Characterisation and Evaluation of PGR: Principles and Techniques

Ashok Kumar and **Vikender Kaur**
Division of Germplasm Evaluation, ICAR- NBPGR, New Delhi

Plant Genetic Resources (PGR) refers to "germplasm" or "genetic diversity" of actual or potential value that exists among individuals or group of individuals belonging to a species. The full spectrum of PGR consists of diverse type of collections such as those derived from the centres of diversity, centres of domestication and from breeding programmes. PGR broadly includes landraces, farmers' varieties, breeding material, genetic stocks, obsolete and modern varieties, wild and weedy relatives of cultivated plants, and potential domesticates such as wild species. Amongst the total number of species of higher plants which have been identified world-wide (250,000), PGR comprise 40% of these species, while the crop plants (cultivated as agricultural or horticultural species) cover only 2.8% of the species. Nevertheless, it is often stated that only 30 species "feed the world" providing more than 90% of calories or protein to human nutrition (FAO, 2010). Intensive modern breeding efforts in these staple food crops for higher yields have led to a narrowing of the gene pool by concentrating more on favorable alleles. Furthermore, the increasing genetic uniformity of crop varieties combined with climate change effects makes crops more vulnerable to various biotic and abiotic stresses. PGR are therefore important for maintaining genetic diversity for and preventing such losses, which may have serious consequences for food, nutrition and environmental security.

The need of characterization and evaluation

The role of PGR in crop improvement is well recognized. PGR provide the raw materials for crop improvement for productivity enhancement, cytoplasmic male sterile lines for hybrid breeding, nutrition, disease resistance, insect-pest resistance, breeding for non-conventional seasons/area and breeding for adaptation to climate change (Dhillon and Agrawal, 2004). Characterization and evaluation is the key to assess the potential and actual value of germplasm. Therefore, it is gaining importance all over the world due to the utilization of PGR in crop improvement programmes. Such activities are also consistent with the Convention on Biological Diversity under which countries agreed to conserve, sustainable use and benefit sharing from PGR.

Techniques for characterization and evaluation

A number of techniques are available for characterization and evaluation depending upon the need, type and nature of the plant/material available. Amongst these, a) agro-morphological traits b) Biochemical traits, and c) molecular or DNA based markers are the prominent techniques for characterization and evaluation of PGR. The techniques of field-testing for agro-morphological parameters can't be avoided even though these are highly influenced by the environment. The DNA based markers or molecular markers are also gaining importance because of the lack of environmental influence on these molecular makers.

Principles of germplasm characterization and evaluation

Germplasm characterization and evaluation in the broad sense and in the context of genetic resources is the description of a particular accession. It covers the whole range of activities starting from the receipt of the new samples by the curator and growing these for seed increase, characterization and preliminary evaluation, and also for further detailed evaluation and documentation. There is a need for its systematic evaluation in order to know its various morphological, physiological and developmental characters including some special features, such as stress tolerance, insect pest and disease resistance. Newly explored collections, trait specific exotic introductions for location specific character expression and the accessions redrawn

from genebank after long interval form the basic material for characterization and evaluation. The germplasm accessions are usually evaluated for two consecutive years for documentation and preparation of crop catalogue. For effective evaluation of germplasm, a close organization and personal contact between curator and breeder is necessary in the context of breeding objective *vis-a-vis* evaluation programme.

Characterization

The characterization of germplasm deals with the understanding and recording of highly heritable traits which are generally expressed in all the environments, therefore, it can be performed in a single environment. The characterization should be carried out during the initial stage of acquisition of germplasm and may be completed in a suitable environment preferably at a site nearest to the site of collection of the germplasm or under similar agro-climatic conditions. The internationally/nationally accepted descriptors and descriptor states' should be used to record observation such as those developed by UPOV, USDA, Bioversity International (formerly known as IPGRI/ IBPGR) or NBPGR Minimal Descriptors for Characterization and Evaluation of Field Crops (Mahajan *et. al*., 2000). Wherever, descriptors and descriptors' states are not available these should be developed by germplasm curators in consultation with crop advisory committee and crop experts for a given crop species (Bioversity International, 2007). Biological status of the germplasm should also be known in advance to determine the characterisation strategy. With the advancement of techniques, the molecular and biochemical markers are being used. The highly reproducible molecular markers like Amplified Fragment Length Polymorphisms (AFLPs), Simple Sequence Repeats (SSRs) and Single Nucleotide Polymorphisms (SNPs) should be used for characterization. The field experiment should be conducted with statistically sound experimental design depending upon the quantity and number of germplasm accessions under trial. Augmented block design (ABD) is generally recommended if the planting material is less in quantity or the number of accessions are more than fifty (Federer, 1956). The checks should be replicated in each block after separate randomization of checks within a block in ABD trials. However, for less number of accessions randomized block design (RBD) may be followed.

Characterization data may be used in establishing taxonomic identity as it is not influenced by environment. Wherever, the number of accessions is more, the characterization data is generally utilized to develop core set comprising 10 per cent of the entire collection representing the total variability in the germplasm to bring them to a manageable level (Frankel, 1984; Brown, 1989). However, if the core set is too large to handle, a mini core set comprising 10 per cent of the core set representing the total variability in the core set is developed for facilitating germplasm management in crop improvement (Upadhyay and Ortiz, 2001). Several methodologies may be followed to arrive at the desired core and mini core sets from the entire germplasm collection and the same may be validated through various statistical tools and techniques.

Evaluation

Germplasm evaluation deals with the assessing the agronomic potential of an accession including quality parameters and response to various abiotic and biotic stresses. Evaluation of germplasm resources is necessary to identify the appropriate germplasm with a target trait for their further utilization. Genetic resources are invaluable sources of variation for improving agricultural productivity. However, the conservation of a resource only becomes important if it acquires recognized value which can be assigned only through thorough evaluation of the germplasm for the critical genetic material. Evaluation should be undertaken in germplasm accessions which are already characterized and where enough quantity of planting material is available. They should confirm to standardized and calibrated measuring formats. Evaluation of germplasm is a multi-disciplinary approach and it should be done in collaborative mode involving germplasm curator, plant breeder, physiologist, pathologist, entomologist, biochemist etc. A network/coordinated approach at multiple locations under different agro-climatic zones involving

crop-based institutes, project directorates, AICRP centres is recommended. The accessions should be evaluated in the area of their adaptation or under similar environmental condition considering the breeding behavior and biological status of germplasm. Standard agronomic practices prescribed for raising a good crop including proper plant spacing, fertilizer application, weeding, irrigation, plant protection measures need to be followed.

Evaluation experiments should be conducted with proper experimental design, depending upon the number of accessions to be evaluated. The blocks should be laid out across the soil fertility gradient of the experimental plot. In general, ABD is being practised in large number of accessions. In ABD, the checks should be replicated in each block after separate randomization of checks within a block. The number of checks will depend upon the crop and the parameters under study and representative of the type of germplasm. Three or more checks in which one national as well as one locally adapted check used for comparative assessment of germplasm. For few or promising accessions and less soil heterogeneity, evaluation should be done in RBD wherein the checks should be randomized along with the accessions in each replication. The experimental plot should have at least three rows of 3-5 m length for each accession with recommended gap in between the accessions The number and row length should be more for cross pollinated species than those for self-pollinated ones. The observations should be recorded on the plants from the middle row to avoid the border effects. After evaluation, the promising accessions should be further validated through multi-locational, multi-season and multi-year evaluations. Majority of agronomic descriptors are polygenic in nature and highly influenced by the environment. Therefore, e valuation trails should be carried out in at least three diverse environments to minimize Genotype x Environment (G x E) interaction.

Evaluation for biotic stresses: Evaluation of crop germplasm against diseases is very crucial as degree of expression of diseases depends upon both the germplasm, virulence of pathogen and the environmental conditions. Since, there are number of biotic stresses that are of economic importance, prioritization should be done taking into account the targeted one at a time. Preliminary screening is done for one year with large number of accessions to narrow down the numbers to a manageable extent. Identification of resistance source against a particular race/strain/isolate/biotype within a particular location does not guarantee its resistance response in other locations as race/strain/isolate/biotype may vary depending upon the agro-meteorological conditions. Therefore, screening of germplasm for biotic stresses should be accompanied with identification race/strain/isolate/biotype of the pest and pathogens existing at that particular location. These four factors i.e. susceptible host, virulent pathogen, environment and time, which cause disease called is called "disease quadrangle". Therefore, non-occurrence of symptoms in any germplasm accession or reduced symptom production in any accession does not always imply resistance or tolerance of those germplasm, rather there may be chances of escape from the infection. Under these circumstances, repeated field screening with proper experimental design followed by stringent screening under artificial challenged inoculation condition under field and glasshouse are essential to establish a real resistance or tolerance response of germplasm. For recording of the observations on fungal, bacterial and viral diseases, standard evaluation system (SES) scale should be followed. For recording of the data on defoliators, percentage infestation in each accession should be recorded. In case of sucking insect pests, the number of insects per unit plant/leaves/inflorescence should be counted.

Evaluation for abiotic stresses: Abiotic stresses are gaining importance in context of changing climate. Preliminary screening/phenotyping should be done for one year with large number of accessions under field conditions. To narrow down the large number of germplasm accessions, preliminary screening for abiotic stress should be carried out for one year with large number of accessions under field conditions peculiar to the stress under study. Evaluation of germplasm for tolerance to different abiotic stresses is to be carried out under well-defined controlled conditions so that the optimum stress can be imposed at the desired stage. Like, evaluation for light and temperature stresses should be carried out under phytotron facility and for drought tolerance should be undertaken in drought plots with rain out shelters and with well defined moisture conditions. Descriptors for evaluation like canopy temperature; reflectance etc., at different

phenophases should be used for high temperature and drought stress (Pask *et. al*., 2012 and Reynolds *et al.,* 2012). Microplots with well defined electrical conductivity including specific salt/ions should be used for evaluating germplasm under saline/alkaline conditions. Standard checks identified for specific abiotic stress should be used for proper comparison of the germplasm.

Conventional methods of assessing plant growth under abiotic stress involve destructive analysis of plants but recent developments in digital imaging allow comprehensive and quantitative non-destructive analysis of plants. Image analysis in phenotyping plants for stress tolerance in a high-throughput manner has opened new opportunities for plant biologists. Plant phenotyping combines plant biology, sensor technology, and automation engineering and is gaining increasing importance owing to the need to accelerate progress in plant breeding. These novel facilities enabled the automated imaging of plants in both controlled environments and the fields. Automated phenotyping involves:

i. non-invasive sensors based on spectrometry (Hyperspectral radiometers, Fourier transform infrared spectroscopy (FTIR), Infra-red (IR) Thermometry, Near Infra-red (NIR) meter, etc.) and spectroscopy (Visual imaging, Hyperspectral imaging, IR thermography, NIR image analysis, Chlorophyll fluorescence imaging, bioluminescence imaging, fluorescence imaging)
ii. automated data processing to obtain phenotypic traits of interest,
iii. robotized delivery of plants to sensors or vice versa,
iv. robotized plant culturing, and
v. automated analysis of processed data in a data management pipeline.

National Phenomics Facility is available at IARI (New Delhi), CRIDA (Hyderabad) and NIASM (Baramati) for accelerated and high throughput phenotyping.

Evaluation for quality parameters: Quality evaluation plays an important role for in identification of value rich germplasm keeping in view of the nutritional and health security. Quality parameters are analyzed using standard protocols in a well equipped laboratory. Quality traits like oil, fatty acids, protein, phenols, sugar, amino acids, vitamins, minerals and anti-nutritional factors requires specialized equipments which needs a lot of investment, therefore, strong linkages are needed with referral laboratories. Adequate quantity of pure, clean and dry material free from any infestation and harvested at appropriate stage should be analyzed depending upon the parameters. Properly labeled and packed material containing complete experimental details along with passport information should be made available for quality analysis.

Utilization of trait-specific germplasm

I. ***Development of core-set, mini-core and reference sets:*** The large sizes of many of these ex-situ germplasm collections not only complicate their characterization, evaluation, but also their utilization and maintenance (Rao and Hodgkin, 2002). The concept of core collections was introduced to increase the efficiency of characterization and utilization of collections stored in the gene banks, while preserving as much as possible the genetic diversity of the entire collection (Frankel 1984; Brown 1989). Frankel (1984) defined a core collection as a limited set of accessions representing, with minimum repetitiveness, the genetic diversity of a crop species and its wild relatives. It was envisaged that such collections, which would contain approximately 10% of the collection, or 2000–3000 accessions, whichever is the smaller, would provide the starting material for breeders in search of new variation or specific characters and research workers investigating diversity.

The concept of the core collection appears to offer a number of potential benefits to users of genetic resources. Plant breeders would have a manageable number of accessions to use in the search for new characters or character combinations and a structured way to evaluate whole collections. More than 60 core collections have been identified in a wide range of different crops and wild relatives (Rao and Hodgkins, 2002). A number of references suggest alternative types of collections, or sets of collections, to enhance the efficiency of capturing diversity or addressing utilization, including specialized core collections (Brown and Spillane, 2004), mini core sets (Upadhyaya and

Ortiz, 2001), nested core collections (Mc Khann *et al.*, 2004) and composite collections (Furman, 2006). Despite this diversity of core collection methodology, there seems to be a lack of literature that demonstrates that core collections have had a significant impact on the utilization of genetic resources. Rare and adaptive alleles, most of which are thought to be functional, may even be missed from a core collections (Brown and Spillane, 2004; Polignano *et al.* 2001; Pessoa-Filho *et al.* 2010). The role of NBPGR has been instrumental in unlocking the hidden genetic potential of the plant genetic resources for broadening the genetic base of crop cultivars through identification of core, mini-core, trait specific reference sets as well as genomic resources. The core sets of Brinjal, Chickpea, Wheat, Mung bean, Sesame, Okra, wild *Lens* sp., etc. are available with NBPGR.

II. **Focused Identification of Germplasm Strategy (FIGS):** FIGS is a new tool developed jointly by ICARDA, the Vavilov Institute of Plant Industry in Russia, and the Grains Research and Development Corporation (Australia) to improve the efficiency with which adaptive traits are identified from a PGR collection. Since 1990s, geographic information system (GIS) has been specifically applied to the genetic resources conservation which is a database management system that can simultaneously handle digital spatial data and attached, non-spatial, attribute data. FIGS combines agro-ecological information with data on plant traits and characteristics. By using the eco-geographical data of a reference dataset of accessions with resistance to the sought after adaptive trait, such as resistance to either diseases or pests, the FIGS has successfully helped to identify a number of novel genes in germplasm from environmentally similar sites to those of the reference/template dataset (Mackay, 1995; Mackay and Street, 2004). FIGS datasets identify sets of plant genotypes from large number of collections with a higher probability of containing specific 'target' traits. This strategy allows gene bank managers and agricultural researchers worldwide to screen large plant genetic resource collections more rapidly and accurately than was previously possible using traditional methods.This strategy has helped identify sources of resistance to biotic stresses in wheat such as powdery mildew (Bhullar *et al.* 2009), Russian wheat aphid (El-Bouhssini *et al.* 2010), stem rust (Endersen *et al.* 2011); net blotch disease resistance in barley (Endresen *et al.* 2011) and abiotic stress tolerance such as drought tolerance in *Vicia faba* (Khazaei *et al.* 2013).

III. **Documentation for wider access:** The success of any crop breeding program primarily depends upon the availability of reliable genetic stocks for key characters. Genetic stocks for several important agro-morphological traits have been identified in different crops which can be utilized in the breeding programmes to suit different needs. The utilization of the genetic stocks in the breeding program has been proved of high value in various crops such as wheat, rice, maize, soybean and tomato. Analyzing the phenotypes of gene-bank accessions, thereby enabling researchers to create an internationally accessible informatics infrastructure to catalogue the diversity in the world's seed collections has been recently proposed (Mc Couch *et al.* 2013). Online documentation of the germplasm in the form of online genetic resource database such as NIASGBdb can be easily accessed by researchers in crop improvement programmes to promote the utilization of genetic resources for the development of improved crops (Takeya *et al.* 2011). The publication of the evaluated germplasm through catalogues, proceedings of the workshops, research papers, notification of germplasm registration (NBPGR, 2006; Kak *et al.* 2009), trait specific germplasm identified through multilocation trials (http://www.nbpgr.ernet.in:8080/tsgi/index.htm) have been made available for wider circulation, so that it can be assessed by breeders for utilization in crop improvement programmes. The PGR information has been duly documented by NBPGR in user friendly databases such as PGR Portal and mobile/desktop apps Genebank, PGRMap. NBPGR is the nodal agency (since 1997), for registration of elite germplasm/genetic stocks with unique traits. 1100 unique germplasm accessions have been registered and the Inventory of registered germplasm is available at NBPGR website (http://www.nbpgr.ernet.in:8080/ircg/index.htm), In addition, over a thousand applications have been filed over with Protection of Plant Varieties and Farmers's Rights (PPV&FR) authority for varietal registration.

IV. **Organization of germplasm field days and germplasm supply to the indenters:** One of the important steps in utilization of germplasm is the distribution of germplasm with specific traits for need based research to different crop specific institutes/researchers. Based on the characterization and evaluation work carried out over the years, several accessions have been identified in different crops for desirable traits, which can be of value in crop improvement. During past five years more than 20 field days have been organized for various cereals, oilseeds, pulses and potential crops and more than 15,000 accessions have been supplied to indenters.

Conclusion

The very basis for any crop improvement programme is the extent of variability available for different economically important traits in the germplasm. The germplasm exploration and collection have resulted in accumulation of enormous genetic diversity of crop plants in the gene banks. Therefore, concerted efforts need to be made for its characterization, evaluation and identification of trait specific accessions especially from unexplored/exotic germplasm using field phenotyping coupled with modern genomic tools to trace the underlying gene. Once these alleles identified can be effectively utilized in crop improvement programmes through targeted introgression using molecular marker assisted/genomic assisted breeding.

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