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Conservation of PGR for Effective Utilisation: New Initiatives at NBPGR

RK Tyagi
ICAR-National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi–110012, India

The Indian National Genebank (NGB) comprising four kinds of conservation facilities, namely, Seed Genebank (-18°C), Cryogenebank (-170 to -196°C), *In vitro* Genebank (25°C) and Field Genebank, was established at ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR) to cater the requirement of long-term as well as short-term conservation of plant genetic resources (PGR) of local as well as global importance. Whilst the seed, *in vitro* and cryogenebanks are located in NBPGR, New Delhi, the field genebanks are spread across the 10 regional stations of NBPGR in various agro-climatic zones of India. The NGB is also supported by the active partnership of other crop-based institutions designated as the National Active Germplasm Sites (NAGS), responsible for maintaining, evaluating and distributing germplasm from their active collections to NGB and other user scientists. Till date about 0.48 million accessions belonging to nearly 2,000 species are conserved in the NGB comprising 0.42 million in the seed genebank, 11,200 in cryogenebank, 1,902 in the *in vitro* genebank and 51,000 in the field genebanks of NBPGR.

Genebanks today are not only viewed as instruments important for safeguarding plant genetic diversity for future availability, but also as an active source in providing genetic resources to the research and plant breeding community for immediate and short-term use. Rapid strides in molecular biology (sequencing technologies, synthetic biology and gene editing) and computational science (informatics), is directly impacting operational management and agendas of genebanks. For instance, genebanks need to provide novel services, such germplasm linked with trait-specific data, PGR portals with multiple query systems to select specific germplasm, with data on varied parameters such as agronomy, yield, quality, stress and gene sequences. Accordingly, with the objective to be a source of authentic, quality germplasm to users, several initiatives related to genebanking procedures, protocols and standards have been taken in the recent past at NBPGR, and described hereunder.

Characterisation of Germplasm and Development of Core Collections–Morphological and Molecular Approaches

Characterisation and evaluation of germplasm held in the NGB was undertaken in the past by screening small holdings of each crop, at a time (200-500 accessions). This approach was modified during 'National Initiative on Climate Resilient Agriculture' (NICRA), a network project of the Indian Council of Agricultural Research (ICAR) launched in February, 2011. Under a sub-project entitled 'Acquisition, Evaluation and Identification of Climate Resilient Wheat and Rice Genetic Resources for Tolerance to Heat, Drought and Salt Stresses', a mega characterisation and evaluation approach was adopted, to screen the entire cultivated genepool of wheat (22,469 accessions) and chickpea (14,651 accessions) held in the NGB. In the case of wheat, characterisation for 22 qualitative, and 12 quantitative parameters was undertaken after which a core set (of 2,208 accessions) was developed using PowerCore Software with step-wise approach and grouping method and validated using Shannon-Diversity Index and summary statistics. The core set comprised accessions of *T. aestivum (*1,770), *T. durum (*386), and *T. dicoccum* (52) as a representative of the total diversity recorded in the wheat germplasm (Dutta *et al*., 2013; Bansal *et al*., 2013).

Similarly, for chickpea germplasm held in NGB (14,651 accessions), eight quantitative and 12 qualitative agro-morphological traits were characterised in a single large-scale experiment (Fig.1). Allelic richness procedure was employed to assemble a core set comprising 1,103 accessions, 70% of which were of Indian origin. Comparable values of total variation explained by the first three principal components in the entire collection (51.1%) and the core (52.4%) together with conservation of nine pairwise r values among quantitative traits in the core collection and a coincidence rate around 99.7% indicated that the chickpea core was indeed an excellent representation of the entire chickpea collection in the NGB (Archak *et al*., 2016).

**Author for Correspondence: Email- rishi.tyagi@icar.gov.in*

Fig. 1. Characterisation of chickpea germplasm conserved in National Genebank at ICAR-NBPGR, New Delhi (inset: an accession with upright peduncle suitable for mechanical harvesting)

From the 4,274 accessions of french bean germplasm originating from 58 countries and held in the NGB, large scale characterisation was carried out for 22 phenotypic traits. Based on multivariate analysis, the entire collection could be grouped into 10 genetically diverse clusters irrespective of the origin or place of collection of accessions. First three components obtained through principal component analysis explained 80.44 % of the total variance and it was contributed mainly by pod length, pod width, seed length, seed width, pods/plant and seed weight (Rana *et al*., 2015).

A systematic characterisation of entire genebank collections have been initiated in 2014 to develop the cores and minicores under Consortium Research Platform (CRP) on Agrobiodiversity, with dedicated funding from ICAR. So far more than 50,000 accessions of various crops for 25-32 descriptors have been characterised and data are being analyzed for further use.

Besides agro-morphological data, molecular markers (SNP) are also being used to identify core collections in the NGB. Simple sequence repeat (SSR) and Single Nucleotide Polymorphic (SNP) were assessed for genetic diversity and population structure in rice (375 varieties) conserved in the NGB, using 36 genetic markers. Analysis of molecular variance (AMOVA) indicated that maximum diversity was partitioned between and within individual level but not between populations. The resolution of population was higher with SNP markers, but SSR were more efficient for diversity analysis (Singh *et al*., 2013a). Based on these results, genetic diversity and associated population structure of 6,984 rice accessions, originating from the North-East Region (NER) of India, were assessed using 36 genome wide unlinked SNP markers distributed across the 12 chromosomes. AMOVA analysis showed that maximum diversity was partitioned at the individual accession level (73% for Nagaland, 58% for Arunachal Pradesh and 57% for Tripura). Using POWERCORE software, a core set of 701 accessions was obtained, which accounted for ~10% of the total NER collections, representing 99.9% of the allelic diversity (Choudhary *et al*., 2014)

It is widely accepted that development of core and mini-core collection is an efficient way for effective utilisation genetic resources in crop improvement programmes. The above and other core sets of germplasm identified in the NGB, with well-recorded morphological, agronomical and genetic traits are proving to be valuable resources for genomic studies and crop improvement strategies to utilise further in developing the climate-resilient varieties. Their information can be accessed at http://www.nbpgr.ernet.in/pgrportal.

Identification of Trait-Specific Germplasm

Another recent thrust being accorded to collections held in NGB is the identification of accession(s) with specific trait(s). For instance, wheat germplasm was

Fig. 2. Evaluation of wheat germplasm against rust disease under NICRA project

Fig. 3. Evaluation of wheat germplasm against spot blotch. Inset showing resistant (R) and susceptible (S) lines

screened against major diseases (the three rusts, foliar blight and Karnal bunt) under hot-spot and artificially epiphytotic conditions (Sharma *et al*., 2012). Out of the evaluation of 2,200 selected bread wheat germplasm screened under field condition against leaf rust (*Puccinia triticina*), 1,526 exhibited symptom of leaf tip necrosis (LTN). Ninety-eight per cent of accessions showing LTN revealed reduced leaf rust severities from traces to 40 compared to 80-100 in susceptible infector rows. LTN reduced leaf rust severities independent of its low, medium and high expressions across the accessions. The data indicated presence of durable resistance gene *Lr*34 associated with the trait of LTN, either singly or in complementation with other genes (Kumar *et al*., 2014). In another set of experiments, evaluation of wheat germplasm at rust hot-spots in field yielded identification of 689 accessions being non-race-specific resistant/moderately resistant to different rusts diseases. Out of these, under artificial inoculation conditions, 19 multiple rust resistant to dominant races; 101 accessions resistant to leaf and stem rust; 37 accessions to stem and yellow rust; 23 accessions to leaf and stripe rusts; 50 accessions to leaf rust, were identified (Sharma *et al.*, 2012). Wheat accessions IC5361402 and EC573562 have been registered as genetic stocks for rust resistance, possessing 3 minor/APR genes for rust resistance (*Lr*34/*Sr*57/*Yr*18/*Pm*38; *Lr*46/*Sr*?/*Yr*29/*Pm*39; *Lr*67/*Sr*55/*Ys*46/*Pm*46 (Sivasamy *et al*., 2014).

Entire collection of wheat (19,460) of *Triticum aestivum, T. durum* and *T. dicoccum* available in NGB was evaluated to identify sources of rust and spot blotch resistance during the 2011-14 (Fig. 2). In first round, 4925 accessions were found to be resistant for stripe rust and spot blotch at respective hot-spots. During second round of evaluation, 498 accessions potentially resistant to multiple rusts and 868 accessions resistant to spot blotch were identified (Fig. 3). Evaluation of rust resistant accessions for seedling resistance against seven virulent pathotypes of three rusts under artificial epiphytotic conditions identified 137 accessions with multiple disease resistance. Molecular analysis was done to identify different combinations of genetic loci imparting resistance to three rusts and spot blotch using linked molecular markers; consequently 45 wheat accessions containing resistance genes against all three rusts as well as a QTL for spot blotch resistance were identified. The resistant germplasm accessions can be employed to transfer multiple disease resistance into the background of high yielding wheat cultivars.

Biochemical analysis is also an important tool for identifying important germplasm for nutritional traits. In one project undertaken for evaluating traditional varieties (100) of rice from tribal regions of eastern India, nutritional traits (protein, amino acid, starch, amylose and amylopectin) was assessed to identify superior cultivars. Seeds of tested cultivars exhibited wide variation for nutritional traits. Higher amylose content (>20%) were recorded in 16% cultivars, and accessions GC/JH/ 2007/187 and GC/OR/2007/168 were identified with very high values for protein and amylose contents. Several superior cultivars were identified with better combination of two or more nutritional traits which can be recommended for cultivation by the farmers and tribal communities for their livelihood (Radhamani *et al*., 2014).

In chickpea accession IC436088 has been identified with a unique trait of upright peduncle and podding behaviour. This genetic stock has special significance in breeding varieties amenable to mechanical harvesting of chickpea (Singh *et al*., 2013b). In french bean, screening for bean anthracnose (*Colletotrichum lindemutianum*) disease led to identification of 600 accessions with resistance under field conditions. When these were subjected to screening under artificial conditions against four most prevalent races of *C. lindemutianum* (03, 515, 598 and 529), 16 accessions were identified which have complete resistance and good agronomic superiority (Rana *et al*., 2015).

Another approach for identification trait-specific germplasm in the NGB has been the use of informatics (Agrawal *et al*., 2011). Based on analysis of passport information, crop catalogues and other publications, abiotic and biotic trait-specific germplasm has been identified for collections of rapeseed-mustard (Radhamani *et al*., 2013), pigeonpea (Singh *et al*., 2013c) and cotton (Kak *et al*., 2016). Similar efforts are underway in other important crops. Germplasm identified is available for use by the breeders.

Unlocking the Potential of Crop Wild Relatives

Crop wild relatives (CWR), which are potentially valuable sources of important biotic/abiotic traits, play a very prominent role as donors of genes are important resources for crop improvement for the unpredictable future. Thus, their conservation in the genebanks is of paramount importance for their availability in breeding programmes. It now well acknowledged that most of the world's genebank collections of the CWR are inadequate or under-represented. Gap analysis is an important tool to assess the genetic as well as geographic diversity of the crop and its wild relatives, providing a more defined line of action with respect to collection and conservation strategies. A gap analysis for collected and conserved CWRs in NGB revealed that out of a total of ~2,000 taxa (Anon and Nayar, 1982; Pradeep *et al.*, 2014) CWRs reported from India only 94 species are conserved in NGB (Gupta *et al.*, 2016). Based on the analysis, areas have been identified for future collection to enrich the NGB with CWRs. Thus, priorities and strategies for collection followed by their conservation will be defined based on the economic value of cultivated species, distribution of wild species and its potential use in crop improvement programme for enhancing the food and nutritional security.

Whilst conservation of seeds of crops is now a routine practice to the extent that international guidelines are followed world-wide (Tyagi and Agrawal, 2015), CWR still pose challenge for genebank curators. For instance, *Aegilops* species (wild wheat) exhibit inherent problems caused by erratic and staggered germination due to dormancy imposed by presence of tenacious glumes. At NGB, we devised a method to overcome this impediment by removal of tenacious glume in eight wild species of wheat, before their long-term storage in genebank. Germination in seeds with glume was recorded as 20-70% and in seeds without glume was 72-100%. The tenacious glumes contribute to the physical dormancy and removal of glumes increased the germination and seedling vigour in all eight species namely *Ae. bicornis, Ae. geniculata, Ae. kotschyi, Ae. longissima, Ae. triuncialis, Ae. umbellulata,, Ae. vavilovii,* and *Ae. ventricosa* (Srinivasan *et al*., 2013). Incidentally, from 335 accessions of *Aegilops* spp. evaluated at hot-spots for rusts, 229 accessions with D genome of wheat were found resistant to leaf rust (Vikas *et al.,* 2014).

Why Genebanks Will Continue to Remain in Demand

That genetic resources are important and required for survival of humans is an unquestionable fact. Also what is well-known is the genetic erosion and loss of diversity is a reality of the modern agriculture. And that is why genebanks exists. To safeguard the rapid loss of diversity. In a very recent study, we have used genebank material to demonstrate loss in genetic diversity, using Indian rice varieties, as a model. Trends of genetic diversity and genetic relationship in 729 Indian rice varieties (released between 1940–2013), conserved in the NGB were assessed using 36 HvSSR markers. A total of 112 alleles was amplified with an average of 3.11 alleles per locus with mean Polymorphic Information Content (PIC) value of 0.29. Cluster analysis grouped these varieties into two clusters whereas the model based population structure divided them into three populations. AMOVA study based on hierarchical cluster and model based approach showed 3% and 11% variation between the populations, respectively. Decadal analysis for gene diversity and PIC showed increasing trend from 1940 to 2005, thereafter, values for both the parameters showed decreasing trend between years 2006-2013. In contrast to this, allele number demonstrated increasing trend in these varieties released and notified between1940 to 1985, it remained nearly constant during 1986 to 2005 and again showed an increasing trend. Results demonstrated that the Indian rice varieties harbors huge amount of genetic diversity. However, the trait based improvement programme in the last decades forced breeders to rely on few parents, which resulted in loss of gene diversity during 2006 to 2013. The present study indicates the need for broadening the genetic base of Indian rice varieties through the use of diverse parents in the current breeding programme (Singh *et al*., 2016).

Epilogue

Although many basic conservation principles, organizations and initiatives have persisted almost unchanged for decades, the framing and purpose of conservation have shifted recently. These shifts primarily relate to viewing conservation as the beginning point for utilisation rather than an end in itself. This is to adopt to the ever-changing agro-climatic requirements, development of novel crop varieties in response to new biotic and abiotic stresses and changing consumer preferences. The NGB has been re-orienting its priorities and programmes accordingly. The thrust has been to increase the efficiency in the terms of ensuring PGR security of wider amplitude of species, while not compromising on the genetic diversity captured. Systematic characterisation, regeneration and multiplication of *ex situ* accessions are being undertaken to satisfy needs of efficient conservation, and making diverse genetic material and associated information available to researchers and breeders. We are aiming to provide trait-specific germplasm for breeding more productive and resilient varieties, with emphasis on enhancing nutritional quality, stress tolerance, and resource use efficiency.

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