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Mini Core Germplasm Collections for Developing Climate Resilient Crop Cultivars and Achieving Enhanced Genetic Gains in Plant Breeding Programs

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Crop germplasm diversity contributes to developing improved crop cultivars aimed at increasing crop productivity to meet future food security needs of world. The large size of germplasm collections and lack of reliable information on traits of economic importance has resulted in low use (<1%) of germplasm. To facilitate realization of the potential benefits of germplasm collections to broaden the genetic base of cultivars in many crops, the mini core ($\sim1\%$ of the entire collection) concept was postulated and a development process proposed, to produce resources recognized globally as an "International Public Good" (IPG). Mini core collections have been developed in several crops and new diverse sources of genetic variation identified for biotic and abiotic stresses, agronomic and nutritional traits for use in crop improvement programs. Using genetically diverse sources scientists have developed breeding lines with enhanced trait expression and higher genetic gains. Molecular characterization of germplasm has revealed that phenotypic traits were as effective as molecular markers in sampling allelic diversity to form mini core collections.

Introduction

Germplasm is the total gene pool of a species consisting of landraces, advanced breeding lines, elite cultivars, and wild and weedy relatives. It forms the raw material for any crop improvement program and plays an important role in developing cultivars with the intrinsic genetic potential to mitigate the adverse effects of climate change on global agriculture. Nikolai Ivanovich Vavilov, was an early and exemplary advocate of the importance of genetic diversity for crop improvement and organized extensive germplasm collections of various crops from their 'centers of origin' and distribution for conservation. Since then the germplasm collections of major crop plants continued to grow in number and size. Globally, 7.4 million germplasm accessions are conserved in about 1,750 genebanks. The vulnerability of genetically uniform modern varieties, which are often closely related to one another and planted to large areas, to new pests, diseases, climatic conditions and changes in market needs is widely acknowledged. Diverse landraces, exotics and wild relatives hold a wealth of genes/alleles which, if included in breeding programs, can help raise the yield ceiling as well as enhance stress resilience of agronomically superior cultivars.

Definition and Development of Mini Core Collection

Less than 1% of assembled germplasm has been used in breeding programs globally (Upadhyaya et al., 2006). The elite cultivars of many major crops trace to very few founder genotypes - for example, 50% of wheat cultivars trace to 9 genotypes; 75% of potato to 4, and 50% of soybean to 6 (World Conservation Monitoring Centre, 1992). In chickpea, 41% of cultivars from hybridization have Pb7 as a parent; in pigeonpea 41% have T1 and T190; in urdbean 64% have T9 and in mung bean 35% have T1. Limited numbers of germplasm lines were used in chickpea breeding programs including those of ICRISAT and ICARDA. At ICRISAT, only 91 germplasm lines have been used to develop 3,548 advanced varieties during 1978-2004. L-550 (909 times) and K 850 (851 times) were the most widely used accessions (Upadhyaya et al. 2006). The main reasons for low use of germplasm include large size of collections and lack of reliable data on traits of economic importance, which show high genotype x environment interaction (Upadhyaya et al., 2013). To overcome these problems, Frankel (1984) proposed the concept of core collections (10% of entire collection) that can be evaluated more extensively to identify

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promising germplasm. Core collections of various crops were developed at ICRISAT (Table 1). However, it was soon realized that even the size of core collections was unwieldy for replicated multilocational evaluation to identify sources of variation for traits which show genotype \times environment interaction, as the large field areas required to evaluate thousands of lines inherently added to environmental variation. To overcome this, Upadhyaya and Ortiz (2001) proposed the concept of mini core collections (10% of core or 1% of entire collection)

Upadhyaya and Ortiz (2001) proposed a two stage strategy for developing mini core collections, as follows:

- 1. Development of a core collection (~10%) from the entire collection;
- 2. Evaluation of the core collection for various morphological, agronomic and quality traits or need specific characters and selecting a further subset of about 10% of accessions that sample phenotypic diversity.

At both stages, standard clustering procedures are used to identify groups of similar accessions (Fig. 1), to guide sampling of diversity from the entire collection in the core or mini core entries. Following this strategy mini core collections have been developed at the ICRISAT (Table 1) and several other countries.

Mini Core Collections Meet the Needs of Plant Breeders for Variation in Multiple Traits

Plant breeders often work on many traits simultaneously and prefer sources of variation for multiple traits. The mini core collections developed at ICRISAT have been evaluated for various biotic and abiotic stresses, for nutritional traits and for their agronomic performance in multilocational replicated trials. Genetically diverse sources for individual as well as for multiple traits have been reported in chickpea, groundnut and sorghum (Upadhyaya et al 2013, 2014, 2019). Such diverse germplasm with multiple trait-specific sources in agronomically desirable backgrounds offer breeders opportunities to combine several genes (Upadhyaya *et al.*, 2014).

Mini core collections have helped increase use of germplasm in crop breeding. Pedigree analyses of chickpea breeding lines developed at ICRISAT revealed 47% greater use of germplasm and use of 7 unique germplasm accessions during 2005-08. A similar pattern was observed in groundnut.

Trait Enhancement and Greater Genetic Gains through Use of Mini Core Sources

The use of diverse groundnut germplasm lines identified from a mini core collection resulted in developing exceptionally high oil (up to 63%, compared to \sim 48% in control cultivar) and high-yielding breeding lines,

Crop	Accessions	Traits	Core/mini core	Accessions	Reference
Chickpea	16,991	13	Core	1,956	Crop Sci. 41: 206-210.
	1956	22	Mini core	211	Theor. Appl. Genet. 102: 1292-1298
Groundnut	14,310	14	Core	1,704	Genet. Resou. Crop Evol. 50: 139-148
	1704	47	Mini core	184	Crop Sci. 42: 2150-2156
Pigeonpea	12,153	14	Core	1,290	Genet. Resou. Crop Evol. 52: 1049-1056
	1,290	33	Mini core	146	Crop Sci. 46: 2127-2132
Sorghum	22,473	20	Core	2,247	Crop Sci. 41: 241-246
	2,247	21	Mini core	242	Crop Sci. 49: 1769-1780
Pearl millet	20,766	12	Core augmented	2094	Crop Sci. 49: 573-580
	2,094	18	Mini core	238	Crop Sci. 51: 51 :217-223
Finger millet	5,940	14	Core	622	Genet. Resou. and Crop Evol. 53: 679-685
	622	20	Mini core	80	Crop Sci. 50: 1924-1931
Foxtail millet	1,474	23	Core	155	Plant Genet. Resou. 7: 177-184
	155	21	Mini core	35	Field Crops Res. 124: 459-467
Proso millet	833	20	Core	106	Crop Pastu. Sci. 62: 383-389
Barnyard millet	736	21	core	89	Crop Sci. 54: 2673-2682
Little millet	460	20	core	56	Crop Sci. 54: 2673-2682
Kodo millet	656	20	core	75	Crop Sci. 54: 2673-2682

Table 1. Core and mini core collections developed for ICRISAT mandate crops

Indian J. Plant Genet. Resour. 35(3): 69–73 (2022)





Fig. 1. Flow diagram to establish core and mini core collections in a crop species (adapted from Upadhyaya et al., 2009)



indicating that new germplasm sources contribute to enhancing genetic gains.

Comparison of Mini Core Collections Developed using Phenotypic Traits and those Based on Molecular Markers

In early 2000, when this author (Hari Upadhyaya) started developing mini core collections of the ICRISAT mandate crops and presented outcomes in international conferences, scientists often asked about the efficacy of phenotypic traits vis-à-vis molecular markers in sampling diversity to form the mini core collection. Our collaborative work at ICRISAT with the Generation Challenge Program helped us to genotype a large number of germplasm accessions of chickpea, sorghum (both 3,000 accessions), groundnut, pigeonpea, pearl millet and finger millet (1000 accessions each), and foxtail millet (500) with 20-50 simple sequence repeats (SSR). The aim was to study genetic diversity, population structure and to establish reference sets of 200-400 genetically diverse accessions. To cite an example, the genetic structure, diversity and allelic richness in a world composite collection of chickpea (3,000 accessions), using 48 SSR markers, was assessed and a reference set of 300 accessions was established at ICRISAT (Upadhyaya et al., 2008). The 48 SSR markers detected 1,683 alleles in 2,915 accessions, of which 935 were considered rare, 720 common and 28 most frequent. The composite collections were also characterized for qualitative and quantitative traits at ICRISAT Center, Patancheru, India. Reference sets based on SSR markers, qualitative traits, quantitative traits and their combinations were formed and compared for allelic richness and diversity. In chickpea, for example 48 SSR based reference set captured 78.1% of 1683 alleles in the composite collection compared to 73.5% of alleles in the reference set based on seven qualitative traits. The reference sets based on SSR and qualitative traits captured 80.5% (1,354) of alleles in the composite collection. Similarly, in groundnut the SSR-based reference set captured 95.1% (466) of alleles in the composite collection (490) compared to 93.3% (457) of alleles in the reference set based on 14 qualitative traits. The reference sets based on SSR and qualitative traits captured 95.9% (470) of alleles in the composite collection. In pigeonpea, a reference set based on SSR data and consisting of 300 most diverse accessions, captured 187 (95%) of the 197 alleles in the composite collection.

Another reference set based on qualitative traits captured 87% of the alleles in the composite set. This demonstrated that SSR and qualitative traits were similarly efficient in capturing the allelic richness of composite collections, and thus mini cores that were selected using phenotypic traits were as good as those based on SSRs.

Mini Core Collections as Association Mapping Panel

A sorghum mini core collection has been used as an association mapping panel for various traits such as plant height, maturity, kernel weight and number, resistance to grain mold, rust, anthracnose, germination at low temperature, saccharification yield and other traits.

Conclusion

To meet unprecedented challenges to global agriculture in the 21st century, new genetic variation for developing climate-resilient crops is required. Further, climate resilient cultivars of many taxa, for example pulses, must be nutritionally dense to mitigate adverse effects of malnutrition. Enhanced use of germplasm is needed to develop such cultivars. The concept and process of forming mini core collections, which was recognized as an International Public Good, is very useful in identifying promising sources. Genetically diverse parents provide opportunities to identify transgressive progeny with greater trait expression and enhanced genetic gains.

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