

# Prebreeding Using Wild Species for Genetic Enhancement of Grain Legumes at ICRISAT

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## ABSTRACT

Like many other major crops, ICRISAT's mandate grain legume crops have a narrow genetic base. The production and productivity of these crops is adversely affected by different biotic and abiotic stresses, and high levels of resistance or tolerance to these stresses are not available in the cultivated genepool. In contrast, wild species harbor many useful genes and have potential to thrive well under climatic extremities. However, utilization of these wild species for the genetic improvement of crop cultivars is hindered mainly due to ploidy level differences between cultivated and wild species, cross-incompatibility barriers, and linkage drag. Systematic prebreeding efforts involving wild species of *Cicer*, *Cajanus*, and *Arachis* as donors and popular well-adapted cultivars of chickpea (*Cicer arietinum* L.), pigeonpea [*Cajanus cajan* (L.) Millsp.], and groundnut (*Arachis hypogea* L.) as recipient parents, respectively, have led to the development of new genepools having good agronomic performance and higher frequency of useful genes and alleles introgressed from wild species. Evaluation of a few populations for biotic stresses and yield-related traits resulted in the identification of desirable introgression lines (ILs) that have been shared with NARS for use in breeding programs. Overall, prebreeding ensures continuous supply of novel and diverse genetic variability derived from wild species in readily usable form into the breeding pipelines to develop new climate-resilient cultivars with a broad genetic base.

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**Abbreviations:** AB, ascochyta blight; AB-QTL; advanced backcross quantitative trait loci; BGM, botrytis gray mold; DRR, dry root rot; IL, introgression line; LLS, late leaf spot; QTL, quantitative trait locus.

**N**ARROW genetic base in cultivated agricultural crops is caused by factors such as monophyletic origin, genetic bottlenecks, and repetitive use of elite breeding lines and is the major factor limiting genetic improvement of cultivars. Domestication and breeding has resulted in the loss of adaptive alleles and fixation of deleterious alleles through altered selection, resulting in the reduced fitness of modern cultivars across different environments (Warschefsky et al., 2014). Further, the cultivation of a few genetically uniform cultivars has resulted in vulnerability of the crops to pests and diseases, which causes a huge yield gap between actual and potential yields. Under ever-changing climatic conditions, new insect-pests and diseases are emerging as major threats limiting crop production and productivity globally (Chakraborty and Newton, 2011; Gautam et al., 2013). The semiarid tropics of the world with very little rainfall and degraded soils are most vulnerable to climate change. There is an urgent need to introgress the adaptive gene complexes for disease resistance, abiotic stress tolerance, and other important agronomic and nutrition-related traits into crop cultivars for improving resilience and sustaining agriculture. The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) works for the genetic improvement of three grain legumes, chickpea (*Cicer arietinum* L.), pigeonpea [*Cajanus cajan* (L.) Millsp.], and groundnut (also known as peanut, *Arachis hypogea* L.), and three dryland cereals, pearl

Published in Crop Sci. 57:1132–1144 (2017).  
doi: 10.2135/cropsci2017.01.0033

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millet [*Pennisetum glaucum* (L.) R. Br.], sorghum [*Sorghum bicolor* (L.) Moench.], and finger millet [*Eleusine coracana* (L.) Gaertn.]. All these crops are highly nutritious, and most are drought tolerant, which makes them the best bets for smallholder farmers in the semiarid tropics to survive and improve their livelihoods, and to ensure global food and nutrition security.

Of these mandate crops, grain legumes play a vital role in alleviating protein deficiency and malnutrition prevailing among smallholders farmers in the semiarid tropics. These crops are generally grown under rainfed conditions with minimum inputs and have multiple uses (food, fodder, fuel, medicine), thus offering many livelihood opportunities to farmers and agribusiness entrepreneurs. Amongst these, chickpea and groundnut are self-pollinating diploid ( $2n = 2x = 16$ ) and allotetraploid ( $2n = 4x = 40$ ) food legume crops, respectively, whereas pigeonpea is an often-cross-pollinating diploid ( $2n = 2x = 22$ ) species. Chickpea is cultivated primarily for its protein-rich seeds. Besides protein, chickpea seeds are also rich sources of fiber, minerals (calcium, potassium, phosphorus, magnesium, iron, and zinc),  $\beta$ -carotene, and unsaturated fatty acids (Jukanti et al., 2012). Pigeonpea is primarily grown as a food crop. Dry whole seed and split seed (dehulled) are used for cooking various dishes. Immature tender green seeds and pods are also consumed fresh as a green vegetable. Besides food, pigeonpea is used as forage, fodder, fuel, and medicine and to culture the lac-producing insects. Its deep root system helps to withstand drought, and it is grown on mountain slopes to bind the soil to reduce soil erosion (Gowda et al., 2012). Pigeonpea seed protein content (~21%) compares well with that of other important grain legumes. Groundnut is primarily cultivated as an oilseed crop, and about two-thirds of global production is crushed for extracting vegetable oil. Besides oil, groundnut seeds are rich in protein, minerals, and vitamins and are consumed in a variety of edible products. Like other leguminous crops, these crops also help in improving soil fertility through biological nitrogen fixation. These crops are also vital to the sustainability of mixed crop-livestock farming and provide genetic resources for adaptation to harsh and marginal environments prevailing in the semiarid tropics. Owing to several unique characteristics and benefits, these crops play an important role in sustainable agriculture systems in rainfed areas.

## CONSTRAINTS TO GRAIN LEGUME IMPROVEMENT

Like other major crops such as wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.), etc., these grain legume crops have a narrow genetic base. In chickpea, the narrow genetic base is due to a combination of factors such as restricted distribution of the wild progenitor species *C. reticulatum* Ladiz. in southeastern Turkey, the founder effect associated

with species domestication, and the agricultural selection involved in the shift from a winter to a summer crop (Abbo et al., 2003). In pigeonpea, the narrow genetic base is due to the bottleneck at domestication (Kumar et al., 2004), and in groundnut, it is due to the bottleneck associated with its evolution. Groundnut is believed to have originated by a single hybridization event between two wild diploid species with distinct genome giving rise to a sterile hybrid, followed by a spontaneous duplication of chromosomes producing a fertile tetraploid (groundnut) that remain reproductively isolated from its wild ancestors (Kochert et al., 1991; Jung et al., 2003; Seijo et al., 2004). Further, the displacement of heterogeneous landraces having local adaptation by modern, genetically uniform varieties developed through the frequent use of only a few elite breeding lines in crop improvement programs with intense selection for high yield has resulted in the diminution of genetic variability. The lack of variability is one of the major constraints hindering the genetic improvement of crops and renders the crops vulnerable to biotic and abiotic stresses in different agroecological regions resulting in huge yield losses and sometimes leading to epidemics (Hausmann et al., 2004).

Chickpea production and productivity is adversely affected by several biotic {[fusarium wilt (caused by *Fusarium oxysporum* Schlechtend. emend. Snyder et Hans. f. sp. *ciceri* (Padwick) Matuo et K. Sato), ascochyta blight [AB, *Ascochyta rabiei* (Pass.) Labr.], botrytis gray mold (BGM, *Botrytis cinerea* Pers. ex Fr.), dry root rot [DRR, *Rhizoctonia bataticola* (Taub.) Butler], powdery mildew [*Leveillula taurica* (Lev.) Salmon], and pod borer [*Helicoverpa armigera* (Hübner)]}, and abiotic (drought, heat, cold, and salinity) stresses, and the estimated yield losses by individual pests or diseases vary from 5 to 10% in temperate and 50 to 100% in tropical regions (van Emden et al., 1988). Further, chickpea is mainly cultivated in marginal lands under rainfed conditions, which results in low and unstable productivity (Kumar and van Rheenen, 2000). Similarly, in pigeonpea, the production and productivity in India and elsewhere is adversely affected by several biotic {wilt (*Fusarium udum* Butler), sterility mosaic disease (*Pigeonpea sterility mosaic virus*), phytophthora blight (*Phytophthora drechsleri* Tucker f. sp. *cajani*), alternaria blight (*Alternaria* sp.), and pod borers [*Helicoverpa armigera* (Hübner) and *Maruca vitrata* (Geyer)]}, and abiotic stresses (water logging, salinity, and frost/cold). In groundnut also, several biotic and abiotic stresses affect production and productivity globally. Among biotic stresses, rust (*Puccinia arachidis* Spegazzini), early leaf spot (*Cercospora arachidicola* Hori), and late leaf spot (LLS, *Phaeoisariopsis personata* Berk. & M.A. Curtis Van Arx) are the most common and widely distributed foliar diseases, whereas *Peanut bud necrosis virus* in South Asia, rosette disease (*Groundnut rosette virus*) in Africa, and bacterial wilt in Southeast Asia are the major diseases of groundnut, affecting yield and quality. Soil-borne diseases such as collar rot (*Aspergillus niger* van Tieghem) and stem rot (*Sclerotium rolfsii*

Saccardo) are potential threats to groundnut production and productivity worldwide. Insect-pests are of regional importance; for example, leaf miner (*Aproaerema modicella* Deventer) and *Spodoptera* are important threats in South and Southeast Asia, termites (*Microtermes* spp., *Odontotermes* spp., *Macrotermes* spp., and *Ancistrotermes latinotus* Holgren) in Africa, and corn earworm (*Helicoverpa zea* Boddie), lesser corn stock borer (*Elasmopalpus lignosellus* Zeller), and southern corn rootworm (*Diabrotica undecimpunctata howardi* Barber) in North America. Among abiotic stresses, drought is the major stress limiting groundnut productivity worldwide. Drought is also a predisposing factor for aflatoxin production and contamination in groundnut (Waliyar et al., 2003), which adversely affect its quality.

All these factors, either alone or in combination, cause substantial yield losses worldwide (Dwivedi et al., 2003) and result in a huge gap between the potential and actual yields of these crops (Bhatia et al., 2006). To ameliorate losses to crop production caused by biotic and abiotic stresses and to achieve food sufficiency for ever-increasing world population, especially under changing climatic conditions, immediate efforts are required towards broadening the genetic base of crop cultivars and developing improved plant types with high levels of resistance to various biotic and abiotic stresses.

## GENETIC RESOURCES FOR GRAIN LEGUME IMPROVEMENT

For the genetic improvement of ICRISAT mandate grain legume crops, >50,000 germplasm accessions comprising landraces, obsolete varieties, breeding lines, and crop wild relatives have been conserved in the RS Paroda genebank, ICRISAT, Patancheru, India (Table 1), which provide ample natural genetic variations for use in crop improvement programs. Germplasm accessions of these crops are also conserved in different genebanks globally. For chickpea improvements, 98,766 germplasm accessions (consisting of 97,322 cultivated and 1444 wild-type accessions) are conserved in >118 genebanks in 62 countries. Similarly, for pigeonpea, 43,394 accessions (40,247 cultivated and 3147 wild-type accessions) in >104 genebanks in 63 countries, and for groundnut, 128,146 accessions (123,666 cultivated and 4480 wild-type accessions) are conserved in over 70 genebanks in 46 countries (<http://www.fao.org/wIEWS-archive/wIEWS.jsp>). Owing to the narrow genetic base and low levels of resistance or tolerance to biotic and abiotic stresses available in cultivated genepools, it becomes important to exploit new and

diverse sources of variations. Crop wild relatives are of immense importance, as they are the reservoir of many useful gene and alleles, are genetically more diverse, and have natural defense mechanisms to withstand climate extremities. The genetic potential of crop wild relatives in crop improvement is well recognized and documented in various crops such as wheat (Mujeeb-Kazi et al., 1996), rice (Jena and Khush, 1990; Multani et al., 1994), maize (*Zea mays* L.; Maxted and Kell, 2009), peanut (Stalker, 1980), tomato (*Solanum lycopersicum* L.; Tanksley et al., 1996), and potato (*Solanum tuberosum* L.; Jansky et al., 2013).

For chickpea improvement, sufficient genetic variability is present in the genus *Cicer* comprising 44 species (cultivated *Cicer arietinum*, commonly known as chickpea, eight annual, and 35 perennial wild *Cicer* species) belonging to the family *Fabaceae*, subfamily *Papilionoideae*, and tribe *Cicereae* Alef. (van der Maesen 1972, Muehlbauer 1993). In the RS Paroda genebank, 308 accessions belonging to 18 wild *Cicer* species are conserved for use in chickpea improvement. On the basis of the crossability relationship with cultivated chickpea, these 43 wild *Cicer* species are grouped into three genepools: (i) primary genepool, which includes cultivated chickpea, its landraces and the cross-compatible annual wild progenitor species *C. reticulatum*; (ii) secondary genepool, which includes the cross-compatible annual wild species, *Cicer echinospermum* P.H. Davis; and (iii) tertiary genepool, which includes remaining six cross-incompatible annual and 35 perennial wild *Cicer* species. High levels of resistance or tolerance to important biotic and abiotic stresses such as AB (Singh and Reddy 1993; Stamigna et al., 2000; Collard et al., 2001; Rao et al., 2003; Croser et al., 2003; Shah et al., 2005; Pande et al., 2006), fusarium wilt (Infantino et al., 1996; Croser et al., 2003; Rao et al., 2003), BGM (Stevenson and Haware 1999; Rao et al., 2003; Pande et al., 2006), DRR, *Helicoverpa* pod borer (Sharma et al., 2005), drought (Croser et al., 2003; Kashiwagi et al., 2005; Toker et al., 2007), cold (Croser et al., 2003; Toker 2005; Berger et al., 2012), and high seed protein (Rao et al., 2003) have been reported in wild *Cicer* species.

For pigeonpea improvement, sufficient genetic variability is present in the genus *Cajanus*, which belongs to the family *Leguminosae*, subfamily *Papilionoideae*, tribe *Phaseoleae*, and the subtribe *Cajaninae*. Five hundred and sixty-two accessions belonging to 68 wild species in genus *Cajanus* and related genera are conserved in the RS Paroda genebank (Table 1). On the basis of the crossability relationship between pigeonpea and its wild relatives,

**Table 1. Germplasm conserved under the RS Paroda genebank for crop improvement at ICRISAT, Patancheru, India**

Crops	Number of accessions		Number of wild species	Total number of accessions	Number of countries
	Cultivated	Wild type			
Chickpea	20,294	308	18	20,602	60
Pigeonpea	13,216	562	68	13,778	74
Groundnut	15,144	478	48	15,622	93

these species are classified into three genepools: primary genepool containing all cultigens; secondary genepool containing all 10 cross-compatible species *C. acutifolius* (F. Muell.) Maesen, *C. albicans* (Wight & Arn.) Maesen, *C. cajanifolius* (Haines) Maesen, *C. lanceolatus* (W. Fitzg.) Maesen, *C. latisepalus* Maesen, *C. lineatus* (Wight & Arn.) Maesen, *C. reticulatus* (Dryand.) F. Muell., *C. scarabaeoides* (L.) Thouars, *C. sericeus* (Baker) Maesen, and *C. trinervius* (DC.) Maesen; and tertiary genepool containing all cross-incompatible species *C. goensis* Dalzell, *C. heynei* (Wight & Arn.) Maesen, *C. kerstingii* Harms, *C. mollis* (Benth.) Maesen, *C. platycarpus* (Benth.) Maesen, *C. rugosus* (Wight & Arn.) Maesen, *C. volubilis* (Blanco) Blanco, and other *Cajanus* species and related genera such as *Rhynchosia* Lour., *Dunbaria* W. and A., *Eriosema* (DC.) Reichenb (van der Maesen 1990). In wild *Cajanus* species, new and diverse sources of resistance or tolerance against various biotic and abiotic stresses such as alternaria blight, phytophthora blight, sterility mosaic disease, pod borer, and salinity (Sharma et al., 1987; Subbarao et al., 1991; Dodia et al., 1996; Reddy et al., 1996; Mallikarjuna et al., 2005; Kumar et al., 2005; Srivastava et al., 2006; Sujana et al., 2008; Sharma et al., 2009), as well as accessions with agronomically desirable traits such as early flowering, high seeds per pod, high seed protein content, and photoperiod insensitivity (Mallikarjuna and Moss, 1995; Upadhyaya et al., 2013), have been identified.

Similarly, for groundnut, sufficient genetic variability is present in genus *Arachis* comprising 80 species, which are classified into nine sections. About 480 accessions belonging to 48 wild *Arachis* species representing eight sections are conserved in the RS Paroda genebank (Table 1). On the basis of crossability relationships, these species are classified into four genepools: (i) primary genepool comprising *A. hypogaea* landraces and its wild form *A. monticola* Krapov. & Rigoni, (ii) secondary genepool comprising diploid species from section *Arachis* that are cross-compatible with *A. hypogaea*, (iii) tertiary genepool comprising species of section *Procumbentes* that are weakly cross compatible with *A. hypogaea*, and (iv) quaternary genepool comprising remaining *Arachis* species from other seven sections, which are cross-incompatible with *A. hypogaea* (Singh and Simpson 1994). Compared with groundnut, wild *Arachis* species harbor very high levels of resistance to many biotic and abiotic stresses such as rust, early and late leaf spots, nematode, Peanut mottle virus, Peanut stripe virus, Peanut bud necrosis virus, groundnut rosette disease, aflatoxin, corn ear worm, leaf hoppers (*Ernphasca fibae* Harris), and *Spodopetra* (Dwivedi et al., 2008; Upadhyaya et al., 2011).

## PREBREEDING FOR ACCESSING NOVEL GENES FROM WILD SPECIES FOR CROP IMPROVEMENT

Limited genetic variation present in the cultivated germplasm necessitates the exploitation of wild species for

genetic improvement of different crops. Although the potential of wild species in improving the cultivars is well known, these species are not being adequately used in breeding programs. The major limitations are due to cross-incompatibility barriers between cultivated and wild species, ploidy level differences, poor viability and sterility of  $F_1$  hybrids and progenies, and linkage drag. It takes a lot of time and resources to overcome these limitations, which is why breeders are mostly reluctant to use wild species in their breeding programs.

Under such situations, prebreeding offers a unique platform to enhance the utilization of germplasm, especially wild species, and ensures continuous supply of diverse genetic variability into the breeding pipeline to develop new cultivars with a broad genetic base. The prerequisite for prebreeding is to identify useful traits and/or genes from unadapted germplasm (exotic landraces or wild species). The promising germplasm accessions having high intensity of the traits and/or genes are used as donors in crossing program with widely adapted cultivars as recipients to create new populations and genepools with good agronomic performance and high frequency of useful genes and alleles introgressed from new and diverse unadapted germplasm into a cultivated background. Finally, these prebreeding populations are evaluated to identify desirable introgression lines (ILs) with traits and/or genes introgressed from wild species and with acceptable agronomic backgrounds for ready use by the breeders in breeding programs (Sharma et al., 2013) (Fig. 1).

## PREBREEDING FOR GRAIN LEGUME IMPROVEMENT

The progress of prebreeding activities for enriching variability in the primary genepool for each of the three grain legumes is given hereunder.

### Chickpea

Utilization of wild *Cicer* species for chickpea improvement is hindered due to the cross-incompatibility barriers, sterility of  $F_1$  hybrids, linkage drag, and different phenology of wild *Cicer* and cultivated chickpea (Summerfield et al., 1989; Robertson et al., 1997; Abbo et al., 2002; Berger et al., 2005). As chickpea is an annual crop, our major focus is to exploit the variability present in eight annual wild *Cicer* species. However, of the eight annual wild species, only *C. reticulatum* is readily crossable with cultivated chickpea, resulting in a fertile hybrid (Singh et al., 2005). Although *C. echinospermum* is also crossable with cultivated chickpea, resulting hybrids are mostly sterile (Pundir and Mengesha, 1995). Application of growth hormone is needed to ensure proper pod and seed set in interspecific crosses involving crossable species (Singh et al., 2005). Exploitation of the remaining six annual species for chickpea improvement requires specialized techniques such as application of



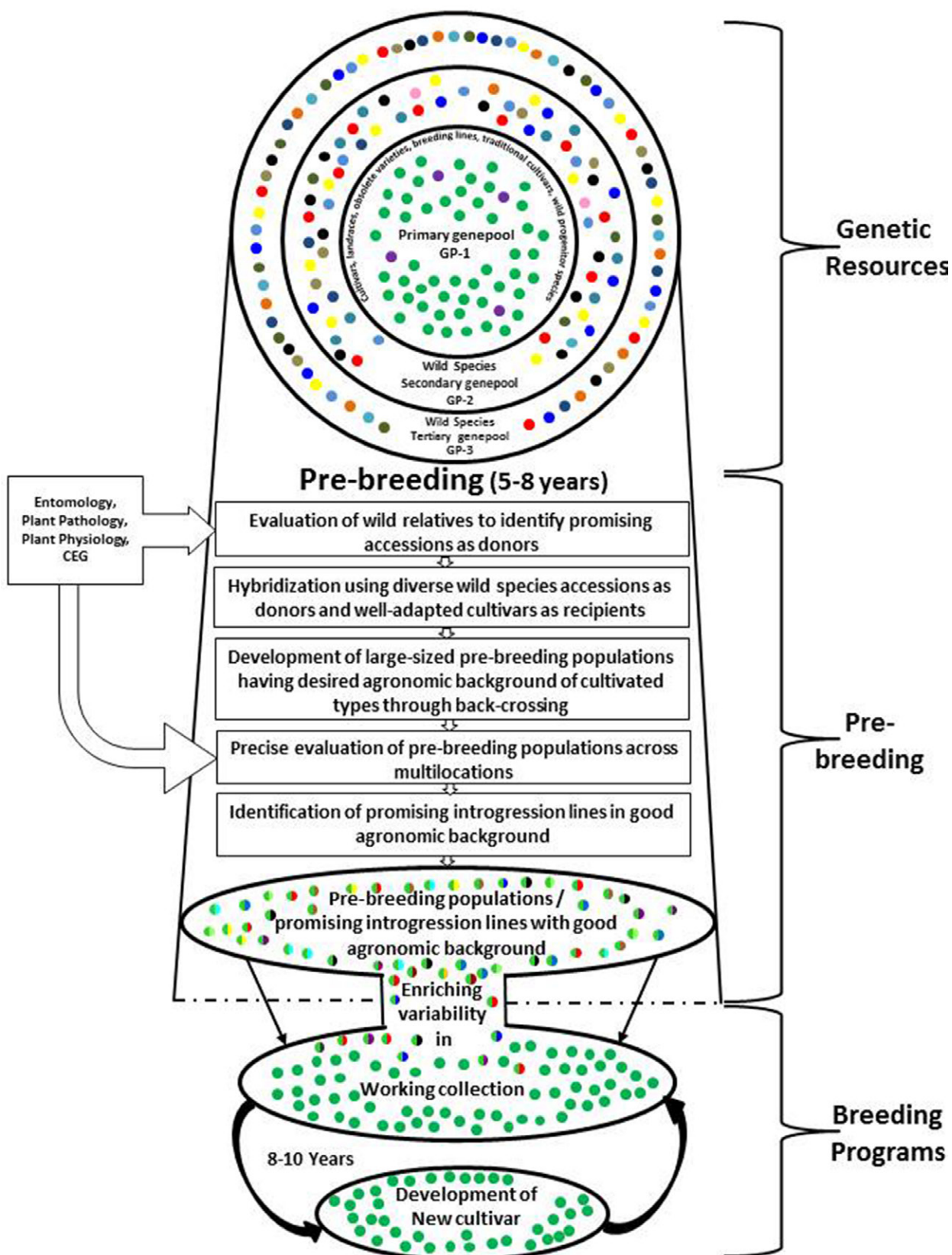


Fig. 1. Prebreeding: a link between genebanks and breeding programs

growth hormones, followed by ovule culture and embryo rescue (Badami et al., 1997; Mallikarjuna, 1999; Mallikarjuna and Jadhav, 2008; Lulsdorf et al., 2005).

The annual wild *Cicer* species are predominantly found in western and central Asia, the eastern Mediterranean, and in isolated populations adjacent to the African Red Sea Coast (Berger et al., 2003), whereas cultivated

chickpea is found in tropical, subtropical, and warm temperate zones. Under natural growing conditions in subtropical regions such as southern India, wild *Cicer* species are generally late in phenology compared with cultivated chickpea and, therefore, cannot be used frequently in crossing programs for chickpea improvement. Use of vernalization and/or extended photoperiod treatment under controlled environmental conditions has been proved successful in reducing the vegetative phase of wild *Cicer* species (Sharma and Upadhyaya, 2015). The study showed that vernalization- and photoperiod-responsive genes or alleles control flowering in wild *Cicer* species, and use of vernalization and extended photoperiod treatments, alone or in combination, can accelerate flowering and facilitate the use of wild *Cicer* species for cultivar improvement by synchronizing flowering between cultivated and wild *Cicer* species (Sharma and Upadhyaya, 2015). In *C. reticulatum* and *C. echinospermum*, which are cross-compatible with cultivated chickpea, response to photoperiod was more significant than to vernalization. Using extended photoperiod treatments and application of plant growth hormone, accessions of *C. reticulatum* and *C. echinospermum* are being used in crossing programs to create novel genetic variability for use in chickpea improvement programs.

Extensive screening of wild *Cicer* accessions belonging to eight annual wild *Cicer* species has led to the identification of accessions with high levels of resistance to AB, BGM, DRR, and pod borer. These promising accessions are being used as donors to introgress useful genes and alleles into popular chickpea cultivars after backcrossing. At ICRISAT, Patancheru, India, efforts are in progress to develop prebreeding populations with high frequencies of useful genes and alleles in good agronomic backgrounds for the following traits:

- introgression of DRR resistance from wild *Cicer* species into heat-tolerant but DRR-susceptible chickpea cultivar(s), especially to expand chickpea cultivation in central and southern India and Myanmar;
- introgression of AB resistance into chickpea cultivar(s) to expand chickpea cultivation in northern regions of India, Nepal, and other growing areas where AB is a major disease of chickpea;
- introgression of BGM resistance from wild *Cicer* species into short-duration chickpea cultivar(s); and
- introgression of pod borer resistance from wild *Cicer* species into elite chickpea cultivar(s).

Using wild *Cicer* accessions as promising donors and popular chickpea cultivars as recipients, introgression of traits in elite material is being pursued by traditional and/

or molecular breeding approaches, and the prebreeding populations are being developed for abovementioned traits following simple (*C. arietinum* × *C. reticulatum* or *C. echinospermum*) and/or complex three-way [*C. arietinum* × (*C. reticulatum* × *C. echinospermum*)] crosses. In simple crosses, the objective is to introgress useful genes and alleles from wild species into elite cultivars, followed by backcrossing to generate advanced backcross populations. Such populations can be used to identify marker-trait associations using an advanced backcross quantitative trait loci (AB-QTL) approach (Tanksley and Nelson, 1996). In complex crosses, the objective is to combine the genes conferring resistance from the two different wild species, *C. reticulatum* and *C. echinospermum*, into a common genetic background using traditional breeding. For introgressing resistance into susceptible cultivars, the complex three-way cross could be better than the simple crosses due to the involvement of two parents contributing resistance to three-way cross progenies. This approach proved useful in wheat, wherein leaf rust severity of three-way cross progenies was lower than that of simple cross progenies (Singh et al., 1998). Further, complex (three- and four-way) crosses allow an increase in the range of traits that can be simultaneously incorporated into elite progeny. However, the frequency of elite progeny from this type of cross is usually very low due to insufficient population sizes (Langridge and Chalmers, 2005; Velu and Singh, 2013). Hence, in complex three-way crosses, the large-sized populations are required to recover the plants with desirable combination of genes compared to simple crosses. Following simple or complex crosses, large-sized advanced backcross populations are being generated using cultivated chickpea as recipient with the objective to recover the maximum genetic background of the cultivated types with small desirable segments introgressed from the wild species. These populations are in different stages of development. Following complex crosses, two advanced backcross populations have been developed; one using desi landrace ICC 4958 as recipient and *C. reticulatum* accession ICC 17264 and *C. echinospermum* accession IG 69978 as donors, and another using kabuli chickpea cultivar ICCV 95311 as recipient, and *C. reticulatum* accession IG 72933 and *C. echinospermum* accession ICC 20192 as donors. In segregating populations (BC<sub>2</sub>F<sub>2</sub> generation) derived from two three-way F<sub>1</sub> crosses {[ICC 4958 × (ICC 17264 × IG 69978)] and [ICCV 95311 × (IG 72933 × ICC 20192)]}, considerable variability was observed for morphoagronomic traits (Fig. 2).

Evaluation of a set of these two BC<sub>2</sub>F<sub>2</sub> populations has resulted in the identification of ILs with early flowering (26 d), high number of pods per plant (up to 460 pods), high pod weight per plant (up to 180 g), high number of seeds per plant (up to 460 seeds), and high seed weight per plant (up to 142.0 g) (Table 2). Besides this, preliminary screening



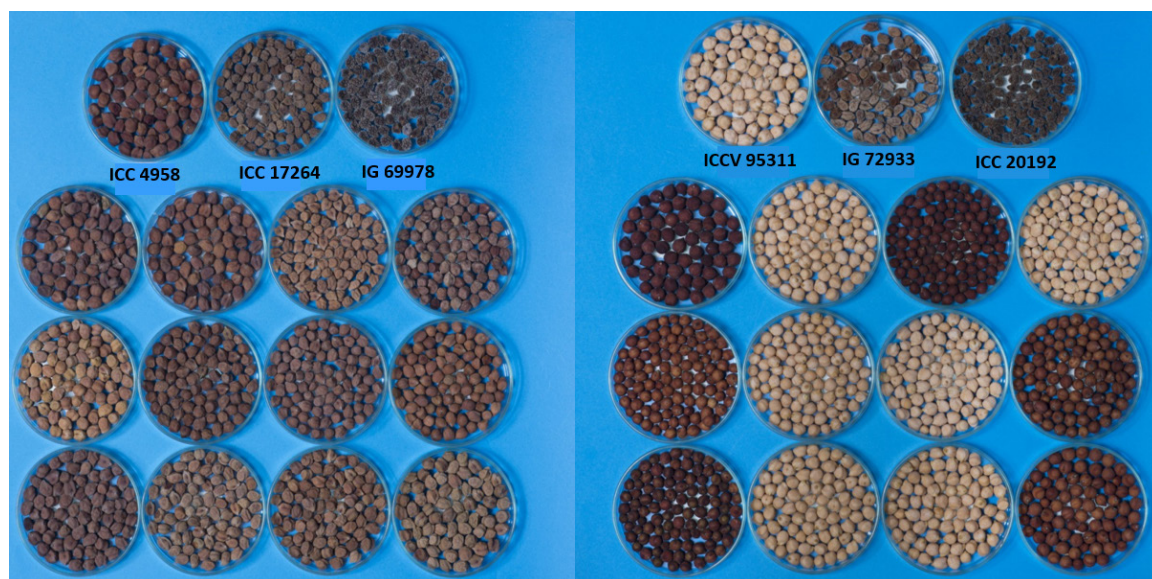


Fig. 2. Variability for seed traits in  $BC_2F_2$  populations derived from two three-way  $F_1$  {left: [ICC 4958  $\times$  (ICC 17264  $\times$  IG 69978)], right: [ICCV 95311  $\times$  (IG 72933  $\times$  ICC 20192)]} crosses at ICRISAT, Patancheru, India.

**Table 2. Introgression lines with good agronomic performance derived from three-way interspecific crosses for use in chickpea improvement.**

Traits	Number of promising introgression lines	
	ICC 4958 $\times$ (ICC 17264 $\times$ IG 69978)	ICCV 95311 $\times$ (IG 72933 $\times$ ICC 20192)
Early flowering (<40 d)	33	–
Number of pods per plant (>200 pods)	63	114
Pod weight per plant (>100 g)	7	3
Number of seeds per plant (>200 seeds)	47	104
Seed weight per plant (>100 g)	3	2

of a few plants in different segregating generations ( $BC_2F_1$ ,  $BC_1F_2$ , and  $3-F_2$ ) derived from two crosses for AB and BGM following the cut-twigg method (Sharma et al., 1995) under controlled environment conditions has identified ILs with high levels of resistance to these diseases (Sharma et al., 2016). These promising ILs will be rescreened further to confirm the resistance. At present, an advanced backcross population derived from an ICC 4958  $\times$  (ICC 17264  $\times$  IG 69978) cross in  $BC_2F_4$  generation exhibiting enormous variability is available for sharing with partners for high-throughput phenotyping across locations for important biotic and abiotic stresses and morphoagronomic traits (Table 3).

Besides ICRISAT, utilization of wild *Cicer* species for chickpea improvement elsewhere has contributed significantly through the development of germplasm lines and interspecific derivatives having cyst nematode (*Heterodera ciceri* Vovlas, Greco and Divito) resistance (Malhotra et al., 2002), resistance to wilt, foot rot (*Operculella padwickii* Kheswalla), and root rot [*Rhizoctonia bataticola* (Taub.) Butler] diseases, and high yield (Singh et al., 2005) from crosses involving *C. reticulatum*. Similarly high-yielding, cold-tolerant lines with high biomass (ICARDA, 1995) and resistance to phytophthora root rot (*Phytophthora medicaginis* Hansen and Maxwell) (Knights et al., 2008) have been developed from interspecific crosses involving

*C. echinospermum*. Similarly, high-yielding lines with good agronomic and seed traits and BGM resistance derived from interspecific crosses involving *C. reticulatum*, and *C. echinospermum* (Singh et al., 1984; Jaiswal et al., 1986; Singh and Ocampo 1997; Singh et al., 2005; Upadhyaya 2008), *C. judaicum* (Chaturvedi and Natarajan, 2010), and *C. pinnatifidum* (Sandhu et al., 2005; Singh et al., 2012a, 2012b; Kaur et al., 2013) have been

**Table 3. Advanced backcross populations derived from wild species for use in chickpea, pigeonpea, and groundnut improvement programs available at ICRISAT, Patancheru, India.**

Cross	Generation	Number of lines
Chickpea		
ICC 4958 $\times$ (ICC 17264 $\times$ IG 69978)	$BC_2F_4$	~1500
ICCV 95311 $\times$ (IG 72933 $\times$ ICC 20192)	$BC_2F_3$	~2000
Pigeonpea		
ICPW 68 $\times$ ICPL 85010	$BC_4F_{12}$	138
ICPL 85010 $\times$ ICPW 004	$BC_1F_{13}$	68
ICPL 87119 $\times$ ICPW 12	$BC_2F_7$	149
ICPL 87119 $\times$ ICPW 29	$BC_2F_7$	183
Groundnut		
ICGV 91114 $\times$ ISATGR 121250	$BC_2F_9$	416
ICGV 87846 $\times$ ISATGR 265-5	$BC_2F_9$	579
ICGV 87846 $\times$ ISATGR 278-18	$BC_2F_8$	250
TMV 2 $\times$ ISATGR 121250	$BC_2F_6$	686

developed for use in chickpea improvement (reviewed in Sharma et al., 2016).

## Pigeonpea

Frequent utilization of wild *Cajanus* species for pigeonpea improvement is hindered due to linkage drag and cross-incompatibility between cultivated and wild species. The promising wild *Cajanus* accessions are being used as donors to introgress useful genes and alleles into popular pigeonpea cultivars for the following traits:

- introgression of phytophthora blight resistance from wild *Cajanus* species into cultivated pigeonpea;
- developing advanced backcross populations using wild *Cajanus* species *C. scarabaeoides* and *C. acutifolius* as donors and pigeonpea cultivars ICPL 87119 and ICP 8863 as recipients; and
- combining components of pod borer resistance from different wild *Cajanus* species into common cultivated pigeonpea background following complex (four-way) crosses.

Due to climate change, phytophthora blight is emerging as a serious threat to pigeonpea production, and high level of resistance is not available in the cultivated genepool (Pande et al., 2011). Four advanced backcross populations derived from interspecific crosses (ICPL 87119 × ICPW 29, ICPL 85010 × ICPW 004, ICPL 87119 × ICPW 12, and ICPW 68 × ICPL 85010) involving cross-compatible secondary genepool species *C. cajanifolius* (ICPW 29) and *C. acutifolius* (ICPW 004, and ICPW 12) and cross-incompatible tertiary genepool species *C. platycarpus* (ICPW 68) as donors and two pigeonpea cultivars ICPL 87119, and ICPL 85010 as recipients were screened for phytophthora blight resistance under controlled environmental conditions at ICRISAT, Patancheru, India. Embryo rescue technique was used to generate population using *C. platycarpus* (Mallikarjuna and Moss, 1995; Mallikarjuna et al., 2005). Only 13 ILs derived from a ICPL 87119 × ICPW 12 cross and five ILs derived from a ICPW 68 × ICPL 85010 cross exhibited high or moderate levels of phytophthora blight resistance under preliminary screening. These ILs are being multiplied to confirm resistance for further use in pigeonpea improvement programs.

Despite tremendous efforts, pod borer remains a major insect causing huge yield losses in pigeonpea. Evaluation of wild *Cajanus* species has identified accessions and species with a diverse combination of morphological and biochemical components associated with expression of resistance to pod borer. A *C. acutifolius* accession was reported to have high levels of antixenosis for oviposition, high expressions of antibiosis, low amounts of sugars, and high amounts of tannins and polyphenols in pods, and a *C. scarabaeoides* accession had high density of Type C and

D trichomes (Sharma et al., 2009). These accessions were used as donors in the crossing program with two popular pigeonpea cultivars, ICPL 87119 and ICP 8863, to generate two advanced backcross populations for mapping QTLs associated with pod borer resistance following an AB-QTL approach. Besides this, efforts are being made to combine these different components of pod borer resistance from different wild *Cajanus* species into a common pigeonpea cultivar. Therefore, two four-way complex crosses in the genetic background of ICPL 87119 [(ICPL 87119 × *C. acutifolius*) × (ICPL 87119 × *C. scarabaeoides*)] and ICP 8863 [(ICP 8863 × *C. acutifolius*) × (ICP 8863 × *C. scarabaeoides*)] were generated (Sharma and Upadhyaya, 2016) and were backcrossed with the respective cultivated parents to recover the genetic background of cultivated types. These four-way BC<sub>1</sub>F<sub>1</sub> populations are being advanced to BC<sub>1</sub>F<sub>2</sub> generation. The BC<sub>1</sub>F<sub>2</sub> populations would be evaluated to identify ILs having enhanced levels of pod borer resistance for further use in pigeonpea improvement programs.

Beside introgressing biotic stress resistance, significant variability for important morphoagronomic traits such as days to flowering and growth habit, as well as for pod and seed traits, was observed in the prebreeding populations derived from wild *Cajanus* species (Fig. 3). Promising ILs having early to medium maturity and high yield derived from *C. cajanifolius* ICPW 29 and *C. acutifolius* ICPW 12 were identified and shared with breeders at ICRISAT and NARS for further evaluation across locations to identify stable, high-yielding ILs. Recently, in the 2016 rainy season, three ILs, ICPL 15028 and ICPL 15036 (both derived from *C. acutifolius* ICPW 12) and ICPL 15084 (derived from *C. cajanifolius* ICPW 29), have been included in the Initial Varietal Testing (IVT) of the All India Coordinated Research Project (AICRP) on pigeonpea across different locations in India (C.V. Sameer Kumar, personal communication, 2016). Besides this, previous studies by various researchers at ICRISAT and elsewhere have reported significant contributions of wild *Cajanus* species for pigeonpea improvement, such as development of different cytoplasmic male sterility systems (Saxena et al., 2010), development of advanced generation populations having resistance to pod borer (Mallikarjuna et al., 2007), and high protein content and seed weight (Saxena et al., 1987; Reddy et al., 1997). At present, the promising ILs and four advanced backcross populations are available for sharing with partners for use in pigeonpea improvement programs (Table 3).

## Groundnut

The frequent utilization of wild *Arachis* species for groundnut improvement is hindered due to both pre- and post-zygotic hybridization barriers between cultivated groundnut and wild *Arachis* species (Halward and Stalker 1987). In the genus *Arachis*, cultivated groundnut belongs to the section *Arachis*, which also contains 29 diploid and one





Fig. 3. Variability for seed traits in advanced backcross population of pigeonpea derived from *Cajanus cajanifolius* (left) and in the late leaf spot- and rust-resistant introgression lines of groundnut derived from synthetics (right) at ICRISAT, Patancheru, India.

tetraploid wild *Arachis* species. Utilization of these species for groundnut improvement is hindered due to ploidy level differences. Groundnut is an allotetraploid ( $2n = 4x = 40$ , AABB), which has originated from two diploid wild *Arachis* species, A-genome species *A. duranensis* ( $2n = 2x = 20$ ) and B-genome species *A. ipaensis* ( $2n = 2x = 20$ ), whereas most of the wild species in the section *Arachis* are diploid ( $2n = 2x = 20$  or  $18$ ), having A, B, D, F, or K genomes (Smartt and Stalker, 1982; Robledo and Seijo, 2010).

For the exploitation of these diploid wild *Arachis* species belonging to the secondary genepool, new sources of tetraploid groundnut (synthetics) were developed for use in groundnut improvement (Mallikarjuna et al., 2012). Diploid wild *Arachis* accessions having A, B, and K genomes were crossed in different combinations, followed by chromosome doubling of the diploid intra- and inter-genomic  $F_1$  hybrids using colchicine treatment to generate tetraploid synthetics (Table 4). These synthetics were screened extensively for resistance to LLS and soil-borne disease such as stem rot and collar rot (Sharma, unpublished data, 2016). The major objectives of prebreeding activities for groundnut improvement include:

- development of prebreeding populations using synthetics as donors and popular groundnut cultivars as recipients to enrich variability for morphoagronomic traits, as well as for introgressing biotic stress resistance; and

- development of new synthetics by using diploid wild *Arachis* species having high levels of resistance to foliar and soil-borne diseases.

Synthetics having high levels of resistance have been identified and are being used to develop prebreeding populations using popular groundnut cultivars. These populations are in different stages of development. Five advanced backcross populations derived from synthetics ISATGR 121250, ISATGR 278-18, ISATGR 265-5, and ISATGR 40 as donors and ICGV 91114, ICGV 87846, TMV 2, and Tifrunner as recipient parents have been developed. These

populations exhibited considerable variability for morphoagronomic traits, as well as for biotic stresses. Precise phenotyping of two populations during rainy seasons over 3 yr (2014–2016) has resulted in the identification of ILs having high levels of LLS and rust resistance (Sharma et al., 2017; Fig. 3). Further, ILs having high levels of stem rot resistance and low preharvest aflatoxin contamination have also been identified and are being rescreened to confirm the resistance for further use in breeding programs. Development of advanced backcross mapping population derived from a Tifrunner  $\times$  ISATGR 40 cross is in progress to map the QTLs for important traits following an AB-QTL approach. Additionally, new sources of resistance for LLS, stem rot, and collar rot have been identified in diploid wild *Arachis* species, which are being used to generate new synthetics to exploit useful variability present in the section *Arachis* for groundnut improvement. The LLS- and rust-resistant ILs (Sharma et al., 2017) and four advanced backcross populations are available for sharing with partners for use in groundnut improvement programs (Table 3).

Table 4. Tetraploid synthetics available for groundnut improvement at ICRISAT, Patancheru, India.

No.	Identity	Species	Genome
1	ISATGR 40	<i>A. ipaensis</i> $\times$ <i>A. duranensis</i>	BBAA
2	ISATGR 184		
3	ISATGR 47	<i>A. valida</i> $\times$ <i>A. duranensis</i>	BBAA
4	ISATGR 48		
5	ISATGR 65		
6	ISATGR 154		
7	ISATGR 168		
8	ISATGR 5	<i>A. magna</i> $\times$ <i>A. batizocoi</i>	BBKK
9	ISATGR 121250	<i>A. duranensis</i> $\times$ <i>A. ipaensis</i>	AABB
10	ISATGR 206	<i>A. duranensis</i> $\times$ <i>A. valida</i>	AABB
11	ISATGR 278-18	<i>A. duranensis</i> $\times$ <i>A. batizocoi</i>	AAKK
12	ISATGR 173		
13	ISATGR 72	<i>A. duranensis</i> $\times$ <i>A. cardenasii</i>	AAAA
14	ISATGR 265-5	<i>A. kempff-mercadoid</i> $\times$ <i>A. hoehnei</i>	AAAA
15	ISATGR 99	<i>A. diogoi</i> $\times$ <i>A. cardenasii</i>	AAAA
16	ISATGR 160		
17	ISATGR 163	<i>A. kempff-mercadoid</i> $\times$ <i>A. stenosperma</i>	AAAA

Previous studies have also showed the importance of wild *Arachis* species for groundnut improvement. Utilization of wild *Arachis* species after interspecific hybridization has resulted in the development of many elite germplasm lines and cultivars such as Spancross (Hammons, 1970), Tamnut 74 (Simpson and Smith, 1975), Coan (Simpson and Starr, 2001), NemaTAM (Simpson et al., 2003), ICGV-SM 85048, and ICGV-SM86715 (Nigam et al., 1998, Moss et al., 1998). Further, the development and utilization of synthetic amphidiploids such as TxAG-6 with high genetic variation (Simpson et al., 1993) in breeding program has resulted in the release of two cultivars (Coan and NemaTAM) carrying genes for root-knot nematode (*Meloidogyne arenaria* Chitwood) resistance from *A. cardenasii* (Simpson and Starr, 2001; Simpson et al., 2003).

## OPPORTUNITIES AND CHALLENGES IN PREBREEDING

Wild species of crops conserved in different genebanks globally provide novel genetic diversity for use in breeding programs and assist the breeders in meeting the growing food demands of an ever-increasing human population by developing new high-yielding varieties with high levels of resistance or tolerance to biotic and abiotic stresses coupled with improved nutrition, thus addressing the issue of hunger and malnutrition prevailing among smallholder farmers, especially in Asia and Africa, and ensuring food security globally. Besides providing useful genes and alleles for existing stresses, wild species hold potential to provide novel sources of variation for new emerging biotic (diseases and insect-pests) and abiotic (erratic rain fall patterns, temperature extremities, salinity, etc.) stresses.

Despite the fact that prebreeding has a great potential in generating variability for crop improvement using crop wild relatives, the success of prebreeding activities is hampered due to several technical and financial challenges. The involvement of unadapted, as well as incompatible, germplasm makes prebreeding a time-consuming and resource-demanding research endeavor, taking several years to develop useful breeding material. It is difficult to acquire long-term assured funding for these activities, and most of them are performed under short-term bilateral projects with no visible success in short time. Technical challenges are due to several factors such as (i) lack of characterization and evaluation data, especially for novel traits in the wild species, which hinders the selection of promising donors; (ii) knowledge of crossability relationships between the cultivated and wild species; and (iii) linkage drag. Utilization of cross-incompatible wild species requires strenuous efforts to understand the barriers and develop the techniques and protocols for the successful introgression of useful genes and alleles into the cultivated background. Linkage drag is the most common problem associated with the utilization of wild species in breeding programs, as the undesirable traits

such as late maturity, poor pod and seed characteristics, photoperiod sensitivity (northern latitudes), and shattering get introgressed along with desirable traits. Though strategies are available to overcome these problems, such as development of large-sized advanced backcross populations and use of molecular markers for precise introgression of useful genes and alleles with minimum linkage drag, these are time consuming and resource demanding. Due to these technical challenges, it takes a long time to introgress desirable traits from wild species into cultivated backgrounds. Prebreeding is a long-term research endeavor, and therefore unrestricted, assured, and long-term funding is required for prebreeding to have a greater impact in improving the resilience of crop cultivars and sustaining crop production and productivity globally. Efforts are in progress to develop innovative approaches to increase the efficiency of introgression of useful genes for important strategic traits from wild species, and to compress the timeframe to introduce novel alleles into breeding pipelines of ICRISAT mandate crops, which are otherwise hidden in the genebank. To harness the full potential of wild species, especially in the era of climate change, there is an urgent need for active engagement with the stakeholders to strengthen the prebreeding programs, which will ensure the continuous supply of new genetic variability into the main breeding programs to accelerate genetic gains and to improve nutrition and resilience of modern crop varieties.

## Acknowledgments

The funding support from the Global Crop Diversity Trust (GCDT) to carry out prebreeding activities in pigeonpea, the Department of Science & Technology (DST), GOI, India for chickpea, and the CRP Grain Legumes for groundnut is duly acknowledged. Author also wish to thank three anonymous reviewers and the Associate Editor, Dr. Linghe Zeng, for their most useful comments and suggestions on this manuscript.

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