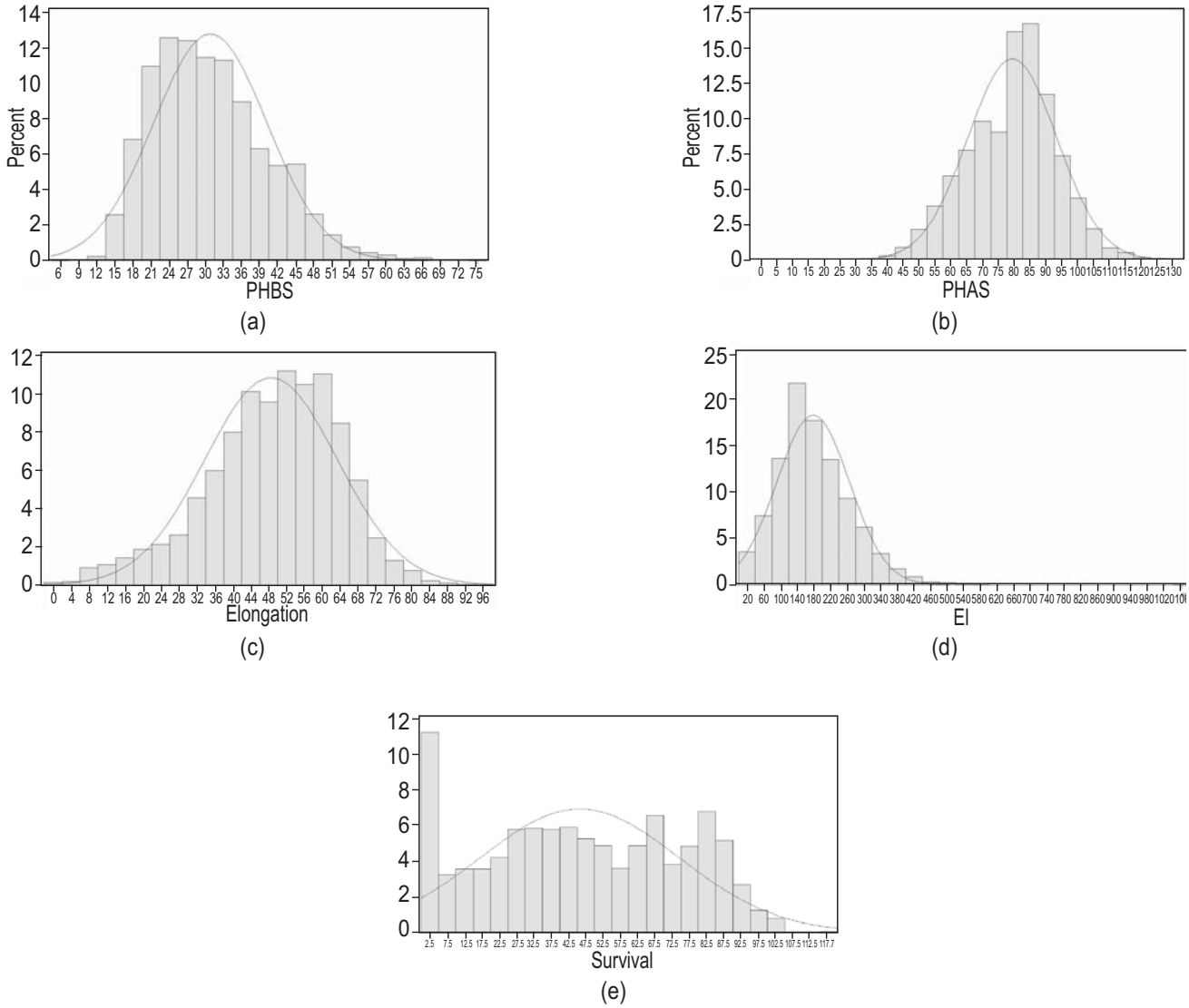

Fig. 1. Distribution analysis of five traits (a) Plant height before submergence (b) Plant height after submergence (c) Elongation (d) Elongation % and (e) Survival %

Table 3. Comparison of range, mean, and variance between the entire set of rice (*Oryza sativa*) collection and the mini-core for 6 phenotypic traits

Traits	Entire set			Core set			Tests	
	Range	Mean	Variance	Range	Mean	Variance	N-K	Lev
PH(BS)	5-75.68	30.82	88.45	5-76	35.38	372.44	**	*
PH(AS)	0-131	79.31	197.11	0-126	78.91	999.18	NS	*
Elongation	0-97	48.54	217.18	0-92	45.05	774.53	NS	*
Elongation (%)	0-1060	178.42	7587.29	0-1060	220.33	66165.97	**	*
Survival (%)	0-118	45.75	823.05	0-118	58.94	1074.29	**	NS
Submergence type	1-4	1.51	0.52	1-4	1.90	0.99	**	NS

PH - Plant Height, BS - Before Submergence, AS - After Submergence
*- significant at 1%, NS - Non significant, ** - significant at 5%

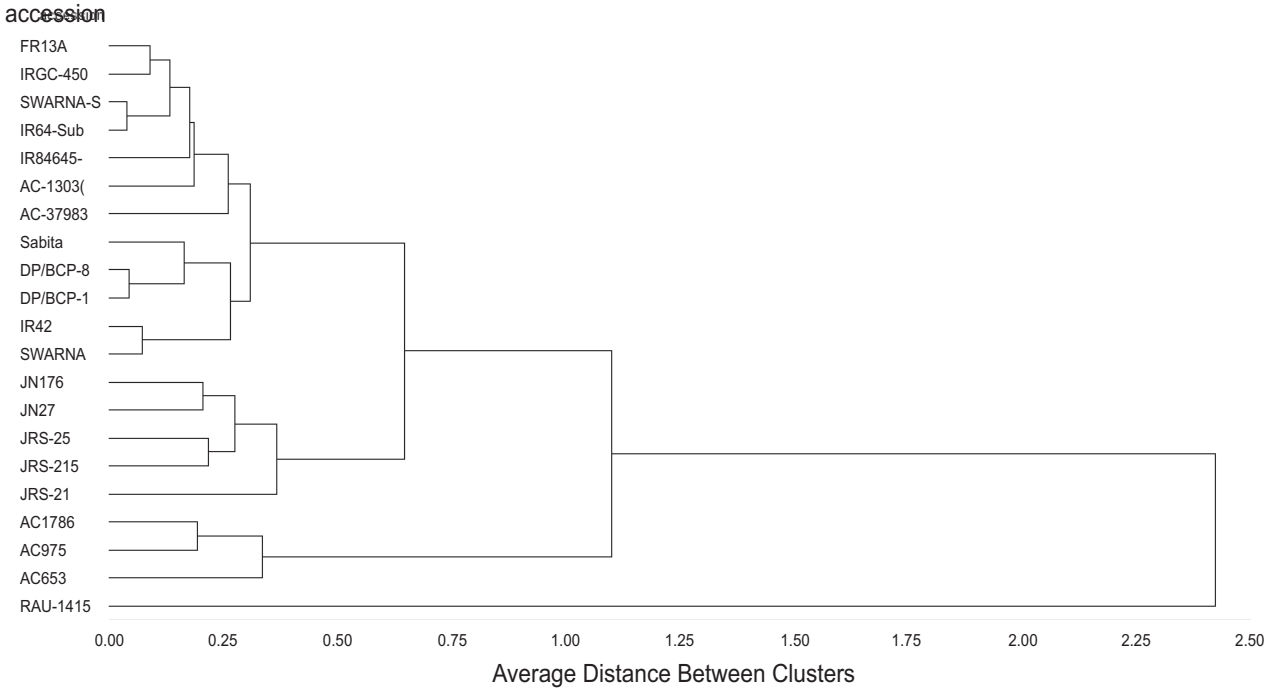

Fig. 2. Dendrogram of 21 selected accessions using UPGMA method

representation. The coincidence rate indicates whether the distribution ranges of each trait in the mini-core subset are well represented when compared to the core collection. The resulting CR% over the 6 traits was 98.21%, indicating homogeneous distribution of the phenotypic traits because it was larger than 80% (Kim *et al*., 2007). The calculated coverage value for the resulting mini-core was 100%, suggesting there is full coverage of all the diversity present in each class of phenotypic traits in the entire set of rice collection.

Cluster Analysis

A cluster analysis was performed using UPGMA method resulted into four clusters. Twelve accessions (FR 13A, IRGC-45059, Swarna-Sub 1, IR64-Sub1, IR 84645-2-11-71-B, AC-1303B, AC-37983, Sabita, Baikoili, Ranjei, IR 42 and Swarna) were grouped together in Cluster 1. Cluster 2 contains five accessions (Kalam, Kankada Bichha, Mugakatia, Bajal and Gitanjali). Three accessions (AC-1786, AC-975 and AC-653) grouped to form cluster 3 and cluster 4 contains only single accession RAU - 1415-12-7-6-4-3-8 (Figure 2).

Discussion

Phenotypic Property of the Mini-core Set

We developed a mini-core subset containing 21 entries of its parental population using a strategy based on heuristic searches. The MD% between the mini-core and core collections for 6 phenotypic traits (12.53%) was higher than the core collections developed by Kim *et al*. (2007), but it is within significance level of 20%. The VD% of the mini-core (68.13%) was higher than that of the reported studies for each of 10 test runs in rice (Kim *et al*., 2007) and 9 test runs in cotton (*Gossypium hirsutum* L.) (Hu *et al*., 2000). The VR% of the mini-core (187.38%) was larger than the test runs in Kim *et al*. (2007) and Hu *et al*. (2000), and the CR% of traits in the mini-core (99.21%) was larger than nine out of 10 test runs in Kim *et al*. (2007) and six of nine test runs in Hu et al. (2000). Core collections with low MD% and VD% and large VR% and CR% are considered to provide a good representation of the genetic diversity of the initial collection (Hu *et al*., 2000; Kim *et al*., 2007). Furthermore, the presented mini-core displayed 100% coverage for 6 phenotypic traits, demonstrating that the PowerCore software is effective in maintaining the diversity present in each class of the traits in the entire set of rice collection. In contrast, Brown (1989a; b) used several statistical models to suggest that at least 70% of the variation in an entire collection could be represented in a core composed of at least 10% of accessions. Therefore, this mini-core should be considered as a sound representation of rice genetic diversity found in the entire set of rice collection.

The diversity estimated by the Nei index (Nei, 1972) of the mini-core subset (0.676) was greater than the original core collection (0.536), as would be expected when genetically similar accessions were removed from the original core. The diversity existing in the mini-core is larger than that in rice landrace populations in Yunnan, China (Zeng *et al*., 2007), Indonesian rice populations (Thomson *et al*., 2007).

Core collections are subsets of the main collections, comprising selective accessions that represent most of the genetic variability contained in the entire collection. The main collection can be stratified into groups (subsets) sharing common characteristics according to taxonomy, geographic or ecological origin, and descriptors followed by sampling within these groups. A core subset provides in a way an entry point to the entire collection for accelerating the utilization of germplasm and should not be looked upon as a substitute of the latter. By maximizing the diversity studied in a reduced number of individuals through the use of core collections, the probability of identifying variants of interest for association studies involving complex traits is increased. Furthermore, the knowledge gained through the core collections allows the choice of optimal crosses for generating quantitative trait loci (QTL) mapping populations.

Cluster Analysis

The mini-core of 21 accession includes 9 susceptible, 7 avoiding, 3 medium tolerant and 2 tolerant accessions. Out of these, cluster 1 contains 2 tolerant and medium tolerant each; 4 avoiding and susceptible each. Cluster 2 includes 2 avoiding and susceptible each and 1 medium tolerant accession. Cluster 3 contains 2 susceptible and 1 avoiding accessions; and cluster 1 includes 1 susceptible accession. Thus, cluster 1 contains 100% of the tolerant, 57% avoiding, 45% susceptible and 67% medium tolerant accessions. Cluster 2 includes 29% avoiding, 22% susceptible and 33% medium tolerant accessions. 14% avoiding and 22% susceptible accessions involved in Cluster 3; and cluster 4 contains 11% accessions of the mini-core set.

The mini-core contains accessions from IRRI, Philippines and five states Odisha, West Bengal, Bihar, Andhra Pradesh, Chhattisgarh of India. These contribute 23%, 43%, 19%, 5%, 5% and 5% from IRRI, Odisha, West Bengal, Bihar, Andhra Pradesh and Chhattisgarh respectively. Cluster 1 includes 80%, 26%, 25% 100% and 100% accessions form IRRI, Odisha, West Bengal, Andhra Pradesh and Chhattisgarh respectively. Cluster 2 contains 22% Odisha and 75% West Bengal accessions; cluster 3 includes 20% IRRI and 22% Odisha accessions while cluster 4 contains 100% accession from Bihar.

Conclusion

The rice germplasm collection of **N**RRI consists of more than 5716 accessions, which were collected from different regions of the country and during different time periods. This study was aimed to establish a smaller set based on collections made during different time period. Enough phenotypic data is not available on all the 5716 accessions for use to construct core/mini core at one go. Hence, the only strategy is to develop a small set/mini core set for phenotypic evaluation which will be the representation of the entire 5716 lines. Five genotypes FR 13A, Sabita, IR 42, Swarna and Swarna-Sub1 were the check varieties so preferentially included in the mini-core. In conclusion, the mini-core of 21 accessions presented in this study is a good representative subset of the submergence tolerance rice core collection of 5716 accessions.

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SHORT COMMUNICATION

Notes on the Distribution of a Rare and Little Known Species: *Allium fasciculatum* Rendle from Sikkim and West Bengal

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Germplasm of a rare and lesser-known species *Allium fasciculatum* Rendle was enriched through exploration undertaken in interior parts of North, South and West districts of Sikkim (1300-2600 m) and Darjeeling in West Bengal where it was reported for use as vegetable and condiment. This species, a rare and economically useful plant species growing in the kitchen gardens/backyards in the area of exploration, forms a new record on cultivation with addition to the germplasm holdings in field genebank at ICAR-NBPGR regional station at Bhowali, Uttarakhand.

Key Words: *Allium fasciculatum,* Distribution, Little Known Species, Sikkim

Indian gene center is considered rich in distribution of about 34 species of *Allium* with concentration of diversity in the western Himalayan and northeastern hill region (Baker, 1882; Stearm, 1947; Nasir, 1975; Pandey *et al*., 2008). For past four decades, the ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR) has taken a lead in assembling this diversity from these areas (Negi, 2006; Negi and Pant, 1992; Pandey *et al*., 2008). Native as well as exotic wild species (30 species) have been maintained, characterized and evaluated at field gene bank (FGB) at Bhowali, Uttarakhand.

In order to enrich germplasm of *Allium,* an exploration was undertaken to interior parts of North, South and West districts of Sikkim (1300-2600 m) and Darjeeling in West Bengal during September 2012. During field survey and collection, the authors collected five accessions of a rare and lesser-known species of *Allium* growing in the kitchen gardens/backyards (Negi *et al.*, 2012). After critical morphological studies and comparison with the herbarium specimens preserved in Northern circle, Botanical Survey of India, Dehradun (BSD) and Forest Research Institute (Council of Forest Research and Education), Dehradun (DD) was confirmed as *Allium fasciculatum* Randle (syn. *A. gageanum* W.W. Smith) and later validated with local flora and other relevant literature available on this genus. This species was reported from higher altitude of Sikkim and adjoining parts of Bhutan, Nepal and China (Das Gupta, 2006; Noltie, 1994; Hara *et al*., 1978). In earlier distributional records there was meager information on occurrence of this species from India (Chandel and Pandey, 1992; Arora and Pandey, 1996; Hazra and Verma, 1996; WOI, 1985; Das Gupta, 2006; Devi *et al.,* 2014; Pradheep *et al*., 2016, 2017). There were no reports on edibility of the species for the reported (now) use from India (Arora and Pandey, 1996; WOI, 1985; Devi *et al.,* 2014). In area of collection *Allium fasciculatum* was reported for use as vegetable and condiment.

Allium fasciculatum Rendle (Alliaceae) was previously collected by Smith and Cavanilles, White, JC and Prain, D during 1809 to 1909 from Lhonk, Dongbong and Kambajong respectively (Herbarium no. CAL2130, 2132). Earlier collections on *Allium fasciculatum* Rendle were made from Dongdong, Kambajong, Lhonk and Nathula of Sikkim during 1809-1909 as per herbarium specimens available at Central National Herbarium, Kolkata, West Bengal; for more than a century, there were no records on the collection of this species from north-eastern region of India, specially from Himalayan region.

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Allium fasciculatum forms a new addition to the germplasm holdings in *Allium* FGB at ICAR-NBPGR regional station at Bhowali, Uttarakhand (Pandey *et al.*, 2008). This species is a rare and economically useful plant germplasm from Sikkim and parts of Darjeeling, West Bengal. Live material (vegetative propagule) collected from different locations were transplanted, multiplied and maintained in FGB (Negi, 2006).

Plant characters – leaf shape, size, inflorescence-shape, size, perianth – shape and curling nature at maturity were clearly matching with the protologue description. Observations recorded by the first author in field and during experimental fields at RS Bhowali showed no fasciculate roots at maturity and no evident vertical fibers on basal part which confused the identity and warranted further validation. In order to authenticate the correct identity the first and second authors undertook periodic observations at different growth stages under variable soil conditions during 2014-16 using original material from RS Bhowali and in pots under Delhi conditions. Presence of fasciculate roots were observed from two months up to pre-flowering stage; however the fibres were very scarce and were only visible at drying stage of plant. These characters were in complete agreement with the protologue description. The diverse material available in other national and International herbarium sources (FRI, CAL, P, K, E; *Allium fasciculatum*, Flora of China Illustrations vol. 24, fig. 168, 1-3 [line diagramme]. http://www.efloras.org/object_page.aspx?object_id=60223&flora_id=2.) also confirmed these observations. Herbarium vouchers of *A. fasciculatum* were deposited in NHCP (collector no. NH21818a, b; NG3165; 15.9.2012).

Description: ***Allium fasciculatum*** Rendle in J. Bot. 44: 42, 1906; Stearn in Herbertia 12:83, 1947 and Bull. Brit. Mus. Nat. Hist. 2: 183, 1960. Syn. *A. gageanum* W.W. Smith in Rec. Bot. Surv. India IV: 247-8, 1911.

Annual herb, upto 50 cm high; bulbs absent; roots 6-7 in number, fleshy, fusiform or cylindric, 3-4.5×0.5 cm long; leaves basal, 1.0 to 1.5 cm above the base, flat, linear, 6-9 no, 25.0-40.0 × 0.8-1.0 cm., margin scabridulous. Umbles globose to sub-globose, 3.0-3.5 cm diameter, compact, numberous flowered; spathe broadly ovate, scape fistular, shorter than leaves (10.0-30.0× 0.5-0.6 cm), acute; flowers campanulate, sweet-scented, pedicels 1.0-1.5 cm long; perianth linear-lanceolate, 0.5-0.7 × – 0.2-0.3cm, acute, tepal white-greenish white, irregularly coiled at maturity; filaments linear 0.2-0.5 cm long, sub-equal; anthers yellowish-green, oblong, 0.3 to 0.5 cm; ovary obovoid, base narrowed to form a stipe, conspicuously 3-furrowed; style 1-2.5 mm. long; capsule obovoid, 0.3-0.4 × 0.3- 0.4 cm., trilobed, light brownish yellow, pericarp papery, locules 2-seeded; seeds ovoid-obovoid, 0.2-0.3 × 0.1 × 0.2 cm., concavo-convex, reticulated.

Specimens Examined: Tibet, Phari, July 1879, Dungboo s.n. (BM lecto., CAL); Sikkim – Lhonak 4500 m, 05.08. 1909, Smith & Cave 2130 and 2132 (K, CAL); Ribu & Rhomoo, 2771 (CAL); Sikkim: Dongdong, 10.7.1906, JC White s.n. (CAL); Kambajang, September 1903; Prain s.n. (CAL); 15.08.1882, King's collector (CAL); Nathula 5500 m, September-October, 1809; Nagaland: Kohima, July 1886, D Prain (CAL).

Distribution Reported (now): details of collected germplasm are as follows: (i) NG-3165, Payal Kyong Upper, Tingbong, Passingdong, North Sikkim, 15.09.2012, alt. 1300m; (ii) NG-3176 and NG-3179, Namachi, South Sikkim, 16.09.2012, alt. 2100 m; (iv) NG-3183, Uppermuk, West Sikkim, 17.09.2012, alt. 1930 m; and (v) NG-3189, Tiger Hills, Darjeeling, West Bengal, 19.09.2012, alt. 2600 m.

Ecology: reported (now) under cultivation in kitchen gardens, backyards (altitude of 1300-2600 m).

Phenology: Flowering and fruiting in July-September

Uses: Locally called as ***"Shanu Dungdunge:, "Romu or Rombu"*** ; young leaves are cooked as pot- herb, aromatic vegetable is prepared mixing leaves with potato. Leaves are sun dried and used as condiment for later consumption.

The present paper contains detailed taxonomic description along with live photographs (Fig. 1) to facilitate field identification and collection of germplasm.

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Fig. 1. *Allim fasciculatum* (Shanu dungdunge) collected from Sikkim: (top row, left to right) plants growing in field margin, Sikkim; roots of two month old plants; roots at pre-flowering stage with fasciculate and normal roots; (bottom row, left to right) flower with narrow perianth; herbarium specimen deposited in NHCP; close up of vertical fibres

for carrying out experimental study; FRI and CAL for study on this species.

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Plant Germplasm Registration Notice*

The Plant Germplasm Registration Committee of ICAR in its XXXIVth meeting held on May 24th, 2016 at the ICAR-National Bureau of Plant Genetic Resources, New Delhi approved the registration of following 23 germplasm lines out of 35 proposals considered. The information on registered germplasm is published with the purpose to disseminate the information to respective breeders for utilization of these genetic stocks in their crop improvement programmes. Upon request, the developer(s)/author(s) is/are obliged to distribute the material for crop improvement programme of National Agricultural Research System.

1. RP 5448-RIL-501 (IC0617119; INGR16001), a Rice (*Oryza sativa)* Germplasm with Novel Dual Donor for Resistance to both Brown Plant Hopper (BPH) and White Backed Plant Hopper (WBPH). Possesses Resistance at Vegetative and Reproductive Stages

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Brown planthopper (*Nilaparvata lugens* Stal) and whitebacked planthopper (*Sogatella furcifera*) are the most devastating planthoppers causing 100% yield losses under hopper-burn in rice. Resistant varieties are becoming susceptible as the new virulent insect populations are evolving. So it is essential to develop or search for novel donor sources to cope up with infestation. In this context, a genetic stock designated as RP 5448 - RIL-501 was developed at ICAR-Indian Institute of Rice Research, Hyderabad. It was found to be a unique dual donor possessing resistance to two planthoppers namely BPH and WBPH at seedling and reproductive stages. It is a recombinant inbred line (RIL) generated from the cross TN1 (susceptible)/ Ptb 33 (resistant) following single seed descent method of pedigree selection.

Morpho-agronomic Characteristics: Upon screening the RILs (F_9) derived from the cross TN1/Ptb 33 at a natural hot spot namely APRRI, Maruteru in AP during 2012 and thirty RILs showed resistant reaction during reproductive stage. The insect pressure was so high that one of the resistant RILs designated as RP 5448-RIL-501 could withstand the infestation of around 500-567 WBPH insects and 35-40 BPH insects per hill during *kharif*, 2012; 347-415 BPH insects and 10-15 WBPH insects/ hill during *rabi*, 2012 with a damage score (DS) of 3 as per SES of Rice (IRRI, 2002, IIRR Annual report, 2012-13).

In 2013, the same set of 30 resistant lines were rescreened for another 2 seasons. Heavy infestation of 350-515 BPH and 80-130 WBPH insects/hill while 500-600 BPH and 5-10 WBPH insects/hill occurred for a period of >10 days during *Kharif* and *rabi* respectively (Table 1). The same RIL showed resistance for plant hoppers (DS3.9) which survived and produced seed. (IIRR Annual report, 2013-14). Resistant reaction was on par with the resistant check, Ptb 33 (DS: 3) in both years.

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Table 1. Reaction of RP 5448 - RIL - 501 to BPH and WBPH insects under natural and artificial epiphytotics during 2012 and 2013

	Year & Season	BPH/WBPH		Damage Scores of Resistant check		Damage scores of susceptible check	
			Damage scores of RP 5448-RIL-501	Ptb 33 (BPH)	MO1 (WBPH)	TN1 (BPH)	TN1 (WBPH)
Natural conditions (Field)	2012 Kharif and Rabi	BPH + WBPH	3.0 (R)	3 (R)	3 (R)	9 (S)	9 (S)
	2013 Kharif and Rabi	BPH + WBPH	3.9 (R)	3 (R)	3 (R)	9 (S)	9 (S)
		Mean scores	3.45 (R)	3 (R)	3 (R)	9 (S)	9 (S)
Artificial conditions (Green house)	2013 Kharif and Rabi	BPH	2.85 (R)	3 (R)	-	9 (S)	-
		WBPH	3.0 (R)	-	2.9 (R)	-	9 (S)

R: Resistant S: Susceptible

For confirmation, the field resistant RILs were subjected to greenhouse screening simultaneously during 2013 in two seasons at ICAR-IIRR, Hyderabad. The RILs showed varied levels of resistance in greenhouse. However, RP 5448-RIL-501 showed more or less similar kind of resistance against BPH (DS: 2.8) and WBPH (DS: 3.0) during *kharif* and *rabi* seasons of 2013 (Fig.2) as was recorded in field assays. The resistant checks, Ptb 33 and MO1 for BPH and WBPH recorded damage scores of 3 and 2.9 respectively (IIRR newsletter, 2015). Thus the overall resistance reaction was observed to be stable in field and greenhouse.

Associated Characters and Cultivated Practices: RP 5448-RIL-501 is an intermediate, mid duration culture (140 days), possessing medium slender grains and intermediate plant height (120 cm) and non-lodging. It displayed good cooking quality traits such as high milling recovery (61.6%) coupled with medium head rice recovery (53%) and desirable alkali spreading value (4). It recorded intermediate desirable amylose content (25.1 %) with hard GC (22 mm).

Apart from good cooking quality, it exhibited consistent resistance reaction continuously for 4 seasons in 2 years (2012 to 2013) in field and two seasons in glass house for one year (2013). Hence this elite genetic stock could be utilized as a novel donor for developing rice varieties with combined resistance to planthoppers.

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2. DWRB 128 (IC0617170; INGR16002), a Two Row Feed Barley (*Hordeum vulgare* L.) Germplasm with Immune to Yellow Rust (*Puccina striiformis* f. sp. *hordei*)

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DWRB128 (BK1229-DWRUB54/DWRUB75) is two-row high yielding malt barley genotype, which was evaluated for disease reactions at multi-locations for three years viz. *rabi*, 2012-13 to 2014-15 under Initial Barley Disease Screening Nursery (IBDSN) and National Barley Disease Screening Nursery (NBDSN),

respectively (Anonymous 2013; Anonymous 2014; Anonymous 2015). During *rabi*, 2012-13, DWRB128 was evaluated at 4 locations namely, Bajaura, Durgapura, Dhaulakuan and Ludhiana and showed resistant reactions for stripe rust under artificial inoculations (IBDSN). DWRB128 was evaluated in timely and late sown initial malt barley varietal trials (IVT-MB-TS and IVT-MB-LS) during 2013-14 and again confirmed resistance (0=immune response) for barley stripe rust under artificial inoculations in NBDSN at 7 locations (Table 1). The two-row check varieties namely DWRUB52, DWRB92 and DWRB91 exhibited the stripe rust reactions of 10S, 20S and 40S, respectively. During 2013-14, DWRB128 depicted grain yield of 51.7 q/ha and was significantly superior to both the checks viz. DWRUB52 (49.4 q/ha) and DWRB92 (49 q/ha) under timely sown malt barley trial (IVT-MB-TS). In third year of stripe rust resistance confirmation, the DWRB128 again showed of resistant reactions at Bajaura, Durgapura and Almora locations in NBDSN. Whereas, the check varieties *i.e.* DWRUB52, DWRB92 and DWRB91 depicted the yellow rust reactions of 5S, 30S and 10S, respectively (Kumar *et al*., 2015) (Table 1).

On the basis of two years data viz. 2013-14 and 2014-15 the genotype DWRB128 also depicted superiority for Beta-glucan (10.6 %) and grain protein content (10.0%) over malt barley check DWRUB52, respectively.

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Table 1. Multi-location stripe rust reactions (artificial inoculations) of DWRB128 and checks for three years

Year	Location	DWRB128 (2R-BK1229)	DWRUB52 (2R-check)	DWRB92 (2R-check)	DWRB91 (2R-check)	Infector
First year (2012-13)	Bajaura	0	0	0	5S	80S
	Dhaulakuan	0	0	5S	0	60S
	Durgapura	tMR	tMS	10MS	10S	100S
	Ludhiana	0	0	5S	5S	60S
Second Year (2013-14)	Bajaura	0	0	0	5MR	100S
	Dhaulakuan	0	0	0	0	100S
	Durgapura	0	10S	20S	40S	100S
	Ludhiana	0	0	tS	0	40S
	Karnal	0	0	0	0	80S
	Hisar	0	0	0	0	80S
	Almora	0	0	5MS	tR	80S
Third year (2014-15)	Bajaura	0	0	30S	0	100S
	Durgapura	tMR	5MS	20MR	10S	100S
	Almora	0	5S	20S	5S	60S
Over all HS		tMR	10S	30S	40S	100S

3. DWRB 1143 (IC0617171; INGR16003), a Six Row Malt Barley (*Hordeum vulgare* L.) Germplasm Immune to Highly Resistant for Yellow Rust (*Puccina striiformis* f. sp. *hordei*)

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DWRB143 (BK1333) is a six-row high yielding genotype, which was developed by pedigree method (DWRB73/DWR83) at ICAR-IIWBR, Karnal. It was screened for disease reactions at multi-locations for consecutive two years viz. *rabi*, 2013-14 and 2014-15, under Initial Barley Disease Screening Nursery (IBDSN) and National Barley Disease Screening Nursery (NBDSN), respectively. During *rabi*, 2013-14, the genotype DWRB143 was evaluated by code BK1333 at five locations namely, Bajaura, Durgapura, Dhaulakuan, Ludhiana and Karnal in IBDSN. The genotype BK1333 exhibited immune response (0 reaction) for yellow rust under artificial disease epiphytotic conditions (Anonymous, 2014). The check varieties of NEPZ namely Lakhan exhibited susceptible yellow rust reactions of 100S followed by K603 (80S), whereas, NWPZ *elite* feed barley varieties BH902 and BH946 also showed susceptible reactions of 30S and 10S, respectively in NBDSN at the same locations (Table 1). Similarly, During *rabi*, 2014-15, DWRB143 was evaluated in initial varietal rainfed coordinated trial in NEPZ (IVT-RF-NEPZ) for grain yield and vis-à-vis for disease resistance under artificial inoculations in NBDSN. DWRB143 again showed immune response to yellow rust (0 reaction) at all the centres namely Durgapura, Bajaura and Almora (Anonymous, 2015; Kumar *et al*., 2015) (Table 1).

Table 1. Multi-location stripe rust reactions (artificial inoculations) of DWRB143 and checks for two years

Year	Location	DWRB143 (6R-BK1333)	Checks of NEPZ (same trial)		Recent checks of NWPZ		Infector
			Lakhan (6R-check)	K603 (6R-check)	BH902 (6R-check)	BH946 (6R-check)	
First year (2013-14)	Bajaura	0	30S	40S	30S	0	100S
	Dhaulakuan	0	20S	60S	0	0	100S
	Durgapura	0	100S	80S	0	10S	100S
	Ludhiana	0	5S	5S	0	0	40S
	Karnal	0	40S	40S	10MS	0	80S
Second Year (2014-15)	Durgapura	0	100S	60S	tMR	10MR	100S
	Bajaura	0	60S	20S	0	10S	100S
	Almora	0	80S	80S	5S	10MS	60S
Two years HS		0	100S	80S	30S	10S	100S

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4. PPMI 904 (IC0617290; INGR16004), a Pearl Millet (*Pennisetum glaucum* L.) Germplasm with High Iron Content of 91 mg/kg High Zinc Content of 78 mg/kg

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Pearl millet is a major warm-season cereal, grown in more than 29 million ha in the arid and semi-arid tropical regions of Africa and Asia (Kannan *et. al*., 2014). India is the largest producer of this crop in Asia. During 2013-14, pearl millet in India being grown in 7.91 m ha with a production 9.19 m ton and productivity 1161 kg/ha (DES-DAC, 2014). In Pearl millet growing regions 35% of total intake of calories, protein, iron and zinc is from this millet only and it is the cheapest source of iron and zinc content compared to other cereals such as rice and wheat (Rao *et al.,* 2006). Biofortification approach involves enchancing the levels of specific, limiting micronutrients in edible tissues of crops by combining crop management, breeding, and genetic approaches (Hirschi, 2009). Micronutrient-enriched or biofortified pearl millet serves as the logical vehicle for providing Fe and Zn in the diets of the people and also shall be a cost-effective and sustainable approach to alleviate micronutrient deficiencies. Bashir *et al*. (2014) observed a wide range of variation for iron and zinc content (Fe:19.7 mg/kg to 86.4 mg/kg and Zn : 13.5 mg/kg to 82.4 mg/kg). Rai *et al*., (2012) reported iron content as high as 135 ppm and zinc being 92 ppm in pearl millet grains, whereas Govindaraj *et al.,* (2011) reported a little narrower range of these micronutrients (iron: 30.1 to 75.7mg/kg and zinc: 24.5-64.8mg/kg) in improved populations obtained from India and Africa.

We are proposing pearl millet line named PPMI 904 for registration as pearl millet lines with high iron and zinc. PPMI 904 is unique because it is rich in Iron and Zinc, 91 mg/kg and 78 mg/kg of grain respectively. As per the harvest plus millet strategy (2010), the target levels of iron are 77 mg/kg grain of pearl millet. The proposed pearl millet line was developed at Division of Genetics, IARI, New Delhi from the recombinant inbred line population from cross between PPMI 683 x PPMI 627 parents initiated in *kharif*, 2009. This line was tested in All India Coordinated Pearl millet improvement programme's high iron or elite joint biofortification trial in multilocations in *kharif*, 2013.

The same material was tested in 8 diverse locations in *Kharif*, 2014 for grain iron and zinc content (Tara Satyavathi *et al*., 2015)

Morpho-agronomic characters: Morpho-agronomic characters of line PPMI 904 are its height is approx. 120 cm with erect plant type with days to 50% flowering around 65 days. The nodes are green in colour. Anthers are yellow in colour. Panicle exertion is complete and length of panicle is 24 cm in length and girth is 2.74 cm. The spike is cylindrical in shape with semi compact nature. The 1000 seed weight of PPMI904 is 8.23 grams. Seed colour is grey and seed is globular to obovate in shape.

Table 1. Mean performance of the proposed pearl millet genotype along with checks in multi-locational test conducted during Kharif, 2013 in High iron joint biofortification trials of All India Coordinated Pearl millet improvement Programme

Entry	Coimbatore		Delhi		Durgapura		Gwalior		Jamnagar		Ludhiana		Mandor		Grand Mean	
	Fe	Zn	Fe	Zn	Fe	Zn	Fe	Zn	Fe	Zn	Fe	Zn	Fe	Zn	Fe	Zn
PPMI 903	46	66	98	99	54	68	92	52	100	64	58	83	85	72	76	72
PPMI 904	93	107	130	121	48	48	143	81			29	33	105	78	91	78
PPMI 905	27	48	50	61	44	48	35	37	72	54	41	75	35	37	44	51
PPMI 906	54	81	94	97	57	71	97	59	88	61	50	69	56	52	71	70
841 B	32	42	45	46	42	50	38	38	62	46	27	42	33	37	40	43
D 23	33	46	48	44	69	51	46	39	59	49	28	37	34	36	45	43
ICTP 8203 imp																

Source: Annual Report of All India Coordinated Pearl millet Improvement Project 2013-14

Table 2. Mean data for grain Fe & Zn(ppm) and grain yield (kg/ha) of 8 pearl millet genotypes grown at 8 locations during *Kharif*, 2014

Genotype	Grain iron content (ppm)									Grain Zinc content (ppm)								
	L1	L2	L3	L4	L5	L6	L7	L8	Geno Mean	L1	L2	L3	L4	L5	L6	L7	L8	Geno Mean
PPMI 903	67.67	98.00	65.00	92.00	91.00	67.00	85.33	65.33	78.92	63.67	99.00	69.00	52.00	75.67	83.00	72.00	60.00	71.79
PPMI 904	93.00	126.7	78.00	111.0	111.0	73.67	105.00	87.00	98.17	107.00	114.33	48.00	80.00	82.00	33.00	78.00	103.00	80.67
PPMI 905	29.33	50.00	44.00	34.67	43.00	41.00	38.00	34.33	39.29	46.33	61.00	48.00	36.33	39.67	74.67	37.00	43.67	48.33
PPMI 906	74.33	94.00	60.33	97.00	66.00	65.00	66.00	87.67	76.29	80.67	97.00	70.00	59.00	55.00	69.00	52.00	77.67	70.04
841B	32.00	45.00	42.00	38.67	38.67	27.00	33.00	26.33	35.33	42.00	46.00	50.67	38.00	39.67	42.00	37.00	39.33	41.83
D 23	33.00	48.00	69.00	46.00	39.67	28.67	34.33	27.67	40.79	46.00	44.33	50.00	39.00	41.00	37.33	36.00	41.00	41.83
ICTP 8203	47.00	97.00	76.00	95.67	83.33	74.33	77.00	40.67	73.87	54.00	74.00	69.00	52.00	57.33	62.67	54.00	50.67	59.21
Loc. Mean	46.56	68.50	59.31	66.17	58.69	47.03	53.53	43.61	55.42	56.56	68.67	59.11	48.03	49.39	54.14	45.81	52.97	54.33

Source: Tara Satyavathi, *et al.* (2015).

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5. Cotton Queen (SIMA) (IC0618569; INGR16005), a Tall and Perennial Cotton (*Gossypium barbadense*) Germplasm

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Cotton Queen (SIMA) is 37 feet tall cotton Plant, yield above 2000 bolls per plant with good fobre quality as tested by CICR Nagpur.

6. DOGR-1549-Agg (IC0616539; INGR16006), an Onion (*Allium cepa* var. *aggregatum*) Germplasm with Unique Early Multiplier; Suitable for both *rabi* and *kharif* Seasons; Early Maturing with Six Uniform Bulblets per Bulb

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Multiplier onion (*Allium cepa* var. *aggregatum*) is mainly grown in Tamil Nadu, Andhra Pradesh and Karnataka. It is famous for its use in *Sambar* preparation, an important South Indian dish and have high export potential also. It produces small size bulbs, many in number, to form an aggregated cluster. It is normally propagated by bulblets. There are no early maturing multiplier onion varieties which could be suitable for both *kharif* and *rabi*. Hence, there is need to develop early maturing varieties in multiplier onion. The work in this direction led to development of an early maturing multiplier line DOGR-1549-Agg.

Background

Genotype DOGR-1549-Agg (IC-0616539)were developed through clonal selection from a sample collected from Kagati, Chintamani, Karnataka. It is better than parental population in respect of uniformity, earliness and yield parameters. It is an unique early maturing multiplier onion suitable both for *kharif* and *rabi* seasons. It matures in 85 days after planting i.e. about one week earlier than popular variety CO-5. It has six uniform bulblets per bulb which are attractive pink and oval in shape tapering towards neck [1, 4 & 5].

Table1. Performance of DOGR-1549-Agg in comparison to controls

A. Days to Maturity

Entry	*Rabi* 2012-13	*Rabi* 2013-14	Mean	*Kharif* 2013	*Kharif* 2014	Mean	Overall Mean
1549-Agg	94.00	89.67	91.84	68.33	84.00	76.17	84.00
CO-4 (C)	99.00	90.00	94.50	77.00	85.00	81.00	87.75
CO-5 (C)	97.33	95.00	96.17	78.00	87.00	82.50	89.33

B. Total Yield (q/ha)

Entry	*Rabi* 2012-13	*Rabi* 2013-14	Mean	*Kharif* 2013	*Kharif* 2014	Mean	Overall Mean
1549-Agg	213.30	253.30	233.30	240.22	143.00	191.61	212.46
CO-4 (C)	180.56	180.76	180.66	185.62	129.50	157.56	169.11
CO-5 (C)	176.05	237.52	206.79	177.05	114.00	145.53	176.16

C. No. of Bulblets/bulb

Entry	*Rabi* 2012-13	*Rabi* 2013-14	Mean	*Kharif* 2013	*Kharif* 2014	Mean	Overall Mean
1549-Agg	6.20	6.20	6.20	5.19	6.07	5.63	5.92
CO-4 (C)	5.40	6.00	5.70	4.21	6.40	5.31	5.50
CO-5 (C)	5.47	5.40	5.44	5.23	5.00	5.12	5.28

D. TSS (%)

Entry	*Rabi* 2012-13	*Rabi* 2013-14	Mean	*Kharif* 2013	*Kharif* 2014	Mean	Overall Mean
1549-Agg	12.25	16.20	14.23	13.27	14.11	13.69	13.96
CO-4 (C)	11.60	12.88	12.24	13.76	15.16	14.46	13.35
CO-5 (C)	12.25	12.68	12.47	13.32	13.56	13.44	12.95

* Compiled by Amar Jeet Gupta, Vijay Mahajan, S. Anandhan and Jai Gopal

Morpho-agronomic Characteristics

DOGR-1549-Agg was evaluated along with multiplier onion germplasm maintained at DOGR during *rabi* 2012-13 and 2013-14, and *kharif* 2013-14 and 2014-15 at Rajgurunagar (Table 1) [1, 2, 3 & 4]. It has erect foliage, plant height 25-30 cm with 5-7 green leaves per plant. Compound bulb diameter is 3.0-3.5 cm and bulblet diameter 1.2-1.5 cm. Bulblets are pink oval tapering towards neck.Total soluble solids are 13.5-14.5^0 brix and average yield is 20-22 t/ha.

The uniqueness of this genetic stock is that it is about one week earlier than popular variety CO-5 and is suitable for both *rabi* and *kharif*. It has six uniform pink bulblets per bulb (Fig.1) [5].

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7. MGMS-7 (IC0618568; INGR16007), a Non-Spiny-Marker-Linked Genetic Male Sterile Line in Safflower (*Carthamus tinctorius* L.)

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MGMS-7 (IC0618568; INGR16007) is a genetic male sterile (GMS) in which male sterility is linked to non-spiny trait; the linkage is visible when plant is around 40-45 days old. Inherently, the GMS system segregates into male sterility and fertility in 1:1 ratio (Heaton and Knowles, 1982) and they are distinguishable only after flower opening, which is an impediment for utilization of GMS lines in breeding and hybrid seed production programmes for occurrence of female-selfs in hybrid seed lot. However, MGMS-7, a non-spiny-marker-linked genetic male sterile line developed for the first time in safflower at the Indian Institute of Oilseeds Research, Hyderabad, can distinguish male sterile and fertile plants at about 40-45 days prior to flowering and provides ample time (40-45 days) for roughing-off of fertile plants prior to flowering itself thus facilitates production of genetically pure F_1/cross seed. MGMS-7 was derived from a cross between a spiny GMS parent '13-137' and 'A1'. The 1:1 segregation of male sterile and fertile progenies in MGMS-7 confirmed GMS nature of the line. Absence of male sterility in F_1 and 3:1 segregation of male fertile and male sterile in F_2 confirmed the recessive nature of male sterility in MGMS-7. In MGMS-7, the non-spiny trait is strongly linked to genetic male sterility. The recombination value of 0.15-0.4% over years and 0.16% over seasons confirmed stability of tight linkage between non-spiny marker and male sterility in MGMS-7. This line would be useful to safflower breeders for producing pure hybrid seed as well as in population improvement and gene pyramiding programmes where multiple crossing is a prerequisite. By using MGMS-7, the tedious and time taking emasculation by hand can be avoided and absolute cross seed can be ensured.

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8. CoM7601 (IC0618560; INGR16008), a Sugarcane (*Saccharum officinarum* L.) Germplasm with Resistance to Whip Smut

and

9. MS7604 (IC0618562; INGR16009), a Sugarcane (*Saccharum officinarum* L.) Germplasm with Resistant to Whip Smut

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Achieving durable resistance to important diseases is the prime objective of sugarcane breeding. Screening genotypes for smut resistance with artificial inoculation is being carried out from last 42 years at Central Sugarcane Research Station, Padegaon. Two genotypes bred by this center *viz*., CoM 7601and MS 7604 have shown consistently resistant reaction to smut from the last 28 years. These resistant genotypes could be of immense utility in breeding programme for developing smut resistant sugarcane varieties.

Whip-smut caused by *Ustilago scitaminae* Sydow. is a serious disease of sugarcane in Tamil Nadu, Karnataka and Maharashtra (Alexander, 1975). Management of plant diseases through host resistance is the best option for crop protection. Development of resistant varieties is followed by coevolution of the pathogen resulting in breakdown of resistance and hence achieving durable resistance is important. Screening sugarcane clones for resistance to whip-smut with artificial inoculation of smut teliospore suspension is being done from 1970 at Central Sugarcane Research Station, Padegaon.

The resistant genotypes were tested every year in *Suru* season (Jan-Feb. planting) against whip-smut from 1970 to confirm the consistence of resistance. Two eye budded setts of each genotype were artificially inoculated by soaking them for 30 minutes in fresh viable (90 to 95% viability) smut teliospore suspension (@ 10 g teliospore powder per 50 lit of water) having spore load of 10^6 to 10^8 teliospores ml^{-1} (Shinde *et al.,* 1985 and Chirme *et al.,* 1998). Treated sets were planted in moist soil in the field @ 15 sets per row of 6 m length. The incidence of smut was recorded at fortnightly interval. Based on the cumulative smut incidence, the genotypes were categorized as resistant (R= 0.0%), moderately resistant (MR= 0.01 to 10.0%), moderately susceptible (MS= 10.01 to 20.0%), susceptible (S= 20.01 to 30.0%) and highly susceptible (HS= >30.0%) as per Shah *et al*. (1997).

Two resistant genotypes bred by this center *viz*., CoM 7601 and MS 7604 have shown consistently resistant reaction to smut from the last 28 years which indicates that these genotypes have durable resistance to smut under artificial inoculation condition.

The usual method of identifying resistant clones through inoculation test is still the reliable method (Flores *et al*., 2009). The method of artificial inoculation by immersion of sugarcane cuttings in the suspension of smut spores was found to be effective to evaluate the resistance against smut in experimental material of sugarcane (Briceno *et al*., 2005).

Thus, it is confirmed that the sources of durable resistance against whip smut are available in sugarcane which can be utilized in breeding programme for evolution of new high yielding sugarcane varieties with in-built resistance to whip smut (Begum *et al*., 2007).

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10. JEX/A 911(IC0618556; INGR16010), a Potato (*Solanum tuberosum* L.) Germplasm with Suitable for Making Multicolor Chips; Tuber Flesh Attractive Multicolored

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Of late, there is an awareness and interest in the presence of antioxidants in the diet. Purple potato colour is caused by the presence of flavonoids. Among these flavonoids, anthocyanins are found to have antioxidant properties in potatoes, fruits and vegetables. Antioxidants in the diet are good and have been associated with a reduced risk of quite a number of diseases, ailments with aging, and so on. Recently, antioxidant rich purple coloured potatoes are being marketed for table consumption in Japan, Canada and US and chips made from these potatoes are also being sold by several companies in the US [EijckPaul van 2008]. In India there is need to develop potato which contain high antioxidants. In order to provide antioxidant rich processed products to the Indian consumers, primitive cultivated andigena germplasm collection (about 770 accessions) which is known for its diversity was screened to identify sources for coloured tuber flesh (Marwaha RS, Kumar Raj, Pandey SK 2008). JEX/A 911 with multicolour flesh tubers, was found to be useful as its tubers were suitable for processing into multicolor chips with attractive chips having deep yellow colour mottled with purple patches and good taste.Tubers of JEX/A 911 had very high dry matter content (24.8%), contained reducing sugars (41.0 mg) in acceptable limit for producing quality chips. JEX/A 911 is a clonal selection from segregating progeny of the accession CIPC700939 of cultivated tetraploid species *Solanum tuberosum* ssp. andigena. This germplasm accession can be very useful in developing pigmented flesh varieties for producing coloured chips with attractive color pattern

Morpho-agronomic Characteristics: Tubers of JEX/A 911 are oval shape, purple skin, medium sized with medium deep eyes. Tuber flesh colour is yellow mottled with purple secondary colour. The flower corolla colour is blue-violet. Plant is medium tall. Leaflets are ovate lanceolate with wavy margin. Sprout colour is purple. The yield of this accession is significantly lower than commercial cultivars Kufri Chipsona-1 and Kufri Chipsona-3.

Associated Characters and Cultivated Practices: This accession is late maturing and susceptible to late blight and early blight diseases.

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11. PDA-01 (IC0618554; INGR16011), a Sweet Bamboo/Asper Bamboo (*Dendrocalamus asper* (Schult.) Baker ex Heyne) Germplasm Tolerant to Water-Logged Conditions

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Dendrocalamus asper (Schult.) Baker ex Heyne, commonly known as sweet bamboo is a multi-purpose tropical sympodial bamboo of high economic value. The young shoots of asper are sweet and are used as food. The food industries based on this edible species are expanding quite fast.Variegated Leaves line PDA-01, a natural mutant was found in the planted area in agroforestry research centre at Pantnagar in 2006.

Three plants showed variegation in their leaves, out of which one plant was selected and maintained in nursery conditions. Then plants were macroproliferated by separating the tillers with rhizomes into more plants and these plants were planted in field. The retention of variegation may be attributed to vegetative propagation which maintains the genetic identity and stability of the character. Trait is stable even after 7 years. There was no significant difference in mutant and normal plant for height, culm diameter and number of culms. In bamboos, leaf variegation has also been reported in *Bambusa multiplex* and *B. heterostachya* but report on variegated asper plant has not been found in the literature elsewhere. Reported variegated *D. asper* has huge potential to be used as ornamental plant and as marker in genetical and breeding studies.

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12. PBN 12-01 (IC0618555; INGR16012), a Malla Bamboo (*Bambusa nutans* Wall. Ex Munro) Tolerant to Water-Logged Conditions

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Bamboos are one of the fast growing plants on the earth. They are known to grow under dry conditions. Particularly,stagnated water is found very fatal to their survival and growth of the bamboos. Among promising bamboo species which are preferred by the growers, problem of growing under wet-land is much severe. Among preferred species, a line of *Bambusa nutans* Wall. ex Munro has been found to tolerant to water stagnation at Pantnagar conditions. Nutans bamboo commonly known as malla bamboo, is a multi-purpose tropical sympodial bamboo which may attain a height of 20 meters. This bamboo is used in house construction, basketry and crafts. It grows best at altitudes of between 500-1500 m. Thrives on moist hill slopes and flat uplands, and well-drained sandy loam to clayey loam soils.

A line from the germplasm of *Bambusa nutans* was selected from natural areas near Raipur–Dehradun, plants were found to be viable even after long period of 4 months of water logged conditions (line was identified in 2008 and selected in 2012). Later this line was evaluated under experimental water stagnated conditions where it showed survival even after 6 months of water stagnation. Line was named as PBN 12-1. Generally, bamboos are said to be recommend only in dry conditions with well

Table 1. Growth of *Bambusa nutans* PBN 12-1 under water-logged conditions (Planted in July 2008 and data taken from one year age in July every year)

Age (year)	Culm ht (m)	Diameter (cm)	No of new culms	Total No of culms	Fresh Culm weight (kg)
1 yr-2009	4.4	2.9	6.9	6.9	1.5
2 yr-2010	7.5	3.8	7.2	12.2	3.2
3 yr-2011	9.2	4.5	7.5	18.5	7.3
4 yr-2012	9.8	5.7	8.2	22.1	9.1
5 yr-2013	10.1	6.4	11.4	30.8	10.2
6 yr-2014	11.9	6.8	12.8	38.1	14.3

drained soils but this particular line was found tolerant to water logged conditions during rainy season almost for 4-6 months for over six years of testing (2008 to 2015). Line PBN 12-1 will have high practical applicability to be grown in low lying areas with problem of soil erosion. Therefore, farmers and tree growers may like to plant this particular line on their lands for economic and ecological benefits.

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13. LBRIL 189 (IC0611476; INGR16013), a Wheat (*Triticum aestivum*) Germplasm with Resistance to Spot Blotch

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Spot blotch or foliar blight caused by *Bipolaris sorokiniana* of wheat is the major disease and its occurrence is higher in north eastern plains zone (NEPZ) due to favourable conditions (hot and humid weather). The disease is spreading to other regions of the country and thus requires attention of researchers. Improvement of host resistance against spot blotch disease and widening the genetic base of the model genotypes through introduction of new alleles for resistance are the major activities being carried out at Indian Institute of Wheat and Barley Research, Karnal. This way, integration of conventional breeding with molecular tools will help in mitigating the problem of spot blotch disease in wheat through incorporation of useful QTLs in susceptible yet popular genotypes that are adapted to a particular region (Singh *et al*., 2016). In a set of breeding material developed to meet the these challenge, 215 recombinant inbred lines (RILs) derived from the cross between agronomic parent (disease susceptible genotype) "Kanchan" (HUW-202//K-7537/ HD-2160-N) and highly resistant genotype Chirya 1 (CS/*Ag.cu*//Glennson-81/3/ Alondra/ Pavon-76/ 4/ Ningmai-4/ Olesen//Alondra/Yangmai-4) and advanced to F_{9-10} generation. Complete set of this population of RILs (F_{9-10}) were phenotyped for assessing spot blotch disease during two consecutive crop seasons (2012-13 and 2013-14) under natural high disease pressure (two hot spot) locations namely, Coochbehar, Kalyani (West Bengal) in eastern India and also at IIWBR, Karnal under field and epiphytotic conditions (poly house) having 03 replications. Phenotypic data and disease score recorded on double digit scale at dough stage (taking disease reaction of top two leaves, flag and flag-1) at three different stages was for the study. Out of the potential material, one promising line LBRIL-189 that showed comparatively higher level of resistance against spot blotch as compared to even resistant parent (Chirya-1) consistently across the locations and over the two years (Table 1) was shortlisted as has been approved for registration as genetic stock.

Morpho-agronomic Characters: In addition to the better resistance, some of the morphological features like; days to heading (76-97), days to maturity (102-128) and plant height (94-113) that varied in different environments were also comparable. Besides, the average 1000-grain weight (TKW) of the genotype LBRIL 189 (40.0 g) was also comparable to its best check i.e. Chirya-1 (donor parent) which had TKW of 42.0 g and much higher than the agronomic parent.

Associated Characters and Cultivation Practices: LBRIL 189 was also observed to be leaf rust resistant

Table 1. Performance of LBRIL-189 for resistance and agronomic traits evaluated under four environments over two years (2012-13 & 2013-14)

Genotype	Condition	LB Score	Days to heading	Days to maturity	Plant height	1000-grain weight
LBRIL-189	Field	02	85	111	103	39
	Polyhouse	02	84	103	103	40
Chirya 1 (Resistant Parent)	Field	13	85	115	92	42
	Polyhouse	13	74	107	91	43
Kanchan (Agronomic parent)	Field	67	82	116	101	32
	Polyhouse	89	81	106	95	32

and thus can be grown in spot blotch affected regions by adopting normal wheat cultivation practices. Diagnostic markers reported earlier *Xgwm*371, *Xgwm* 425 (Kumar *et al.*, 2010) were also used to validate the promising line for spot blotch resistant (Singh *et al*., 2014).

The identified genetic stock possess high degree of resistance along with desired agronomic background thus will serve as potential donor for improving spot blotch resistance in wheat.

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14. GPHCPB 45 (IC0614156; INGR16014), a Finger Millet (*Eleusine coracana*) Germplasm with High Calcium Content 452.8mg/100g

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Finger millet is an important staple in most parts of India and Africa and is a good source of nutrients especially calcium with an average value of 344 mg 100 g^{-1} (Gopalan *et al*., 2004). Finger millet grains are known to contain high calcium in comparison to all important food grains. GPHCPB 45, an elite finger millet line has been identified as unique germplasm with respect to grain calcium. It has highest grain calcium (452.80 mg/100 g seed) in comparison to check varieties VL 149 (360 mg/100 g seed) and VR 708 (370 mg/100 g seed). This line is a local collection from Uttarakhand hills. The germplasm was collected by erstwhile hill campus Ranichauri (Now a campus of UUHF, Barshar, Uttarakhand) of GB Pant University of Agriculture and Technology, Pantnagar, India.

In addition to high calcium content, GPHCPB 45 also possesses larger ears in comparison to the checks. GPHCPB 45 has erect growth habit and droopy leaves. The details of morphological traits are given in Table 1. The germplasm line recorded higher incidence of finger and neck blast in comparison to elite lines and improved varieties. However, it can be used as a donor for the development of new varieties with high calcium content and in basic studies for identification of molecular markers /QTL's for grain calcium content. Diversification of germplasm with the involvement of this high calcium line in hybridization would be useful for enhancing the nutritional value of the crop in particular and nutritional security of the masses in general.

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Table 1. Distinguishing characteristics of GPHCPB 45 compared to check as recorded at Almora location

Character name	GPHCPB 45	VL Ragi 149	VR 708
Pigmentation on node	Absent	Present	Absent
Growth habit	Erect	Erect	Erect
Leaf blade pubescence	Present	Present	Present
Leaf attitude	Droopy	Droopy	Droopy
Ear shape	Open with finger straight at the time of flowering and curved at the time of maturity	Open with top curved finger	Compact fist type
Number of fingers/ear	8-10	8-10	7-9
Ear length (cm)	10-12	8-10	5-7
1000 Seed weight (g)	2.58	2.71	2.83
Days to flowering	70-75 days	65-70	60-65
Days to Maturity	103-107 days	100-105	95-100
Plant height (cm)	125-135	120-125	115-120
Grain color	Dark Red Color	Copper color	Light copper color

15. Ogu 3A (IC0614417; INGR16015), a Cytoplasmic Male Sterile Line of Cauliflower (*Brassica oleracea* Var. *botrytis* L.) with Good Seed Yield; Cream Coloured Flower

and

16. Kt-3B (IC0614418; INGR16016), a Male Fertile Maintainer Line of Ogu 3A Cauliflower (*Brassica oleracea* Var. *botrytis* L.)

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Cauliflower is an important vegetable crop and more than 90% area is covered by F_1 hybrids. Snowball cauliflowers are grown throughout the north Indian plain during rabi season and almost throughout the year in hilly regions of India. In cauliflower, F_1 hybrids are advantageous especially in uniform maturity, high early and total yield, better curd quality with respect to compactness and colour, resistance to insect pests, diseases and unfavorable weather conditions (Kucera *et al.,* 2006). Two pollination control mechanisms *viz.* self-incompatibility (SI) and male sterility (particularly CMS) are used widely for production of F_1 hybrid seed However, the contribution of indigenous hybrids is negligible and bulk of the seeds is imported from outside (Sharma *et al.,* 2004). Development indigenous hybrid seed industry in vegetable crop like cauliflower is the need of the hour. It will not only save revenues spent in import of their seeds but will also create several employment in rural India. Development of indigenous pollination control mechanism like CMS holds the key for indigenous hybrid seed production. The snowball cauliflower CMS line, Ogu3A is one indigenously developed CMS line. It has refined Ogura male sterile cytoplasm which if free from any floral deformities (Dey *et al.*, 2011).It has partially covered snow white-curd. The line was developed by transferring refined *Ogura* male sterile cytoplasm into nuclear background of snowball cauliflower variety, Pusa Snowball K-1 after 13 generation of backcrossing (BC_{13}).

Morpho-agronomic characteristics: This is one of the first snowball cauliflower CMS line with refined Ogura male sterile cytoplasm. This line belongs to late group of cauliflower (December onwards maturity) with a maturity period of 120-130 days at temperate regions and 70-80 days in North Indian plains. This line has normal floral traits with good nectar development. Thus, good honeybee activity was observed in this line. This line has seed yield of 26.8 g /plant when allowed

Table1. Important horticultural and seed yield of the CMS line Ogu33A as compared to the fertile maintainer line

Traits	Ogu3A	Ogu3B
Days to 50% maturity	132.8	131.3
Marketable curd weight (Kg)	0.8	0.8
Nos. of pods/plant	1007.5	1418.8
Seed yield/plant (g)	26.8	29.7

to set seed under natural isolation along with fertile maintainer in the ratio of 1:1. Thus, seed yield was at par with the fertile maintainer line and this line can be used in commercial hybrid breeding programme. The net individual curd size of the line was 0.80 Kg with a total yield of 35.5 tonnes/ha.

Associated characters and cultivated practices: The line Ogu3A belongs to the snowball group of cauliflower and cultivated mainly during the winter season. This male sterile line has cream coloured flowers with normal ovary and good nectar development. Because of normal seed yield this line can be used in commercial hybrid seed production programme. For hybrid seed production it is recommended to follow a planting ratio of 1:1 with fertile male parental lines.

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17. Ogu 33A (IC0614419; INGR16017), a Cytoplasmic Male Sterile Line of Cauliflower (*Brassica oleracea* Var. *botrytis L.*) with Good Seed Yield and Cream Coloured Flower

and

18. Kt-33B (IC0614420; INGR16018), Maintainer Line of Ogu 33A Cauliflower (*Brassica oleracea* Var. *botrytis* L.)

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Cauliflower is an important vegetable crop and more than 90% area is covered by F_1 hybrids. However, the contribution of indigenous hybrids is negligible and bulk of the seeds is imported from outside. Non-availability of indigenous pollination control mechanisms mainly Self-incompatibility and cytoplasmic male sterility (CMS) is the main bottleneck in development of indigenous F_1 hybrids (Sharma *et al.*, 2004). There is an urgent need to develop indigenous CMS lines in crop like cauliflower for development of hybrid seed industry in India. It will not only save a huge amount of revenues spent every year in import of cauliflower hybrid seeds but will also create several employment opportunities in rural India. It was earlier reported that the superior Ogura based CMS lines in cauliflower are highly suitable for cauliflower hybrid development (Dey *et al.*, 2011a and Dey *et al.*, 2011b). The mid-late (November maturity) cauliflower CMS line, Ogu33A is one indigenously developed CMS line. It has refined Ogura male sterile cytoplasm which if free from any floral deformities (Dey *et al.*, 2014). This line has shriveled anther with no viable pollen development.It has partially covered snow white-curd. The line was developed by transferring refined *Ogura* male sterile cytoplasm from Snowball-16 into nuclear background of November maturity cauliflower line, 33B after 12 generation of backcrossing (BC_{12}).

Morpho-agronomic characteristics: This is one of the first cauliflower CMS line with refined Ogura male sterile cytoplasm in November maturity group. This line belongs to mid-late group with a maturity period

of 90-100 days at temperate regions and 60-70 days in North Indian plains. This line has normal floral traits with good nectar development. Thus, good honeybee activity was observed in this line. This line has seed yield of 28.6 g /plant when allowed to set seed under natural isolation along with fertile maintainer in the ratio of 1:1. Thus, seed yield was at par with the fertile maintainer line and this line can be used in commercial hybrid breeding programme. The marketable individual curd size of the line was 1.03 Kg with a total yield of 45.2 tonnes/ha (Dey *et al.,* 2014).

Table1. Important horticultural and seed yield of the CMS line Ogu33A as compared to the fertile maintainer line

Traits	Ogu33A	Ogu33B
Days to 50% maturity	100.8	94.7
Marketable curd weight (Kg)	1.03	0.82
Nos. of pods/plant	1123.5	1224.2
Seed yield/plant (g)	28.6	33.2

Associated characters and cultivated practices: The CMS line Ogu33A has milky white flower colour with straight stigma and normal ovary. This line has dark green slightly puckered leaves and compact curd. As this line has been developed after 12 generation of back-crossing there is significant difference with fertile maintainer line. For development of hybrids it is recommended to plant the CMS line with the fertile male parental line in the ratio of 1:1.

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19. NRCG CS 281 (IC0616376; INGR16019), a Spanish Bunch Genotype of Groundnut (*Arachis hypogaea* L.) with Extra-large Kernel Size (HPS type)

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Groundnut (*Arachis hypogaea* L.) is a major oilseed and food crop of the world. It is cultivated approximately in an area of 25.44 m ha producing 45.22 m tons of nut in shell (FAOSTAT, 2014). Although groundnut is primarily a source of fat, it is also a rich source for protein (~25%), micronutrients and secondary metabolites. Current figures show that ~50% of groundnut produce is crushed for extraction of oil and about ~40% is used for various food purpose. Improved groundnut varieties that meet processing and or export quality are needs of the food industry. Varieties that combine 'large kernel size' with other processing quality traits like higher protein, blanchability, shelling ease, flavor attributes are chosen for table purpose and or by the industry. The groundnut, which is used for direct consumption is generally referred as large seeded groundnut or hand-picked selection (HPS) groundnut and confectionary groundnut depending upon the quality attributes (Dwivedi and Nigam, 2005). Indian groundnut is very popular in the international market for the table purpose, due to its characteristics natural nutty flavour, taste and crunchy texture. In spite of the immense export potential, only limited genotypes have been bred with an aim to obtain early maturing HPS or confectionary groundnut in India. Considering these facts, attempts were made to develop a short duration large seeded groundnut genotype.

The groundnut genotype NRCGCS 281, an interspecific derivative with extra-large kernel size has been developed through pedigree selection from a cross between '(Deep Red Mutant x Purple Variegated Mutant) x *Arachis duranansis*' at ICAR-Directorate of Groundnut Research, Junagadh, Gujarat. It has erect growth habit, produces 50% flowering in 25 days after sowing (DAS) and matures in 100 to 110 DAS during

Table 1. Mean pod yield of NRCGCS 281 (kg/ ha) and check variety over two years (2008 & 2009) at four AICRP on groundnut centres*

Genotype / Year	Junagadh, Gujarat	Rahuri, Maharahtra	Shirgaon, Maharastra	Vridhachalam, Tamil Nadu	Pooled
NRCGCS 281	3341	4644	2633	1737	3089
TPG 41 (Check)	3813	5507	2631	1726	3419
CD at 5%	453.4	1156.4	476.8	407.9	674.2

Table 2. Mean hundred kernel weight (g) of NRCGCS 281 and check variety over two years (2008 & 2009) at four AICRP on groundnut centres *

Genotype / Year	Junagadh, Gujarat	Rahuri, Maharahtra	Shirgaon, Maharastra	Vridhachalam, Tamil Nadu	Pooled
NRCGCS 281	72	87	71	86	79
TPG 41 (Check)	36	84	55	66	60
CD at 5%	51.8	16.8	16.7	24.8	30.1

*Data mentioned in Tables 1 and 2 are reproduced from the Annual report, 2009-10 of Annual *Rabi*-Simmer Groundnut Researchers Group meeting held at BCKV, Mohanpur, West Bengal during November 18-19, 2010.

rainy season. NRCGCS 281 produces an average pod yield of 3000 kg/ hawith72% shelling out turn (Table 1). Pods are moderately beaked with deep constriction and without reticulation; mostly two seeded with rose colour testa. Kernels are extra-large in size with hundred kernel mass ranging of 80-90 g (Table 2) and contain 50.5% oil. This is the first report of a Spanish Bunch early maturing groundnut genotype (NRCGCS 281) with extra-large kernel size in India (Fig. 1). Besides, it is also resistant to Rust disease and suitable for cultivation both in *rabi*/Summer as well as *kharif* seasons. It could be well promoted for table purpose and export with premium price which could fetch higher return to the farmers. In addition to interspecific breeding lines with HPS quality could be a potential donors (Bera *et al*. 2008) for breeding groundnut with extra-large kernel size and has also been used effectively in marker assisted breeding (Bindu *et al*., 2013).

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20. JOR LAB L-8 (IC0619026; INGR16020), a Lemon grass (*Cymbopogon flexuosus*) Germplasm with High Herbage Yield with High Essential Oil Content

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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. The prefix 'Lemon' owes to its typical lemon-like odor, released from 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. 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 needs and great prospect of cultivation of this crop successfully in various region of India. It is necessary

Table 1. Average morphological and quality data of Jor lab L-8 lines with checks in four locations

Variety	Plant height (cm)	Tillers/plant	Herbage yield/tones/ha	Oil % w/w	Oil yield/kg/ha/year	Citral %
Jor Lab L-8	126-138	78	30.32	1.00	303.20	78
Jor Lab L-2	118-132	42	26.80	0.45	120.60	82
Krishana	106-121	56	27.60	0.90	248.40	81

to evolve high 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 three hundred of cymbopogon species. As per record this germplasm banks is second position after the Oddakali germplasm bank. We screened the genotypes based on high oil yielding and high herbage yield and find a high herbage and high oil genotypes named as Jor Lab L-8. Then, these superior selected clones were evaluated in fourdifferent locations of NE region such as Jorhat (Assam), Lakhimijan (Assam), Imphal(Manipur) and Gaurigaon (Sikkim)where these were compared to all the checks.

As per data from Table 1 the herbage yield and tillers per plant for Jor lab L-8 was significantly higher than the checks. As well as the oil yield is higher than the check variety Krishna. The oil yield per kg/ ha/ year was significantly higher in the variety Jor lab-8. The agronomic trait plant height is not significantly different between the elite lines and checks. The higher oil yield is a desired character in lemon grass as it is more economically beneficial. This elite clone Jor Lab L-8 maintained its superiority over all the checks for herb and oil yield and the elite strain was named as variety 'Jor Lab L-8. Plants of this variety are propagated through only vegetative means using the slips, and the plants are stable for commercial cultivation.

Morpho-agronomic Characteristics

S.No.	Characters	Range
1	Plant height (cm)	126-138
2	Growth habbit	Semi compact
3	No of tillers/plant	68-87
4	Herbage yield /Qt/ha/year	287 to 313
5	Oil content %	1
6	Citral %	78

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21. JOR LAB C-5 (IC0619027; INGR16021), a Java Citronella (*Cymbopogon winterianus* Jowitt) Germplasm with High Herbage Yield with High Essential Oil content

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Java citronella (*Cymbopogon winterianus* Jowitt.) is an aromatic grass belonging to Poaceae family which gives essential oils upon steam distillation (Saikia *et al*., 2015). One of the important essential oils extracted from aromatic grasses is citronella oil obtained from citronella grass. This oil is used extensively as a source of important perfumery chemicals like citronellal and geraniol, which finds it extensive use in soap, perfumery, cosmetic and flavoring industries throughout the world The use of active secondary chemical compounds such as alkaloids and terpenoids in the medicinal plants holds a great promise in the field of medicine in ancient as well as modern era (Lal *et al*., 2016).It is cultivated in parts of tropical and subtropical areas of Asia, Africa and America (Shasany *et al*., 2000). In the view of above needs and great prospect of cultivation of this crop successfully in various region of India, It is necessary to evolve high 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 (Saikia

Table 1. Morphological and oil quality data of four locations (average) of Jor Lab C-5 with check variety

Variety	Plant height cm	Tillers/plant	Herbage yield tonnes/ha	Oil percentage w/w	Major oil content Citronellal	Superior % over oil yield	Total oil yield/ kg/ha/year
Jor Lab C-5	118	148	36.5	1.20	35 %	33%	438
Jor LabC-2 (check)	110	127	32.2	0.9%	32.5%	-	289

et al., 2015). Therefore, there is a need to develop high oil and herbage yielding genotypes of Java citronella. Jor Lab C-5 genotype was developed through mutation breeding using EMS as a mutagenic agent and the parent material was a variety of Java citronella (Jor lab C-2). In order to fulfill this gap we developed this high oil and herbage genotypes. Jor lab C-5 was planted along with check variety in four locations; it gives the high oil yield up to 1.2% and citronellal content up to 35%. This variety is 33% better in oil yield in comparison to the checks over the check.

Morpho–agronomic characteristics: The selected clone was planted in four different locations in Northeast India along with check variety Jor Lab C-2. This new genotypes contains 33 % more oil yield and 12 % more herbage yield and mean total oil yield was 468 kg/ha/year, as compared to the check variety yielding 287.60 kg/ha/year. The plant height was 118 cm in Jor Lab C-5 where as check variety Jor Lab C-2 was 110 cm.The tillers /plant is significantly superior in Jor lab C-5 with 149 compared to check variety with only 126. This elite clone Jor Lab C-5 maintained its superiority over the check for herb and oil yield and named as line Jor Lab C-5'. Plants of this variety are propagated through only vegetative means using the slips, and the plants are stable for commercial cultivation.

Agro Morphological Description of the Jor Lab C-5 Germplasm

S. No.	Characteristics	Description
1	Plant height cm	118
2	Growth habit	Bushy and droopy leaves
3	Stem	Culm, Moderately bold rudimentary, leaves coming out in whorls
4	Colour of leaf	Green
5	Inflorescence	Panicle (rare)
6	Oil content %	1.20
7	Citronellal content %	35
8	Herbage yield qt/ha	36.5
9	Major oil content	Citronellal
10	Tillers/plant	149

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22. SM/00-120 (IC0616580; INGR16022), a Photo-Insensitive High Yielding Potato Hybrid (*Solanum tuberosum* L.) with High Resistance to Late Blight both in Hills and Subtropical Plains

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Potato (*Solanum tuberosum* L.) originated in the Andes about 8000 years ago and evolved as long day, temperate, summer crop. British or Portuguese traders most probably introduced it into India during early 17th century. Initial efforts to introduce and acclimatize European varieties were unsuccessful due to long day adaptability of these cultivars. The organized breeding to develop indigenous varieties began in 1935 for Indian hills. Till date, CPRI has developed and deployed 53 commercial cultivars suitable for growing under different eco-geographical locations but Kufri Jyoti, released in 1968 and Kufri Himalini released in 2006 are the only two successful examples

Table 1. Mean performance of advanced hybrid SM/00-120 at hills (Kufri) and sub-tropical plains (Modipuram)

Genotype/ controls	Kufri					Modipuram			
	Total yield (t/ha)								
	2011-12	2012-13	2013-14	Overall Mean	% Increase over	2012-13	2013-14	Overall Mean	% Increase over
SM/00-120	11.11	19.36	11.0	13.83	-	39.73	37.4	38.6	-
Kufri Girdhari	8.31	18.54	9.67	12.17	13.6	-	18.6	18.6	107.3
Kufri Jyoti (Sprayed)	9.12	13.45	8.53	10.37	33.4	20.58	28.40	24.50	57.50
Kufri Jyoti (Unsprayed)	9.79	10.08	7.73	9.20	50.2	-	-	-	-
Kufri Himalini	9.39	15.44	8.92	11.25	22.9	-	34.8	34.8	10.8
Kufri Bahar	-	-	-	-	-	27.13	38.1	32.6	18.2
Kufri Sadabahar	-	-	-	-	-	23.62	-	23.62	63.3
$CD_{0.05}$	19.65	17.6	12.9	-	-	6.96	4.5	-	-

in the sub-continent which performs considerably well both under short and long day conditions.

Among biotic stresses affecting potato, late blight caused by oomycetes *Phytophthora infestans* is the most devastating for potato cultivation. In India, late blight acts as serious impediment in increasing potato production and affects most of the potato growing regions in varying degree depending upon the variety grown and control measures adopted (Singh *et al*., 2007). It appears in epiphytotic conditions in the hills every year and once in 2-3 years in devastating form, in the plains. Losses caused by this disease may go as high as 85% in the hills, if the varieties grown are susceptible and are not chemically protected, while losses up to 60-70% can occur in the plains in some years. Historically, resistance against late blight is not durable because of development of matching virulences in the pathogen thereby ensuing an everlasting battle between the breeders and the pathogen. Consequently, development and deployment of new, resistant varieties became the only viable option alongwith other blight management strategies to check this menace. Late blight resistance is a desired trait both in hills and plains but higher the level of resistance longer is the crop duration thus rendering such varieties difficult to fit in short crop duration in the plains.

Disease resistance and adaptability of a genotype to varied environmental conditions is an added advantage for its success. Endogenous plant hormones mediate photoperiod response and day neutral varieties have no or low hormonal-photoperiod response thus perform well both under long and short day conditions. Although, varieties like Kufri Jyoti and Kufri Himalini were bred for long day conditions but now are well adapted for shorter day conditions of plains and plateau regions. However, these varieties have now become susceptible to late blight resulting in lower yields. Consequently, new short duration varieties with high resistance to late blight and photo-in-sensitivity needs to be developed and deployed in these areas to sustain profitable potato production.

Hybrid, SM/00-120 is a promising clone selected from the segregating generation of the cross EB/A-304 × Ex/A-680-16 made in year 2000. It is a day neutral, late blight resistant advanced hybrid with medium maturity and produces yellow skinned ovoid tubers with shallow eyes and cream-yellow flesh. The hybrid yields high both under hills and sub-tropical plains conditions. At Kufri, hybrid out yielded the control variety, K. Girdhari by 13.6%, K. Himalini by 22.9% and Kufri Jyoti (sprayed) by 33.4% for total tuber yield over last 3 years at 100 days crop duration (Table 1.). Under sub-tropical plains at Modipuram, hybrid out yielded the local control, K. Bahar by margin of 18.2%, K. Himalini by 10.8% and Kufri Jyoti by 57.5% for total tuber yield over last 2 years. At Kufri, though the AUDPC of the hybrid is higher (131) than highly resistant control variety, Kufri Girdhari (33) but it is much lower than susceptible control varieties viz., K. Jyoti (799) and K. Himalini (520). The hybrid possesses high level of resistance against late blight along with medium dormancy and good keeping quality under country store conditions. With these parameters, SM/00-120 can be helpful in expanding the potato area under kharif cultivation and can suitably supplement Kufri Jyoti, the leading cultivar of the country.

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CPRI Annual Report, 2014-15, pp. **74.**

23. SS 1652-09 (IC0616581; INGR16023), a Wild Potato (*Solanum Jamesii*) Clone Possessing High Resistance to Late Blight and Low Cold Induced Sweetening

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The discovery and incorporation of resistance and other genes of economical value to the cultivated germplasm is the key for sustaining crop productivity. In potato, late blight is caused by oomycete *Phytophthora infestans*, and is the most important biotic stress afflicting potato crop worldwide. Host resistance is an important component for the management of late blight on potato.To reduce the cost of the disease and the environmental damage, it is important to identify resistance that can be used inbreeding programs. In the past, 11 major resistance genes(R-genes) were introgressed from hexaploid *S. demissum* into cultivated potato and all these genes confer race-specific resistance.Thus, the resistance based on these geneswas quickly overcome by the pathogen. Hence, newsources of resistance are required to develop late blightresistant potato varieties. Utilization of *S. demissum* as donor parent for late blight resistance breeding led to high genetic similarity among many potato cultivars released and results in vulnerability of crop to the pathogen and necessities search for new resistance source in the wild potato species in order to broaden the genetic base of future cultivars.Keeping this view wild potato species were screened for late blight resistance by detached leaf method for two years in laboratory condition and one year screening in open field under natural epiphytotic condition. For detach leaf method six leaflets of top 4-5th leaf from 2-4 plants/ clone were artificially inoculated with complex races of *P. infestans* using 6×10^6 sporangia per ml. For field screening the clones were graded on 1-5 scale (1-highly resistant, 5-highly susceptible). Based on results obtained accession SS1652-9 of *S. jamesii* was reported highly resistant (lesion area <1.0 cm^2). This identified resistance source can be used to further diversify the source of resistance to late blight in cultivated potatoes.

Similarly, producing light-colored chips is a significant challenge to the potato industry. Over 90 % of the annual potato crop in India is grown in sub tropics during short days of winter season and harvested in the month of February-March when the temperature starts rising. This necessitates the storage of potato at low temperature (2-4 °C) for round the year availability. Low-temperature storage is highly beneficial because it extends storage duration by minimizing pathogen activity, reduces water loss and prevents tuber sprouting. But low temperature storage stimulate cold-induced sweetening (CIS), which is characterized by an accumulation of sucrose and conversion of sucrose to reducing sugars by vacuolar acid invertase. Cold stored potatoes produce dark colored chips (Malliard reaction) that are carcinogenic and disliked by consumers. Resistance to CIS, or cold chipping, is the ability of a genotype to produce light colored potato chips directly following cold storage. An ideal reducing sugar content should be below 100 mg per 100 g of tuber fresh weight (0.1 %).

For resistance to cold-induced sweetening there is a need of introgression genes from wild *Solanum* species as till date no cultivar has been reported resistant to CIS.Despite the presence of heritable variation for cold chipping, no commercial cultivars are currently available that can chip acceptably directly from 4 °C storage. This necessitates the potato growers to use post harvest agrochemicals to reduce storage losses. The identification of cultivars that chip directly from 4 °C will allow growers to reduce storage losses without the use of post harvest agrochemicals. The biggest impediment towards

development of cold resistant potato cultivars through conventional breeding is lack of suitable germplasm to be used as parents. Therefore, to identify genotypes resistant to cold induced sweetening, 72 accessions of 15 wild and cultivated species were screened for glucose content before and after cold storage (cold stored at 2-4 °C for nearly six months, without reconditioning) during 2007-08 and 2008-09. The glucose estimation was done by analyzing the potato tuber juice through YSI Biochemistry Analyzer.

Wild species accession namely SS1652-9 (*S. jamesii*) maintained low glucose level (glucose content <50 mg/100 gram fresh tuber weight) before and after cold storage over the years. This accession can be used to diversify the genes of resistance to cold induced sweetening in cultivated potatoes as most of the wild species can be crossed to cultivated types following ploidy manipulations. This wild potato accession has medium plant height,hollow stem,open canopy, open leaf structure with narrow lanceolate leaflets,white flowers, moderate flowering and high pollen fertility. The tubers are white-cream,smooth, roundwith shallow eyes.

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GUIDELINES TO AUTHORS

GENERAL

The Indian Journal of Plant Genetic Resources (IJPGR) is the official publication of the Indian Society of Plant Genetic Resources. For publication in the journal, the authors must be a member of the Society. IJPGR publishes full-length papers or short communications of original scientific research in the field of plant genetic resources. Review articles (with prior consent or invitation only) summarizing the existing state of knowledge in topics related to plant genetic resources will also be published.

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

ORGANIZATION OF THE MANUSCRIPT

Full-length papers

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

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

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

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

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

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

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

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

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

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

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

Line drawings of high quality, preferable in the desired final size would be accepted. The inscriptions should be clearly legible.

Photographs for publication should be of high contrast, black and white, glossy print, trimmed at right angles. Magnification should be indicated with a bar scale on the photo. Authors need to indicate colour reproduction of photographs (cost of colour printing borne by the authors).

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

References in text should be cited by author, year of publication (e.g. Joshi, 1995) and multiple citations should be in chronological order (Withers and Englemann, 1998; Rao *et al.*, 2001). References should be listed in alphabetical order under the first authors' name. The names of journals should be abbreviated according to the latest edition of the World List of Scientific Periodicals (eds. P Brown and GB Stratton), Butterworths, London. The following examples may be used for citations:

1. Bisht IS, RK Mahajan, TR Loknathan and RC Agarwal (1998) Diversity in Indian sesame collection and stratification of germplasm accessions in different diversity groups. *Genet. Resour. Crop Evol.* **45**: 325-335.
2. Withers LA and F Engelmann (1998) *In vitro* conservation of plant genetic resources. In: A Altman (ed.) *Agricultural Biotechnology*. Marcell Dekker Inc., New York, pp 57-58.
3. WOI (1985) *The Wealth of India - Raw Materials. A Dictionary of Indian Raw Materials and Industrial Products - Raw Material* Vol 1: A (Revised). Publications and Information Directorate, Council of Scientific and Industrial Research, New Delhi, 513 p.
4. Engels, JMM and V Ramanatha Rao (eds) (1988) *Regeneration of seed crop and their wild relatives*. Proceedings of a Consultation Meeting, 4-7 December 1995. ICRISAT, Hyderabad, India and IPGRI, Rome, Italy, 167 p.

Short Communications

The style and format as mentioned for full-length papers should be followed for Short Communications. However, the abstract should be restricted to not more than 50 words and the remainder text should be continuous (without headings). Illustrative material should be kept at minimum, usually not more than one table or figure and only few references should be included (not more than 10).

Submission of the Manuscript

Submission of an article will be held to imply that it has not been previously published or submitted for publication elsewhere. A cover letter including a statement to this effect should be submitted with the manuscript. Three copies of the manuscript written in English and typed in double space on one side of A4 bond paper with the pages numbered consecutively (starting with the title page and through the text, reference list, tables and figure legends, and should be submitted (preferably in floppy or CD or through email) to:

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Publication of a paper in the Journal does not imply the responsibility for an agreement with the statements or view written therein, and rests entirely on the authors thereof.

The authors will receive page proofs, which should be corrected and returned without delay. Corrections must be kept to the minimum, and the proof stage should not be regarded as an opportunity for further editing and additions. Although, every effort is made by the editors to correct proofs of all the papers they assume no responsibility for errors that may remain in the final print.

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