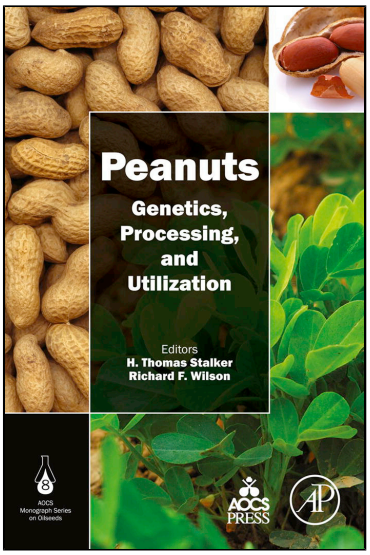

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Chapter 3

Global Resources of Genetic Diversity in Peanut

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OVERVIEW

Peanut or groundnut (*Arachis hypogaea* L.) is an annual herb, with geocarpic fruits, and an indeterminate growth habit. It is classified as a legume in the plant family Fabaceae. Carl Linneaus first described the cultivated species in 1753, as *A. hypogaea* which was derived from the word "arachos" meaning a weed and "hypogaea" which means an underground chamber (Stalker and Simpson, 1995). Cultivated peanut can be classified into two subspecies, *fastigiata* and *hypogaea*, based on the presence or absence of floral axes on the main stem. They can be further divided into six botanical varieties (subspecies *hypogaea*: var. *hirsuta*, and var. *hypogaea*; subspecies *fastigiata*: var. *aequatoriana*, var. *fastigiata*, var. *peruvian*a, and var. *vulgaris*) based on a range of morphological characteristics such as growth habit, trichomes, and pod morphology. Many intermediates exist among these botanical types, therefore, the taxonomy is not always clear (Stalker and Simpson, 1995). In 1969, Krapovickas postulated that the variety *hypogaea* was the most ancient due to its runner type habit, no floral spikes, and branching patterns which is similar to the characteristics of the wild *Arachis* species (Krapovickas, 1969).

Cultivated peanut is a self-pollinating allotetraploid (AABB genome, $2n=4x=40$) putatively derived from the natural hybridization of two wild diploid species *Arachis duranensis* Krap. & W.C. Gregory (AA genome, $2n=2x=20$) and *Arachis ipaënsis* Krap. & W.C. Gregory (BB genome, $2n=2x=20$) (Kochert et al., 1991, 1996; Moretzsohn et al., 2012; Seijo et al., 2007; Stalker, 1997). Interspecific hybridization and polyploidization significantly affects plant

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evolution and plays an important role in the origin of many crops (Hilu, 1993; Singh, 2003). It has been demonstrated that polyploids maintain and combine diploid chromosomes which allows them to be fertile, well adapted, genetically stable, and occur frequently in nature; however, genome restructuring can occur to stabilize the genome (Leitch and Bennett, 1997; Seijo et al., 2007; Soltis and Soltis, 1999). It is well documented that cultivated peanut has a narrow genetic base which was likely due to a single polyploidization event that isolated it from the wild species and created small founder populations and a significant genetic bottleneck in the cultigen (Kochert et al., 1996). Genetic variability is known to decline in proportion to the severity of the bottleneck. The smaller the population and the longer it remains small, the more the allelic diversity erodes with the low frequency alleles being most at risk during a bottleneck (Rao and Hodgkin, 2002). Nucleotide substitution rates suggest that the A, B, and K (*Arachis batizocoi* Krap. & W.C. Gregory) genomes diverged fairly recently, between 2.3 and 2.9 million years ago (Moretzsohn et al., 2012).

DISCUSSION

Origin of the Genus *Arachis*

The earliest archeological record of peanut comes from Peru and dates back to 3900–3750 years ago (Hammons, 1994). The genus *Arachis* is thought to have originated in the southwestern part of Mato Grosso do Sul, Brazil, or northeast Paraguay because the most ancient species of the genus still grow in that area (Simpson et al., 2001). *Arachis* contains 81 described species that have been classified in nine distinct taxonomic sections based on cross-compatibility, morphological characters, and geographic origin (Krapovickas and Gregory, 1994; Valls and Simpson, 2005). Even though the wild species in this genus are important due to harboring genes for disease resistance and other important agronomic traits, the most economically valuable *Arachis* species is the cultivated peanut (*A. hypogaea*). Cultivated peanuts are produced as seed for edible oil and food in more than 100 countries worldwide (Huang et al., 2012). Currently, peanuts are ranked fifth in the world in terms of oil production. The only other known species in this genus cultivated for seed are *Arachis stenosperma* Krapov. & W.C. Greg. and *Arachis villosulicarpa* Hoehne (Stalker and Simpson, 1995; Stalker, 1997).

Due to the economic importance of cultivated peanut, germplasm is preserved and maintained around the world in ex situ collections. Preservation of agricultural resources in the form of collecting and storage of seeds or plant materials in gene banks is the conservation method of choice to prevent extinction of natural plant populations (Benz, 2012). One shortcoming of maintaining these collections ex situ is that genetic variation is thought to be eroding as modern cultivars replace traditional ones and the natural habitats of wild species are being destroyed (Upadhyaya et al., 2008b). On the other hand, in situ preservation has

certain advantages such as adaptation to local conditions, natural gene flow, and preservation of genetic diversity. However, due to the loss of natural habitats and the exponential growth and expansion of human populations, the habitat of many plant species in the modern world is threatened or already has been demolished. Agriculture systems over time encounters ecological driven and other challenges (i.e., biotic and abiotic stressors, culinary preferences, increased demand for a particular product as the human population increases) many of which are overcome by breeding and selection via resorting to the genes found in landraces, breeder stocks, and wild relatives (Benz, 2012).

The largest collections of *Arachis* germplasm are housed in India (International Crops Research Institute for the Semi-Arid Tropics, ICRISAT), United States (United States Department of Agriculture, USDA), China (Oil Crops Research Institute, Chinese Academy of Agricultural Sciences (OCRI-CAAS)), and Brazil (Empresa Brasileira de Pesquisa Agropecuaria, EMBRAPA), although smaller collections do exist in many countries around the world. The holdings, preservation strategies, and evaluation of the largest collections (in ICRISAT, USDA, and OCRI) will be discussed. Conservation of genetic diversity provides a library of different traits that can be mixed in various combinations in order to produce new varieties needed to thwart environmental challenges. Normally, gene banks play an important role in providing genetic diversity for interrelated research and are responsible not only for the integrity of the samples, but also for all of their associated data (Richards and Volk, 2010). Conservation and utilization of genetic resources is critical for ensuring sustainable increases in production of healthy food for mankind and increased resilience of agricultural ecosystems (Dulloo et al., 2010).

Germplasm Collections and Preservation

USDA Germplasm Collection

The USDA Agricultural Research Service (ARS) Plant Genetic Resources Conservation Unit in Griffin, Georgia (USA) maintains a large *Arachis* germplasm collection. At the time of this writing, this collection consisted of 9976 accessions of which 9321 are cultivated peanut and 655 are wild species. This collection is derived of lines collected from 102 different countries around the world. Approximately 44% of the collection was collected in South America, where peanut originated. The majority (>95%) of the USDA peanut germplasm collection is backed up at a secondary location (National Center for Genetic Resources Preservation in Fort Collins, Colorado, USA); thus, in case of a natural disaster in Griffin, Georgia, this valuable collection could be recovered. The cultivated peanut collection is solely maintained as seed. Each seed accession is split into two samples which are stored at 4 °C and −18 °C. The wild species, on the other hand, are either maintained clonally (since some do not produce seed under cultivated conditions) or as seed collections in 4 °C and −18 °C temporal storage. The 4 °C samples are maintained at 25% relative humidity and are

primarily used to distribute seeds to requestors worldwide, whereas the −18 °C are stored in heat-sealed bags and are for long-term storage/preservation.

The USDA germplasm collection not only preserves valuable germplasm, but also serves the national and international communities by providing plant cuttings and seeds to requestors for research and education purposes. From 1993 to 2013, over 6000 peanut lines were distributed to over 52 countries reaching all continents except Antarctica. China, Israel, Bolivia, and South Korea were the most frequent requestors of peanut germplasm outside of the United States. In this same time period, over 26,600 seed samples has been sent to 46 of 50 states in the USA with Georgia being the top receiver of peanut germplasm with 15,645 samples shipped to scientists in Georgia during the past 20 years. The number of accessions distributed per year ranges from 500 to 9720. The most frequently distributed cultivated peanuts are "Florunner," "New Mexico Valencia A," "Guanajuato," "Pronto," "US 1260," "Georgia Red," "Early Bunch," "Spanco," and "Chico." The most requested wild species are *A. ipaënsis*, *Arachis pintoi* Krap. et W.C. Gregory, *A. duranensis*, *Arachis diogoi* Hoehne, *Arachis monticola* Krap. & W.C. Gregory, *Arachis cruziana* Krap., W.C. Gregory, & C.E. Simpson, *Arachis paraguariensis* Chodet & Hassl., and *Arachis glandulifera* Stalker. The reasons for the germplasm requests vary widely but have included studies on molecular genetics, cytogenetics, physiology, disease resistance, breeding, biochemical evaluation, drought stress, sustainable gardens, and education purposes.

The USDA collection currently conserves 66 distinct *Arachis* species (Table 1). Preservation of wild species is difficult even under the best conditions because they occupy many different, highly specialized habitats in their sites of origin. Wild species have been discovered in a range of habitats from semiaquatic to extremely arid conditions occurring in transition zones between forests and grasslands. It is possibly that the evolution of the species under harsh conditions (i.e., drought, fires, etc.) may account for the geocarpic habit protecting valuable seeds underground (Stalker, 1997). Therefore, many of the wild species that have been conserved are propagated vegetatively and maintained as clonal plants due to poor or nonexistent seed set (Stalker and Simpson, 1995). Regeneration of wild species tends to be fairly intensive and challenging with either few pods produced in a greenhouse setting versus fairly good seed set in the field, but labor intensive because the soil has to be sifted and cleaned by hand to recover the pods.

Critical genetic gaps need to be filled in the USDA wild species collection and acquisitions are being pursued when possible to fill these voids. The wild species are vitally important to any peanut germplasm collection due to the wide range of diverse traits that are part of the genetic makeup, but not typically found in the cultivated species. Extremely high levels of resistance or immunity have been observed in many of the wild *Arachis* species (Stalker et al., 2013), making them highly valuable genetic resources. Utilizing the wild species in breeding strategies can improve the narrow range of diversity in cultivated peanut. A comprehensive review of the different *Arachis* species, their resistance to important diseases and pests can be seen in Stalker et al. (2013). Introgression

TABLE 1 List of the Species in the USDA Peanut Germplasm Collection

Taxon	Section
Arachis batizocoi	*Arachis*
Arachis benensis	*Arachis*
Arachis cardenasii	*Arachis*
Arachis correntina	*Arachis*
Arachis cruziana	*Arachis*
Arachis decora	*Arachis*
Arachis diogoi	*Arachis*
Arachis duranensis	*Arachis*
Arachis glandulifera	*Arachis*
Arachis gregoryi	*Arachis*
Arachis helodes	*Arachis*
Arachis herzogii	*Arachis*
Arachis hoehnei	*Arachis*
Arachis hypogaea	*Arachis*
Arachis hypogaea var aequatoriana	*Arachis*
Arachis hypogaea var fastigiata	*Arachis*
Arachis hypogaea var hirsuta	*Arachis*
Arachis hypogaea var hypogaea	*Arachis*
Arachis hypogaea var peruviana	*Arachis*
Arachis hypogaea var vulgaris	*Arachis*
Arachis ipaënsis	*Arachis*
Arachis kempff-mercadoi	*Arachis*
Arachis krapovickasii	*Arachis*
Arachis kuhlmannii	*Arachis*
Arachis lignosa	*Arachis*
Arachis linearifolia	*Arachis*
Arachis macedoi	*Arachis*
Arachis magna	*Arachis*
Arachis microsperma	*Arachis*

Continued

TABLE 1 List of the Species in the USDA Peanut Germplasm Collection—cont'd

Taxon	Section
Arachis monticola	*Arachis*
Arachis palustris	*Arachis*
Arachis praecox	*Arachis*
Arachis simpsonii	*Arachis*
Arachis stenosperma	*Arachis*
Arachis trinitensis	*Arachis*
Arachis valida	*Arachis*
Arachis villosa	*Arachis*
Arachis williamsii	*Arachis*
Arachis pintoi	*Caulorrhizae*
Arachis repens	*Caulorrhizae*
Arachis archeri	*Erectoides*
Arachis benthamii	*Erectoides*
Arachis cryptopotamica	*Erectoides*
Arachis hermannii	*Erectoides*
Arachis major	*Erectoides*
Arachis oteroi	*Erectoides*
Arachis paraguariensis	*Erectoides*
Arachis paraguariensis subsp. *capibarensis*	*Erectoides*
Arachis paraguariensis subsp. *paraguariensis*	*Erectoides*
Arachis porphyrocalyx	*Erectoides*
Arachis stenophylla	*Erectoides*
Arachis burchellii	*Extranervosae*
Arachis lutescens	*Extranervosae*
Arachis retusa	*Extranervosae*
Arachis villosulicarpa	*Extranervosae*
Arachis dardanoi	*Heteranthae*
Arachis pusilla	*Heteranthae*

TABLE 1 List of the Species in the USDA Peanut Germplasm Collection—cont'd

Taxon	Section
Arachis seridoensis	*Heteranthae*
Arachis sylvestris	*Heteranthae*
Arachis appressipila	*Procumbentes*
Arachis chiquitana	*Procumbentes*
Arachis kretschmeri	*Procumbentes*
Arachis matiensis	*Procumbentes*
Arachis pflugeae	*Procumbentes*
Arachis rigonii	*Procumbentes*
Arachis subcoriacea	*Procumbentes*
Arachis burkartii	*Rhizomatosae prorhizomatosae*
Arachis glabrata	*Rhizomatosae* series rhizomatosae
Arachis glabrata var. glabrata	*Rhizomatosae* series rhizomatosae
Arachis glabrata var. hagenbeckii	*Rhizomatosae* series rhizomatosae
Arachis nitida	*Rhizomatosae*
Arachis pseudovillosa	*Rhizomatosae* series rhizomatosae
Arachis guaranitica	*Trierectoides*
Arachis triseminata	*Triseminatae*
Arachis hybrid	Mixed sections
Arachis species	–

of genes from wild species into cultivated peanut is a long process of breeding and selection; however, great gains can be made in improving cultivated types using this strategy. Peanuts developed from the introgression and selection of genes from a wild species such as, root-knot nematode (*Meloidogyne arenaria)*, leaf spots, and *Sclerotinia minor* resistance have been highly successful at delivering improved cultivars (Holbrook et al., 2008; Isleib et al., 2011; Simpson and Star, 2001; Simpson et al., 2003).

The lifespan of peanut seeds is limited. Overall, there is limited information on the particular attributes of seed that affect its storage performance (Walters et al., 2005), and thus, more research is needed to improve the longevity of peanut seeds. However, it is generally thought among the peanut

community that 10–15 years is the maximum amount of time to store peanut seeds in cold storage after which the viability dramatically declines. This timeframe means that approximately 665–1000 lines need to be regenerated annually from the USDA collection to avoid loss of valuable genetic resources. Research of storage longevity in peanut suggests that storing cultivated peanut seeds at −18 °C prolongs the lifespan and increases the time that a lot of seeds can be stored (Walters et al., 2005) while still maintaining high viability which in turn reduces the number of times a line needs to be regenerated. The P50 (rate of time in which germination was reduced to 50%) was 25 years when seeds were stored at −18 °C (Walters et al., 2005). Given the size of this collection and an approximately 25 year life span of seeds for germination to drop to 50%, then approximately 400 accessions would need to be regenerated yearly in order to keep the collection in good shape.

The primary function of ex situ conservation sites is to maintain viable germplasm as long as possible and reduce the frequency of regeneration of the genetic resources which can cause the loss of genetic diversity through genetic drift (Dulloo et al., 2010). Unfortunately, since peanut does not remain viable in storage for a long period of time compared with other crops, frequent regeneration is generally necessary. Regenerations become necessary for ex situ collections in order to increase the quantity of seed when the accession has become depleted or to restore the viability to a particular seed lot (Upadhyaya et al., 2008b). This is a critical and a necessary process that tends to be costly in terms of resources and does involve certain risk to the genetic integrity of an accession (Upadhyaya et al., 2008b) especially in cases of species that outcross regularly. For the USDA peanut collection, accessions are chosen to be regenerated based on the age of the seed, germination information, lines with low seed counts, or ones that are frequently requested. Most lines that are regenerated for a seed increase are grown in standard two row plots that are 10 ft (3 m) long with 100 seeds planted at a depth of 1.5–2 inches (3.8–5 cm) and sprayed to control the major diseases to ensure the best possible yield and recovery of seeds. All harvested pods are dried for several days to obtain 7–8% moisture content in the seeds. Pods are shelled to reduce the footprint in storage and to recover the seeds that are subsequently placed in cold storage. In 2012, an average of 1050 seeds per accession were harvested using this planting regime with a mean of 80% germination.

ICRISAT Germplasm Collection

The world's largest peanut collection of 15,446 accessions from 92 countries is housed at "The RS Paroda genebank" in ICRISAT, Patancheru, India. The ICRISAT collection represents 14,968 accessions of cultivated peanut from 92 countries and 478 accessions of 48 wild *Arachis* species from six countries. A total of 12,669 accessions were assembled through donation from 84 institutes in 41 countries and 2777 accessions were collected in 67 collection missions from 29 countries. The cultivated peanut accessions represent

7172 traditional cultivars/landraces, 979 advanced or improved cultivars, 4986 breeding lines/research materials and 1831 genetic stocks (mutants and experimental germplasm). The cultivated peanut collection represents all the six botanical varieties: 44.0% var. *hypogaea* (6791 accessions), 35.6% var. *vulgaris* (5494 accessions), 15.2% var. *fastigiata* (2353 accessions), 0.1% var. *aequetorania* (14 accessions), 0.1% var. *hirsuta* (20 accessions), and 1.6% var. *peruviana* (251 accessions). Three hundred fifty-two wild species accessions belonging to 45 species are seed-producing and 100 accessions of two species are vegetatively propagated. One hundred ninety-five accessions of 16 species are annual and 232 accessions of 17 species are perennial.

At ICRISAT, each seed accession is split into two samples stored at 4 °C as an active collection in medium-term storage and at −20 °C as a base collection in long-term storage. Two wild species, on the other hand, are maintained as live plants under controlled environment facility and 46 as seed collections in active and base collections. The active collections are maintained at 30% relative humidity and are primarily used to distribute seeds to requestors worldwide, whereas the base collections are stored at 7% moisture content in re-sealable laminated aluminum foil packets. This germplasm is freely available for distribution providing the requisitioned signs a standard material transfer agreement with ICRISAT. Additionally, 13,900 peanut accessions have been deposited for safety duplication at the Global Seed Vault at Svalbard, Norway. The ICRISAT gene bank has repatriated 6049 peanut accessions to India. Demand for seeds is met by distributing the samples from the medium-term storage until the seed is about to be exhausted or when seed viability begins to decline below 85%; then the accession is regenerated.

ICRISAT has supplied more than 100,400 seed samples of 14,465 unique accessions to researchers in 96 countries worldwide, involving 93.6% of the entire peanut collection conserved at ICRISAT gene bank. This includes 39 sets of the peanut mini core collection to the researchers in 13 countries. The most frequently requested accessions were ICG 799 (Robut 33-1), ICG 221 (TMV 2), and ICG 156 (M 13), ICG 2738 (Gangapuri), and ICG 1697 (NCAc 17090). TMV 2 is an early maturing Spanish type released in India. Robut 33-1 is a Virginia bunch type, and M 13 is a large-seeded Virginia runner type released in India. Gangapuri is an early-maturing Valencia type released in India. NCAc 17090 is resistant to rust and late leaf spot (LLS). India is the most beneficiary country receiving 47,773 seed samples followed by Malawi (18,757 seed samples), Indonesia (7007 seed samples), Niger (6446 seed samples), Thailand (1496 seed samples), and China (1354 seed samples). Additionally over 97,600 seed samples were supplied to researchers within ICRISAT for crop improvement.

The regenerated seeds are stored in medium-term storage for further distribution. For regeneration, germplasm lines are grown in standard four row plots that are 4 m long with about 160 seeds planted at a depth of 5–7 cm and sprayed to control the major diseases and pests to ensure the best possible yield and recovery of seeds. The critical accessions that are not adapted to the Patancheru environment

are regenerated in an *Arachis* greenhouse under control environment facility. All harvested pods are dried for several days in air dryer at 15 °C and 15% relative humidity (RH) to obtain 8% moisture content in the seeds for medium-term and 7% moisture for long-term storage. Each accession is monitored for seed viability at the interval of 5 years in medium-term storage and 10 years in long-term storage.

OCRI Germplasm Collection

There has been a long history of peanut cultivation in China where the natural conditions are much more diversified than most other peanut producing countries. In China, systematic collection and preservation of peanut germplasm materials was first initiated in the early 1950s (Sun, 1998). By 1959, a total of 1239 accessions of cultivated peanut were collected from various provinces and then the number of peanut accessions increased to 2378 in 1963 (Sun, 1998). The collection of peanut land races in the 1950s and early 1960s also facilitated the research on the classification of cultivated peanut in China (Sun, 1998). Compared to the peanut germplasm collection in other countries, more Dragon type (var *hirsuta*) genotypes were collected in China as varieties that were most extensively cultivated until the early twentieth century (Sun, 1998). In 1978, the List of Chinese Peanut Genetic Resource was published in which 1577 varieties (including 44 Valencia, 481 Spanish, 151 Dragon, 713 Virginia, 25 intermediate type accessions, and 160 introduced lines) were described (Sun, 1998).

Although most peanut landraces grown in China had been collected and preserved before 1978, extensive collection and characterization of peanut germplasm have been conducted since the late 1970s (Jiang and Ren, 2006; Jiang et al., 2008b, 2010b, 2014; Liao, 2014; Yu, 2011). During the past four decades the collection and characterization of peanut germplasm have been coordinated by the OCRI-CAAS located in Wuhan with extensive participation of various research institutions throughout the country (Sun, 1998). Besides complementary collection of landraces from all the peanut growing provinces, many improved cultivars and breeding lines were assembled and added to the collection. Meanwhile, more germplasm lines were introduced from other countries. By 2013, 8439 accessions of cultivated peanut including landraces, improved cultivars, breeding lines, and special genetic resources have been collected and assembled in China, and among the current holdings 8439 accessions, 4638 are landraces collected from 22 provinces. With the introduction of the mini core collection developed at ICRISAT, the peanut germplasm collection in China consists of all the six botanical types of *A. hypogaea* (Jiang et al., 2008b, 2010a). In addition to the cultivated peanut germplasm, 246 accessions of wild *Arachis* species have been introduced from the USA and ICRISAT and characterized for various traits since 1979. With the support of the Ministry of Agriculture, a National *Arachis* Nursery was established at the OCRI for preserving these wild materials. A branch of the wild *Arachis* nursery was also set up in Nanning, Guangxi in south China (Sun, 1998).

The Institute of Crop Science of CAAS in Beijing is responsible for long-term conservation of crop germplasm and active in managing the National Crop Germplasm Genebank. The crop germplasm accessions in various crops including peanut are conserved in the gene bank at −5 to −10 °C. Based on various studies, the germination of peanut seeds was 75% after storage for 12 years under conventional temperature if the moisture is not higher than 3.5% (Yu, 2011); thus, peanut germplasm could be maintained cheaply under extra low moisture. In order to provide germplasm to peanut breeders and other researchers, a mid-term gene bank was established at OCRI in Wuhan. OCRI is also responsible for regular regeneration, dissemination and exchange of the peanut germplasm in the country.

Challenges with Ex Situ Genebanks

Genebanks are tasked with maintaining the diversity found in seeds of the collection. These seeds can be highly heterogeneous, homogenous, cultivars, genetic stocks, composed of maternal lines or even a mixture of multiple populations and these diverse types present multiple challenges for the management of these collections (Richards et al., 2010). Very few studies have looked at the variability within single peanut accessions; however, accessions with mixed morphological traits (Figure 1) have been observed and noted or in some cases split into separate accessions to preserve unique morphological differences. One study evaluated key single nucleotide polymorphisms (SNPs) in *ahFAD2* genes linked to oleic and linoleic acid accumulation and found a few heterozygous lines from a set of peanuts that had been purified (Chen et al., 2013; Wang et al., 2011). This was unexpected in a self-pollinating, purified set of germplasm. Whether to keep, split, or purify out this genetic variability is often difficult to determine. In certain cases, purification and genetic homogeneity is important for the ease of molecular genomic studies. However, for breeding and preservation of genetic diversity the heterogeneity is advantageous and often desired to maintain. If accessions are split into separate accessions due to apparent heterogeneity then the size of the germplasm collection grows and can become unmanageable if a large number of accessions are variable. It is unknown how much variability there is within each accession in the USDA, ICRISAT, or OCRI collections, and thus, more research is needed to understand and evaluate the extent of this variability.

There are many challenges with maintaining ex situ collections. One of the largest challenges is having adequate funding (labor, supplies, etc.) to support the maintenance and evaluation of any germplasm collection. In the United States, the National Plant Germplasm System (NPGS) has steadily grown in size since its inception; however, funding for the germplasm collections has steadily decreased in recent years making the task of maintenance and evaluation very challenging. Another limitation is the practice of patenting plants, material transfer agreements (MTA), and international treaties which have been put in place to protect breeders or genetic resources originating from a particular

country. This has ultimately limited acquisition and distribution of germplasm to all users. Another challenge is being able to simulate the conditions that are similar to the plant's native origin (i.e., drought, day length, etc.) for adequate seed set in regenerating and maintaining plant germplasm collections. Lastly, significant genetic drift and allele loss also can occur by maintaining plants ex situ and strategies need to be considered to avoid this loss in diversity.

Maintaining germplasm collections requires a series of sampling for regeneration purposes over time to ensure viability of the germplasm. Factors such as seed deterioration, range in allele frequencies, and timing of regeneration affect the extent of genetic drift (Richards et al., 2010). The regeneration frequency in ex situ collections will depend on the conditions of storage and the rate at which the species loses viability. A computer simulated modeling study demonstrated what

FIGURE 1 Heterogeneity that exists among some peanut accession demonstrated by different testa colors.

may occur in germplasm collections when considering genetic drift, frequency of allele loss over time, germination rates, and the evaluation of different regeneration strategies (Richards et al., 2010). This study showed a greater effect on the loss of allelic richness in wild species seed lots than in homozygous cultivars that are self-pollinating when performing serial regenerations (serial regenerations are characterized by sampling seed lots when the next generation is derived solely from the last regeneration of a particular accession). According to the modeling, the largest loss in allelic richness occurs early on in ex situ collections and especially in the case of seed lots with low viability. Therefore, based on this simulation, it is imperative to increase the first generation of seed that will be produced from the original seed source by planting out a large number of seeds (500–1000 seeds as opposed to a small sample with 50–100 seeds) whenever possible to avoid the loss of rare alleles. On the other hand, parallel regenerations in which the source of seeds to produce the next generation is always obtained from the original source of seeds does not result in significant loss in allelic richness. Allelic richness of seed lots could be significantly mitigated in serial or parallel regeneration strategies when germination ratios and the overall longevity of seed lots are high (Richards et al., 2010). The main difficulty with parallel regeneration in peanut conservation is that the life span of seeds in storage rapidly decays, thus making the original source not an ideal selection after 15 or so years. Therefore, curators are frequently forced to mainly perform serial regenerations. Furthermore, the original seed would have to be obtained in a fairly large quantity for parallel regenerations. In many cases, very few seeds are collected or donated to gene banks which make parallel regenerations impractical.

Prior to the 1990s, collecting native plants and exchanging germplasm of various species was a fairly straight forward process with free and open access to genetic resources (Williams, 2005). Collection trips were organized in areas where a species of interest originated, plant materials were collected (seeds, pods, and/or cuttings), and materials were subsequently taken back into the country where the plant collector was stationed. The materials were then evaluated and incorporated into germplasm collections and breeding programs. Of course, all protocols were followed to prevent the spread of disease when foreign material was acquired. In addition, scientists would often freely exchange germplasm to collaborators or interested parties worldwide. Since 1993, exchanging and collecting germplasm has become a fairly restrictive process. The Convention of Biological Diversity (CBD), which aims to conserve and utilize resources sustainably, changed the landscape of acquisition of genetic resources. CBD recognizes the sovereign rights of nation's genetic resources in all forms (plant, animal, etc.) and requires prior informed consent from the government before a collection trip occurs. In addition, benefits from the potential commercialization or any profit on genetic resources are shared along with technologies and education. Changes in these international laws have had significant effects on the NPGS Plant Exploration Program (Williams, 2005) and have limited acquisition of indigenous materials. Another factor that may limit acquisitions in the future is climate change. The phenomenon could have a significant impact on

wild *Arachis* populations and many wild species are predicted to go extinct by 2055 (Jarvis et al., 2008). Therefore, it is critical that germplasm are collected, exchanged, and preserved prior to the extinction of wild species.

Germplasm collection is a means of preserving the genetic diversity of a crop species before that diversity is lost as a result of implementing high input crop monoculture systems and replacement of traditional varieties by modern high yielding varieties The genetic resources contained in germplasm collections will be the basis for much of the future progress in developing new cultivars. Germplasm collections are constantly expanding, which increases the difficulty of evaluating a collection for traits of interest and resulting in its low use in crop improvement programs.

Germplasm Characterization and Evaluation

The importance of preserving germplasm has been previously discussed. In addition to conservation, characterization (either phenotypic, molecular, or genomic), and documentation of these traits of all materials included in a germplasm collection is just as critical. As the size of a collection grows, it becomes more important that traits are properly cataloged so that the users can focus on what accessions have the unique traits they need for their research and breeding programs. In most cases, germplasm collections are far too large to evaluate numerous traits on each individual line. Thus, genetic resources that are uncharacterized tend to be underutilized. Strategies such as mining core or mini core collections can help narrow down the number of individuals to investigate, help identify more sources of accessions with a particular trait of interest, and indicate regions (clusters of accessions) to concentrate on for additional evaluation of a particular trait. Generally, some stratification/selection process needs to occur to narrow down the number of samples to a manageable sample set.

Different production areas and regions around the world have varying traits that are important for their breeding programs to improve cultivated peanuts. Some of the most important traits that breeders tend to focus on for the improvement of peanut include, but are not limited to, disease resistance, yield, flavor, oil content, protein and mineral content, seed size, seed color, drought tolerance, and aflatoxin mitigation. Characterizing germplasm for these and other traits either phenotypically or molecular methods (i.e., quantitative trait loci analysis, or associating markers with these traits) will greatly help improve the utilization of peanut genetic resources.

Phenotypic Variation

A series of descriptors have been developed for standardizing the characterization of peanut genetic resources using various morphophysiological, reproductive, and biochemical traits (IBPGR and ICRISAT, 1992; Jiang and Duan, 2006; Pittman, 1995). Following these descriptors, 14,952 (99.9%) cultivated peanut accessions and 292 (61.1%) wild *Arachis* accessions have been characterized at ICRISAT (http://www.icrisat.org/), 9000 in the United States, and more than

7000 accessions at OCRI in China. The majority of peanut germplasm showed a large variation for qualitative (Figure 2) and quantitative traits, seed quality traits and resistance to biotic and abiotic stresses (Dwivedi et al., 2007; Upadhyaya et al., 2001a). The evaluation of peanut germplasm at ICRISAT and elsewhere identified a large number of accessions possessing tolerance/resistance to biotic and abiotic stresses (Tables 2–4). Several of these genetic resources have been used in breeding programs to develop improved breeding lines/cultivars resulting in significant economic gains to peanut farmers (Liao, 2014; Yu, 2011).

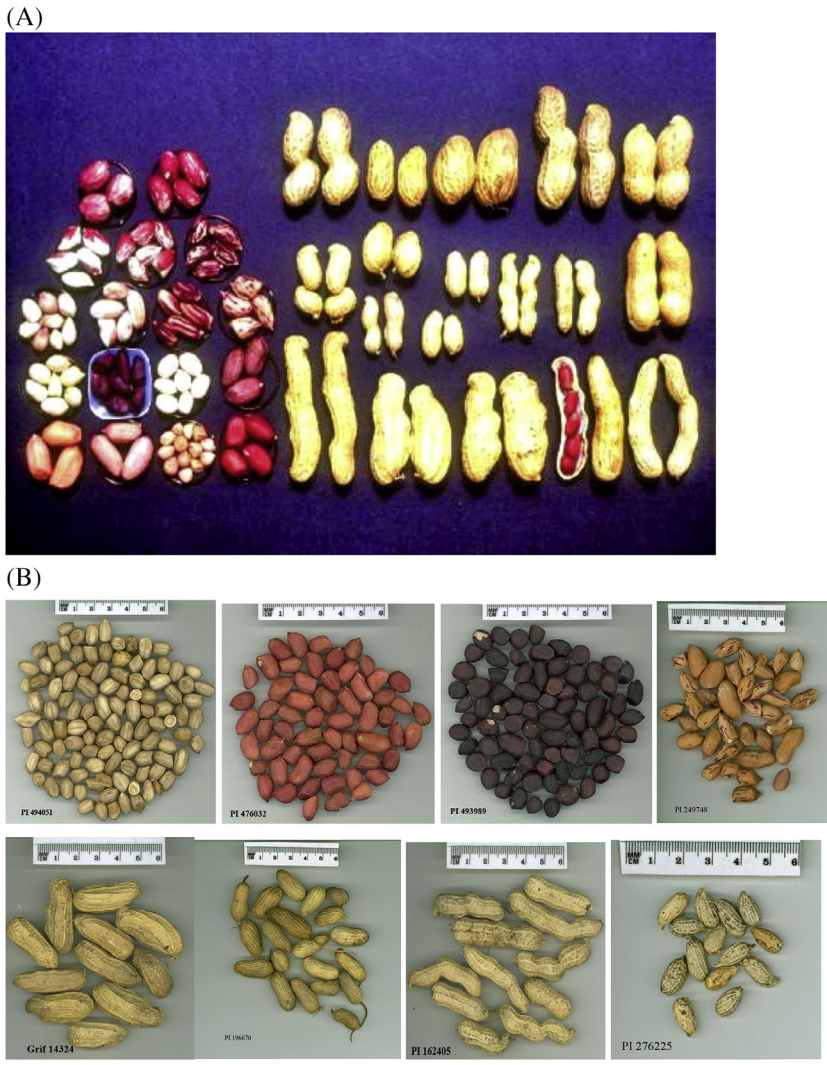

FIGURE 2 Diversity for pod and seed characters in (A) ICRISAT peanut germplasm and (B) USDA germplasm.

TABLE 2 Sources of Resistance to Abiotic and Biotic Stress and Those with Nutrient-Dense Seeds by Various Workers after Evaluating the ICRISAT Peanut Mini Core Collection

Stress	Resistant Genotype		References
Abiotic stresses	Subsp. *fastigiata*	Subsp. *hypogaea*	
Drought	ICG#s 434, 442, 1274, 2106, 3584, 3673, 5475, 6646, 8517, 10554, 11088, 12625	ICG#s 862, 2511, 3053, 5663, 8285, 11855, 14475	Upadhyaya (2005), ICRISAT (2008, 2009, 2010), and Hamidou et al. (2012)
Heat	ICC#s 5236, 6022, 6646, 8517, 9315, 9809, 11088, 12625, 12879, 14985, 15042	ICC#s 862, 1668, 2925, 8285, 9777, 11109, 13982,	Hamidou et al. (2013)
Salinity	ICG#s 442, 2106	ICG#s 862,8285, 9842, 11855	ICRISAT (2008, 2009) and Srivastava (2010)
Low temperature	ICG#s 1274, 5475, 5609, 8517, 10554, 11088, 12625		Upadhyaya et al. (2001b, 2009)
Phosphorus deficiency	ICG#s 442, 646, 3584, 3673, 5609	ICG#s 5663, 9842, 14475	Biradar (2007)
Biotic stresses	Subsp. *fastigiata*	Subsp. *hypogaea*	
Leaf spots	ICG#s 4684, 6022, 12625, 12697	ICG#s 76, 532, 1668, 2857, 2925, 4156, 4412, 6402, 6993, 7243, 8760, 9037, 9777, 9961, 11109, 11426, 12000, 12276, 12672, 13787, 15190	Yugandhar (2005), Ajay (2006), Kusuma et al. (2007), Khalid (2008), Madhura (2006), Sujay et al. (2008), and ICRISAT (2008, 2009, 2010)
Rust	ICG 12697	ICG#s 76, 532, 2381, 2857, 2925, 4412, 6993, 7243, 8760, 9037, 9842, 9961, 9777, 11109, 11426, 12000, 13099, 13787, 14008,	Yugandhar (2005), Ajay (2006), Kusuma et al. (2007), Khalid (2008), Sujay et al. (2008), and ICRISAT (2008, 2009, 2010)

Stress	Resistant Genotype		References
Aspergillus flavus	ICG#s 12625, 12697	ICG#s 76, 2381, 4156, 6402, 8760, 13787	Yugandhar (2005), Kusuma et al. (2007), ICRISAT (2009), Zhang (2010), and Jiang et al. (2010d)
Bud necrosis disease	ICG 4684	ICG#s 76, 1668, 4412, 11109, 12000, 12672, 13099, 14008, 14482, 15190,	Khalid (2008) and ICRISAT (2008, 2009, 2010)
Bacterial wilt	ICG 12625	ICG#s 76, 1668	ICRISAT (2008, 2009), Zhang (2010), and Jianwei et al. (2010)
Seed quality	Subsp. *fastigiata*	Subsp. *hypogaea*	
Oil (%)	ICG#s 3681, 4955, 5475, 12625, 14710, 15309	ICG#s 5827, 6402, 14482	Upadhyaya et al. (2012a)
Protein (%)		ICG#s 5051, 7963, 13982	Upadhyaya et al. (2012a)
O/L ratio	ICG#s 1274, 5221, 5475, 12625	ICG#s 2381, 15419	Upadhyaya et al. (2012a)
Fe (mg kg^{-1})	ICG#s 1274, 4955, 5221, 5475, 14710, 15309	ICG#s 5051, 5827, 6402, 7963, 13982, 14482, 15419	Upadhyaya et al. (2012b)
Zn (mg kg^{-1})	ICG#s 3681, 5221, 14710, 15309	ICG#s 5051, 5827, 6402, 7963, 13982, 15419	Upadhyaya et al. (2012b)

Upadhyaya et al. (2014a).

TABLE 3 Agronomic Performance of Selected Multiple-Trait Specific Peanut Mini Core Germplasm

Identity	Resistances	Yield ($kg\,ha^{-1}$) (Three Rainy and Six Postrainy Seasons Pooled)
ICG 1668	Heat, LLS, PBND, BW	1626
ICG 2381	Rust, *Aspergillus flavus*, O/L	1677
ICG 2925	Heat, LLS, rust	1468
ICG 5475	Drought, low temperature, oil, O/L, Fe	1422
ICG 8285	Drought, heat, salinity	2083
ICG 11088	Drought, low temperature	2506
ICG 12625	Drought, low temperature, LLS, *Aspergillus flavus*, BW, oil, O/L	1953
ICG 14482	PBND, Fe, oil	1830
ICG 11426	ELS, LLS, rust	2034

LLS, late leaf spot; PBND, peanut bud necrosis diseases; BW, bacterial wilt; O/L, oleic/linoleic acid ratio; Fe, iron content in kernels; ELS, early leaf spot.

TABLE 4 Sources of Resistance to Rust, Leaf Spots, *sclerotinia* Blight, Groundnut *rosette* Virus, Aflatoxin, Nematode, Defoliator, Aphid, and Drought Reported in Cultivated and Wild *Arachis* Species

Trait	Peanut Accessions with Beneficial Traits Reported			
	Cultivated Species No. Evaluated	References	Wild *Arachis* Species No. Evaluated	References
Rust	169	Singh et al. (1997)	29	Subrahmanyam et al. (1995)
Late leaf spot	69	Singh et al. (1997)	27	Upadhyaya et al. (2001a)
Early leaf spot	37	Singh et al. (1997)	11	Upadhyaya et al. (2001a)
Groundnut rosette virus	116	Subrahmanyam et al. (1998)	12	Subrahmanyam et al. (2001)
Nematode	21	Holbrook et al. (2000a)	–	–
Seed infection and/or aflatoxin production by *Aspergillus flavus*	21	Singh et al. (1997)	4	Thakur et al. (2000)
Sclerotinia blight	50	Damicone et al. (2003)	–	–
Defoliator (Leaf miner and *Spodoptera*)	9	Dwivedi et al. (1993), Wightman and Rao (1994), Rao and Wightman (1999), and Stalker and Lynch (2002)	38 and 67	Wightman and Rao (1994) and Lynch and Mack (1995)
Aphid	EC 36892 and ICG 12991	Padagham et al. (1990), and Minja et al. (1999)	Wild species not evaluated	
Drought	40	Nigam et al. (2003) and Seetharama et al. (2003)	Wild species not evaluated	
Multiple biotic, abiotic, agronomic, and nutritional traits	82	Upadhyaya et al. (2014a)	20 wild accessions from genus *Arachis* identified for tolerance to drought, superior agronomic, and nutritional traits	Upadhyaya et al. (2011a)

The characterization of diversity in germplasm collections is important to plant breeders to utilize and to the gene bank curators to manage the collection efficiently and effectively. Upadhyaya et al. (2002b) describe the phenotypic diversity in the 13,342 accessions of peanut germplasm contained in the ICRISAT gene bank. Data for 16 morphological descriptors, 10 agronomic traits in two seasons, and for reaction to early leaf spot and peanut rosette virus disease were used to determine the phenotypic variation in different geographical regions. Phenotypic variation was found for most traits in all the regions. The means of geographic regions for different agronomic traits differed significantly. The variances for all the traits among regions were heterogeneous. South America germplasm showed 100% of the range of the entire collection for 12 of the 16 morphological descriptors and on average showed the highest range of variation. The Shannon–Weaver diversity index was variable in different regions for different traits. Of the various geographic regions examined, South America showed the highest pooled diversity index for primary seed color among morphological traits, and leaflet length among agronomic traits, showed highest pooled diversity index. Three of the six botanical varieties, *Arachis aequatoriana, Arachis hirsuta*, *and Arachis peruviana* are poorly represented and need more collection efforts in both primary and secondary centers of diversity.

Molecular and Genomic Variation

Molecular markers (amplified fragment length polymorphism, AFLPs; random amplified polymorphic DNA, RAPDs; restriction fragment length polymorphism, RFLPs; SNPs; and simple sequence repeats, SSR/microsatellites) have direct use for germplasm characterization, trait mapping, and molecular breeding in peanut (Pandey et al., 2012). Assessing the level of genetic diversity in germplasm through fingerprinting with molecular markers provides information on relatedness among individuals, genetic redundancy or diversity, reveal misidentified accessions, population structure, and hybrid origins (Barkley et al., 2006, 2007; Wang et al., 2011) and can help provide insights on the overall management of genetic resources. Molecular markers have been fairly extensively utilized to assess inter- and intraspecific genetic variation in cultivated and wild peanut germplasm (Pandey et al., 2012). Many researchers have found the diversity levels to be low within the cultivated types (Liang et al., 2009; Moretzsohn et al., 2004; Pandey et al., 2012; Stalker and Mozingo, 2001; Yuan et al., 2010) especially when using expressed sequence tag-derived SSRs or diversity arrays technology (DArT) markers. Out of 67 newly developed SSR markers only three were polymorphic in cultivated peanuts (Moretzsohn et al., 2004). In another study, 138 SSR markers were developed and evaluated on four wild genotypes and 20 cultivated genotypes. The majority of the SSR markers revealed allelic polymorphisms in the wilds while relatively few detected genetic variation among the cultivated types (Yuan et al., 2010). Conversely, high intraspecific variability was observed in

a separate study evaluating wild *Arachis* species using intron sequences and microsatellite markers (Moretzsohn et al., 2012; Stalker and Mozingo, 2001).

ICRISAT in collaboration with EMBRAPA and Generation Challenge Program (http://www.generationcp.org) developed peanut composite collections consisting of 1000 diverse peanut accessions, which included the 184 peanut mini core subset and 52 accessions of 14 wild species. This composite collection was molecularly profiled using 21 SSRs. The composite collection showed rich allelic diversity (490 alleles, 23 alleles per locus, 246 common alleles, and 244 rare alleles at 1%), group-specific unique alleles, and common alleles sharing between subspecies and geographical regions (Upadhyaya et al., 2008a,c). Unique alleles are those detected in a group of accessions but absent in other groups. Group-specific unique alleles numbered 101 in wild type accessions of species *A. batizocoi, Arachis cardenasii* Krap. et W.C. Gregory, *A. diogoi, A. duranensis, Arachis hoehnei, A. ipaënsis, Arachis kempff-mercadoi* Krapov., W. C. Gregory & C.E. Simpson, *A. monticola, Arachis villosa* Benth., and *A. stenosperma* in genus *Arachis*, 50 in subsp. *fastigiata*, and only 11 in subsp. *hypogaea*. Accessions from the Americas revealed the highest number of unique alleles (109) while Africa and Asia had only six and nine unique alleles, respectively. The two subsp. *hypogaea* and *fastigiata* shared 70 alleles. Wild *Arachis* in contrast shared only 15 alleles with *hypogaea* and 32 alleles with *fastigiata*. A tree-diagram separated the majority of the *hypogaea* from *fastigiata* accessions while wild *Arachis* accessions clustered with *hypogaea* (Figure 3). A reference set consisting of 300 genetically diverse accessions was formed that captured 466 (95%) of the 490 composite collection alleles, representing diversity from the entire spectrum of the composite collection (Upadhyaya et al., 2008a,c). The genotype-based reference set is an ideal collection of germplasm for allele mining, association genetics, mapping and cloning gene(s), and in applied breeding for the development of broad-based elite breeding lines/cultivars with superior yield and enhanced adaptation to diverse environments.

Integration of genomics tools with ongoing conventional breeding approaches are expected to facilitate development of improved cultivars more efficiently. Therefore, large-scale genotyping data (4597 DArT features and 154 SSRs) and multiple season phenotyping data were generated on the peanut reference set including a mini core collection of ICRISAT. Detailed analyses were undertaken on genetic diversity, population structure, linkage disequilibrium (LD) decay, and comprehensive marker–trait association. As expected, DArT features (2.0/locus, 0.16 PIC) showed lower allele frequency and polymorphic information content (PIC) than SSRs (22.21/locus, 0.71 PIC), but both marker types clearly differentiated the genotypes of diploids from tetraploids. Multiallelic SSRs identified three subgroups ($K=3$) while the LD simulation trend line based on squared-allele frequency correlations (r^2) predicted LD decay of 35 cM in peanut genome. Detailed analysis identified a total of 524 highly significant MTAs with wide phenotypic variance range (5.81–90.09%) using Q-matrix and P3D mixed linear model with optimum compression in addition to most stringent Bonferroni

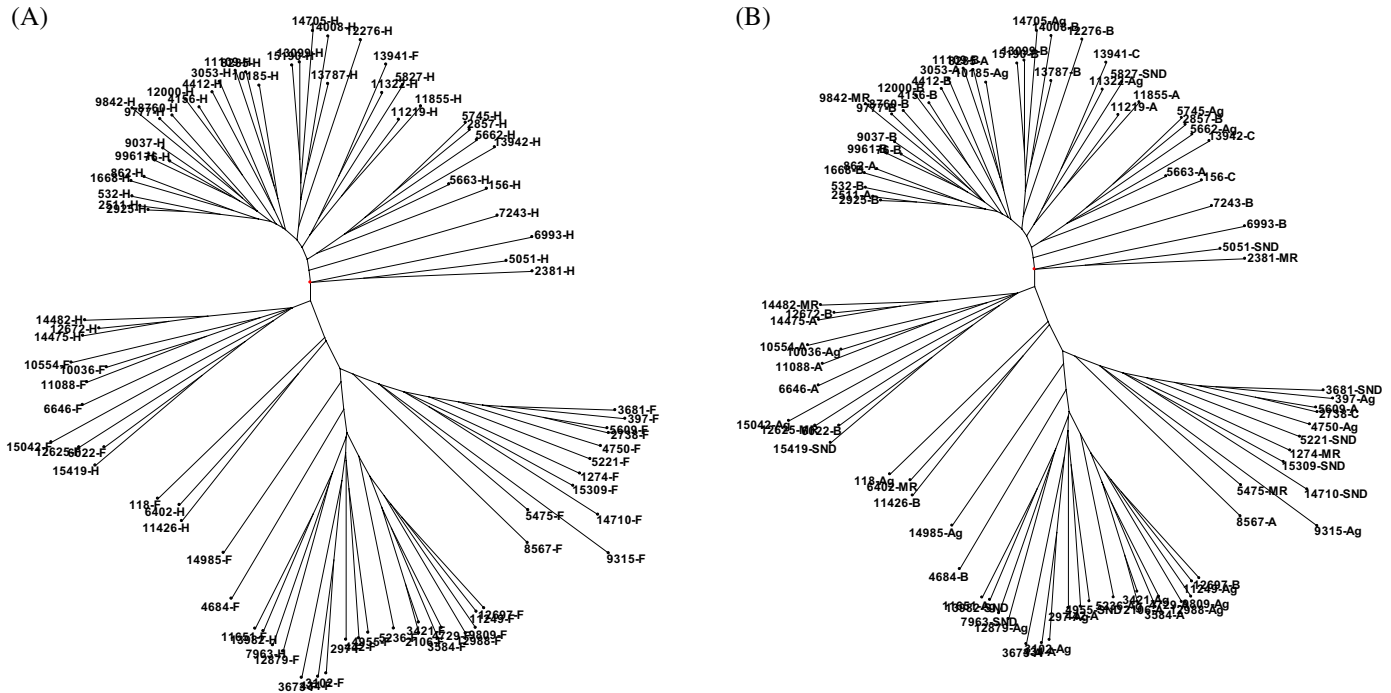

FIGURE 3 Tree diagram based on 21 SSR loci data on 82 peanut mini core accessions and four control cultivars representing two subsp. (*fastigiata* and *hypogaea*) clustered into four groups (abiotic, biotic, seed-nutrient dense, and agronomically desirable). (A) Accession followed by H, represents subsp. *hypogaea* and F, *subsp. fastigiata*. (B) Accession followed by A, represents abiotic stress resistant group; B, biotic stress resistant group; SND, nutrient dense group; Ag, agronomically superior group; MR, multiple resistant to biotic and abiotic stress; C, controls".

multiple test correction for 29 traits (Pandey et al., 2013). After validation, these MTAs may be deployed in improving biotic resistance, oil/seed/nutritional quality, drought tolerance related traits, and yield/yield components.

Core Collections

Large collections of *A. hypogaea* germplasm are maintained in the United States (Holbrook, 2001), ICRISAT (Upadhyaya et al., 2014a), China (Liao, 2014), and other countries. It is an overwhelming task to evaluate the collection for any particular trait of interest. A more efficient way of mining the collection for valuable accessions is needed. One approach is to develop and use subsets of germplasm collections, called "active working collection" by Harlan (1972) and "core collections" by Frankel (1984). A core collection would minimize repetitiveness within the collection and should, to the extent possible, represent the genetic diversity of the crop species. The development of core collections could facilitate easier access to peanut genetic resources, enhance their use in crop improvement programs, and simplify gene bank management.

Holbrook et al. (1993) used this approach to select a core collection to represent the United States peanut germplasm collection that was first stratified by country of origin and then divided into nine sets based on the amount of additional information available for accessions and on the number of accessions per country of origin. Seventy percent of this core collection was stratified by county of origin before using multivariate analysis on morphological data to cluster accessions into groups and then randomly sampling 10% from each group. Because of the lack of morphological data for some accessions, 29% of this core collection was selected using a 10% random sample after stratifying by country of origin. The remaining 1% was a simple random sample. Examination of means and ranges for six morphological variables indicated that this core collection is a representative sample of the entire collection and that the genetic variation expressed for these traits in the entire collection has been preserved in this core collection (Holbrook et al., 1993).

A core collection also was developed specific to the Valencia market type germplasm consisting of 630 peanut (*A. hypogaea* ssp. *fastigiata* var. *fastigiata*) accessions from the USDA collection. These accessions plus a control cultivar, New Mexico Valencia C, were evaluated for 26 descriptors in an augmented design for two seasons (Dwivedi et al., 2008). The accessions were stratified by country of origin, and data on morphological and agronomic descriptors were used for clustering following Ward's method. About 10%, or a minimum of one accession from each cluster and region, were selected to develop a core subset of 77 accessions. Peanut breeders engaged in improving the genetic potential of Valencia peanuts should find this core subset useful in cultivar development (Dwivedi et al., 2008).

To enhance the use of the germplasm collection maintained at ICRISAT, a core collection (1704 accessions) was developed from the entire collection (Upadhyaya et al., 2003). This core collection was selected from a total of 14,310 accessions using an approach slightly different from that used by

Holbrook et al. (1993). The ICRISAT peanut collection was first stratified by botanical variety within subspecies, and then stratified by country of origin. Accessions of the same botanical variety from small and adjacent countries with similar agro-climates were grouped together. This resulted in 75 groups. The accessions within each group (10–1716 accessions) were then clustered using multivariate statistical analysis. Approximately 10% of the accessions from each cluster were randomly sampled resulting in a core collection consisting of 1704 entries which include 910 belonging to subsp. *fastigiata* and 794 belonging to subsp. *hypogaea*.

Upadhyaya (2003) evaluated the ICRISAT peanut core collection for 16 morphological descriptors, oil and protein content in one season, and for 15 agronomic traits in two seasons. The phenotypic diversity was estimated and the importance of different descriptor traits in explaining variation was determined. The results revealed significant variation for morphological and agronomic traits in the peanut core collection. The two groups (subsp. *fastigiata* and subsp. *hypogaea*) differed significantly for all the traits except for trichomes on the leaflet surface and for oil content. The *hypogaea* group showed significantly greater mean pod length, pod width, seed length, seed width, yield per plant, and 100-seed weight than the *fastigiata* group, whereas it was opposite for plant height, leaflet length, leaflet width and shelling percentage (Upadhyaya, 2003).

In addition to the peanut core collection described above, Upadhyaya et al. (2001c) established a core collection for Asia consisting of 504 accessions. To establish the collection, Upadhyaya et al. (2001c) used 4738 peanut germplasm accessions (267 *fastigiata*, 2414 *vulgaris*, and 2057 *hypogaea* types) from 21 Asian countries that were stratified by country of origin within each of three botanical varieties. Data on 15 morphological descriptor traits including growth habit, branching pattern, stem (color, trichomes), leaf (color, shape, trichomes), flower, streak and peg (color), pod (beak, constriction, reticulation), seed (per pod, color) were used for clustering. Ten percent from each cluster or a minimum of one accession per cluster were randomly selected to include in this Asia core collection.

The Asian core collection (Upadhyaya et al., 2001c) which consists of 274 accessions of subsp. fastigiata (29 accessions of var. *fastigiata*, 245 accessions of var. *vulgaris*), and 230 of subsp. *hypogaea* var. *hypogaea*, along with four control cultivars, was evaluated in multienvironments for 22 agronomic traits to select diverse superior germplasm accessions for use as parents in improvement programs. On the basis of superior or equal performance over environments for pod yields per plant and plot, number of total pods, shelling percentage, and 100 seed weight and oil content compared with the respective botanical control cultivars, 15 *fastigiata*, 20 *vulgaris*, and 25 *hypogaea* accessions from 14 countries were selected (Upadhyaya et al., 2005). The selected lines having good combinations of pod yields, total pods, shelling percentage, 100 seed weight, and oil content will provide the germplasm sources that can be used in the peanut improvement programs to broaden the genetic base of cultivars.

A core collection was also selected to represent the *A. hypogaea* collection maintained in China (Jiang et al., 2008b). Jiang selected the core collection based on the 6390 accessions collected before 2005. The entire collection was first classified into five groups (botanical types) and 32 subgroups, and then divided into 258 variety clusters through analyzing the characterization data. The number of lines within each cluster was 21 for the Valencia type, 100 for Spanish type, 100 for Virginia type, 19 for Dragon type and 18 for irregular type (improved cultivars). In each variety cluster, 5–10% was sampled randomly. In total, 576 accessions were selected in the core collection, accounting for 9.01% of the entire collection. In the Chinese entire collection, diversity index was higher in Virginia and Dragon types and lower in irregular type. In the selected core collection, the diversity indexes were also higher in Virginia and Dragon types and lowest in irregular type. The difference of the diversity index between the entire germplasm and the core collection was not significant in each botanical type, indicating that the selected core collection well represents the diversity of the entire collection. Evaluation of the 576 accessions in this core collection indicated that the Chinese collection may be an important source for diversity in var. *hirsuta* and *vulgaris*.

Mini Core Collections

Core collections, reduced subsets consisting of only about 10% of the entire collection that captures most of the species diversity were proposed to help crop improvement scientists obtain reliable information. However, for some traits, the core collection is still too large for a complete evaluation. To overcome this, Upadhyaya and Ortiz (2001) postulated the "mini core" concept, wherein approximately 10% of core collection is subsampled (or 1% of entire collection) to represent global diversity of the species. Following this, Upadhyaya et al. (2002a) developed a mini core collection consisting of 184 accessions from the 1704 accessions of the peanut core collection (Upadhyaya et al., 2003). The mini core collection was developed by evaluating core accessions for morpho-agronomic and quality traits under field conditions at ICRISAT (Patancheru, India). A phenotypic distance matrix was created by calculating differences between each pair of accessions for each of 47 traits. This distance matrix was subjected to hierarchical cluster analysis, which resulted into 77 clusters. The proportional sampling strategy was used, and from each cluster approximately 10% of the accessions were randomly selected for the mini core subset. At least one accession was included from each cluster even if they had 10 accessions or less. The global peanut mini core thus constituted consists of 184 accessions (10.80% of core collection).

A mini core peanut collection consisting of 298 accessions was also selected in China (Jiang et al., 2008b). Genetic diversity of the peanut mini core collection from China and ICRISAT were compared using SSR markers, which revealed considerable genetic difference between the Chinese peanut accessions

and some ICRISAT accessions especially with the *aequatoriana* genotype ICG 12625. The genetic diversity was greater among the Chinese peanut mini core than that among ICRISAT mini core in terms of the similarity coefficient and genetic diversity index (Jiang et al., 2010a,b).

Shortly after developing the US peanut core collection it became evident that an even smaller subset of germplasm was needed for some types of evaluation. Holbrook and Dong (2005) used multivariate analysis of data from accessions in the core collection to select a core of the core (or mini core) collection. The mini core collection consists of 112 accessions making it more amenable for analyzing characters that are expensive to measure. Holbrook and Dong also evaluated the effectiveness of the mini core and showed that it can be used to improve the efficiency of identifying desirable traits in the core collection.

Trait Discovery by Mining Core or Mini Core Collections

The core and mini core approach provides an effective mechanism for proper exploitation of peanut germplasm resources for genetic improvement. The peanut core and mini core collections have been effectively utilized for identifying the sources of various agronomic and nutritional traits, abiotic, and biotic stress tolerance (Tables 2 and 3). Holbrook and Anderson (1995) used data on resistance to LLS (*Cercosporidium personatum* (Berk. & M.A. Curtis)) that was available for the entire collection to retrospectively determine how effective the use of this core collection would have been in identifying sources of resistance in the entire collection. The core collection approach to germplasm evaluation is a two-stage approach. The first stage is to examine all accessions in the core collection for a desired characteristic(s). This information is then used to decide which clusters of accessions in the entire germplasm collection should be examined during the second stage of screening. Theoretically, the probability of finding additional accessions with the desired characteristics should be highest in these clusters. Results presented by Holbrook and Anderson (1995) documented the improvement in screening efficiency from using this core collection and demonstrated the importance of having data available so that multivariate analysis can be used to cluster accessions before random sampling for the development of a core collection. Similar improvements in screening efficiency using this core collection were observed for resistance to the peanut root-knot nematode (*M. arenaria* (Neal) Chitwood race 1) (Holbrook et al., 2000b).

A major benefit of having a core collection has been a great increase in efforts to evaluate peanut germplasm for important traits (Holbrook, 1999). The US peanut core collection has been evaluated for resistance to tomato spotted wilt virus (TSWV) (Anderson et al., 1996), Sclerotinia blight (*S. minor* Jagger.), pepper spot (*Leptosphaerulina crassiasca* (Sechet) C.R. Jackson & D.K. Bell) (Damicone et al., 2010), Cylindrocladium black rot (*Cylindrocladium parasiticum* Crous, Wingefield, et Alfenas), and early leaf spot (*Cercospora arachidicola* Hori) (Islieb et al., 1995); reaction to the peanut root-knot nematode

(Holbrook et al., 2000a); and to yield and aflatoxin contamination under heat and drought stress (Holbrook et al., 2009). The most agronomically acceptable portion of the core collection has also been evaluated for resistance to Rhizoctonia limb rot (*Rhizoctonia solani* Kuhn, AG-4) (Franke et al., 1999). The accessions in the core collection also have been used to evaluate genetic variation for fatty acid composition (Hammond et al., 1997).

Holbrook and Anderson (1993) measured plant descriptors information for all accessions in the core collection. Eight above ground plant descriptors were evaluated using standard procedures (Pittman, 1995) before digging and nine below ground descriptors were similarly evaluated after digging. These data were then used to make inferences about the adequacy of the entire germplasm collection (Holbrook, 2001), and it was concluded that additional peanut accessions should be collected from Columbia, Venezuela, Uruguay, and Bolivia.

Holbrook and Isleib (2001) used disease resistance data from several studies that evaluated accessions in the core collection to examine the geographical distribution of genetic diversity in *A. hypogaea*. The results enable plant breeders to more efficiently utilize the genes for disease resistance that are available in the US germplasm collection.

The development of a mini core also stimulated additional germplasm evaluations. Accessions in the mini core have been evaluated for multiple disease resistances (Chenault Chamberlin et al., 2010; Holbrook and Dong, 2005); total and individual amino acid content, fatty acid content, tocopherols, and folic acid content (Dean et al., 2009); oil content, fatty acid profiles, flavonoid, and resveratrol content (Wang et al., 2013); seed dormancy (Wang et al., 2012); and genetic structure, diversity, and phylogenetic relationships (Barkley et al., 2007; Kottapalli et al., 2007; Wang et al., 2011). Chu et al. (2007) evaluated the US mini core for frequency of a loss-of-function mutation in oleoyl-phosphatidyl choline (PC) desaturase (*ah-FAD2A*), the mutation which results in a dysfunctional desaturase and subsequent high oleic acid content of peanut seed. Kang et al. (2007) also used the US mini core to evaluate the genetic diversity for three peanut allergens.

Some of the accessions of the USDA peanut germplasm collection are heterogeneous (Figure 1). Advances in genomics technology have highlighted the need for collections of homogeneous accessions. To satisfy this need, efforts were undertaken to develop a collection of pure line accessions for the US peanut mini core collection (Chen et al., 2013).

The ICRISAT peanut core collection was evaluated with the aim to identify the diverse early maturity accessions, which resulted in identification of 21 early-maturing lines (Upadhyaya et al., 2006b). The early-maturing landraces produced 12.6% more yield at 75 days after sowing (DAS) and 8.4% at 90 DAS than the mean of three early-maturing control cultivars (Chico, Gangapuri, and JL 24). Four new early-maturing landraces (ICG 4558 (India), ICG 4890 (Argentina), ICG 9930 (Zimbabwe), and ICG 11605 (Bolivia)) with predominantly three to four seeds per pod, were identified as additional sources for breeding confectionery peanut varieties. The diverse early maturity sources

identified from different countries in the peanut core collection are agronomically superior and therefore likely to provide better opportunities in developing early maturity cultivars suitable for different geographic regions.

Tolerance to low temperature is an important prerequisite for optimal performance of peanut in a number of temperate peanut-growing environments. The peanut core collection at ICRISAT was screened for low temperature tolerance for germination at 12 °C for 10 days under laboratory conditions and 25 low temperature tolerance accessions were identified. The selected low temperature tolerant lines can be used in crop improvement program to develop high yielding low temperature tolerant cultivars and to broaden the crop genetic base (Upadhyaya et al., 2001b). Additionally, 158 peanut core accessions belonging to five botanical types that are known to be tolerant to low temperature (12 °C) at germination, were evaluated for phenotypic diversity for 15 morphological, 15 agronomic, and two seed quality traits at Patancheru, India (Upadhyaya et al., 2009).

The main requirement of peanut breeders is to produce genetically diverse, trait-specific, and agronomically desirable germplasm lines from the collections. To meet such requirements, Upadhyaya et al. (2014a) reported new genetically diverse sources for resistance to abiotic and biotic stresses and for agronomic and quality traits in peanut from the ICRISAT peanut mini core collection. The peanut mini core collection has been evaluated for agronomic performance and combined with genotyping information (Upadhyaya et al., 2008a,c), information on biotic stresses (Ajay, 2006; ICRISAT, 2009; Jianwei et al., 2010; Khalid, 2008; Kusuma et al., 2007; Madhura, 2006; Sujay et al., 2008; Yugandhar, 2005; Zhang, 2010), and abiotic stress tolerance (Biradar, 2007; Hamidou et al., 2012, 2013; ICRISAT, 2008, 2009, 2010; Srivastava, 2010; Upadhyaya, 2005; Upadhyaya et al., 2001a,b, 2009), and nutritional traits (oil, protein, oleic/linoleic (O/L) ratio, Fe, and Zn) (Upadhyaya et al., 2012a,b). Researchers identified 28 accessions resistant to abiotic stress, 30 accessions resistant to biotic stress, and 18 accessions that were agronomically desirable, while 16 were seed nutrient dense genetically diverse accessions with agronomically desirable traits for use in peanut breeding and genomics studies (Table 2; Figure 3). Upadhyaya et al. (2014a) further reported a few desirable accessions with multiple beneficial traits: ICG 12625 (resistance to drought, low temperature, LLS, *Aspergillus flavus* Link, bacterial wilt; high oil and good oil quality) and ICG 442 (resistance to drought, salinity, P deficiency); ICG 12625 and ICG 2381 (resistance to rust, *A. flavus*; good oil quality); ICG 12697 (resistance to LLS, rust, *A. flavus*); ICG 6022 (resistance to early leaf spot (ELS, LLS)); ICG 14710 (high oil, Fe, Zn); ICG 7963 (high protein, Fe, Zn); ICG 11426 (resistance to ELS, LLS, rust); and ICG 5221 (high Fe and Zn and good oil quality). Accessions with adaptation to rainy and/or postrainy environments were ICG#s 434, 5745, 8285, 10036, 11088, 11651, 12625, 15042, and 15419. ICG#s 862, ICG 334, 10554 and ICG 3673 were among the abiotic resistant group; ICG#s 11426, 5221, 4684, and ICG 2925 were among the biotic group; ICG#s 3673,

ICG 2381, 14482, and ICG 4955 were among the nutritional-dense seed group; and ICG#s 14705, ICG 3421, ICG 9315, and ICG 5445 were among the agronomic group and genetically most diverse with a genetic distance of 1.00. ICG#s 1668, 2925, 9842, and 12625 were resistant to both abiotic and biotic stresses; ICG#s 2381, 6402, 12625, and 14482 were resistant to the biotic-resistant and had nutrient-dense seeds; and ICG#s 1274, 5475, and 12625 were abiotic-resistant and had nutrient-dense seeds. Similarly, ICG#s 1668, 2381, 2925, 5475, 8285, 11088, 11426, 12625, and 14482 were multiple resistant with desirable agronomic traits (Table 3). These accessions are ideal genetic resources that may be used to develop agronomically superior and nutritionally enhanced peanut cultivars with multiple resistances to abiotic and biotic stresses (Upadhyaya et al., 2014a). The peanut mini core collection has evoked a keen interest from global research community and 39 sets of the peanut mini core have been supplied on request to the researchers in 13 countries, who have identified sources of traits of economic importance through evaluation.

An evaluation of phenotypic traits of the Chinese core collection revealed the diversity of accessions within this collection. The growth period of this collection ranged from 95–180 days with an average of 120 days. Accessions of Valencia types possessed a shorter growth period while those of Dragon type were the longest. Improved cultivars generally possessed a short growth period because early maturity has been a key objective in most breeding programs. The plant height in the Chinese core collection ranged from 4.4 to 107.6 cm with an average as 45.0 cm. The average plant height among different botanic types is largest in Valencia type (63.9 cm) followed by Spanish type (46.0 cm), Virginia type (40.6 cm), Dragon type (40.4 cm), and irregular (36.1 cm). The plant height of improved cultivars was generally shorter. The weight of 100 pods of the Chinese peanut core collection ranged from 27.8 to 355.0 g with an average as 146.4 g. Among various botanical types, the largest average pod weight was in the irregular type as 173.8 g followed by Virginia type (161.3 g), Dragon type (142.3 g), Spanish type (133.5 g), and Valencia type (124.0 g). During cultivar improvement, larger pods or seeds were selected for high yield. The shelling percentage of the core collection ranged from 37.0 to 85.9% with an average as 71.3%. Among various botanical types, the highest shelling ratio was in the Dragon type (73.0%) followed by the Spanish type (72.3%), irregular type (70.7%), Virginia type (70.4%), and Valencia type (70.4%). The oil content of the Chinese peanut core collection ranged from 32.4 to 60.2% with an average of 50.6%, while the protein counted ranged from 12.5 to 36.8% with an average of 27.6%.

Jiang et al. (2008a, 2010a) compared differences between the Chinese peanut core collection to the ICRISAT mini core. No genotype belonging to var. *aequatoriana* or var. *peruviana* was included in the Chinese core, whereas these two types were included in the ICRISAT mini core. In the Chinese core collection, the percentages of Dragon and Spanish types were higher than in the ICRISAT mini core, while in the ICRISAT mini core the percentage of Valencia

types was higher than in the Chinese core collection. Certain characteristics such as plant height, nodes on the main stem, total branches, and shelling percentage in the Chinese core were lower than the corresponding traits of the ICRISAT mini core; but plant yield, pod length, pod width, seed length, 100 pod weight, and 100 seed weight in the Chinese core were relatively higher than in the ICRISAT mini core (Jiang et al., 2008a).

Germplasm Enhancement and Utilization

Various studies have shown scanty use of germplasm in crop improvement programs globally. Only a small proportion of peanut germplasm at ICRISAT and other gene banks have been used successfully in crop improvement programs. For example, peanut scientists at ICRISAT used 986 unique parents (from 1986 to 2002) to develop 8279 advanced breeding lines in peanut, but this work only included 132 germplasm lines and 10 wild *Arachis* species (Upadhyaya et al., 2006a) from the more than 15,400 accessions available in this gene bank. The two most frequently used cultivars were Robut 33-1 (ICG 799) that was used 3096 times and Chico (ICG 476) that was used 1180 times. A similar situation is found in other countries. For example, Jiang and Duan (1998) reviewed the utilization of peanut genetic resources crop improvement in China and concluded that introduced germplasm and wild relatives were seldom utilized in cultivar development. In the United States, the cultivar Dixie Giant was a germplasm source in all pedigrees of runner market-type peanuts and Small White Spanish-1 is in more than in 90% of pedigrees. These two cultivars contributed nearly 50% of the germplasm of runner cultivars (Knauft and Gorbet, 1989).

Plant breeders frequently use parental lines only from their working collections because they make reasonable and steady progress in most cases, and broadening the adapted genetic base generally will dilute agronomic performance (Kannenberg and Falk, 1995). Normally, plant breeders consider elite inbred lines as the best genetic resources because each line contains a combination of genetic traits that satisfies the marketplace (Troyer, 1990). New germplasm, if used in crop improvement programs, can (1) raise the genetic ceiling on improvement, (2) decrease vulnerability to biotic and abiotic stresses, and (3) add new developmental pathways and ecological adaptations (Kannenberg and Falk, 1995). Although plant breeders recognize the limitation of their working collections and the potential value of wild and landrace resources, they are often reluctant to use these resources for several reasons such as lack of reliable knowledge about stable donors for specific traits, linkage load of many undesirable genes, lack of germplasm assessment for economic traits that show high genotype–environment interaction, and require expensive, laborious and replicated multienvironment evaluation (Upadhyaya et al., 2011b). Also, there are assumed risks while dealing with unknown and wild germplasm lines and breeders are apprehensive about the possibility of complete program failures; long timescales, or the value of the new varieties may never allow costs to be

recouped. Additionally, there is the possibility in certain crops of introducing toxic, allergenic or pharmaceutically active plant products into food products, risks that are virtually absent in crossing elite, widely grown germplasm (Heslop-Harrison, 2002). Plant breeders' need for genetically diverse, trait-specific and agronomically desirable parents is not met by the information available in the gene bank databases, and the restricted access due to limited seed availability and regulations governing international exchange. The development of more efficient methods for evaluating germplasm collections should help to speed future breeding progress.

Germplasm Enhancement/Prebreeding

The narrow genetic base of peanut cultivars coupled with the low level utilization of genetic resources is the major factors limiting production and productivity globally. Exploitation and/or creation of novel and diverse sources of variation are needed for the genetic enhancement of crop cultivars. Wild *Arachis* relatives with high levels of resistance/tolerance to multiple stresses provide important sources of genetic diversity for crop improvement. However, their exploitation for cultivar improvement is limited by cross-incompatibility barriers and linkage drag (Sharma et al., 2013). Prebreeding provides a unique opportunity, through the introgression of desirable genes from wild germplasm into genetic backgrounds readily used by the breeders with minimum linkage drag. New and diverse sources of variation for agronomic and nutrition-related traits and resistant/tolerant sources for biotic/abiotic stresses are now available both in cultivated and wild species germplasm and can be utilized to develop new prebreeding populations having greater variability for various traits.

Prebreeding activities using promising landraces, wild relatives, and popular cultivars are in progress at ICRISAT to develop new gene pools in peanut with a high frequency of useful genes, wider adaptability, and a broad genetic base (Sharma et al., 2013). Utilization of wild *Arachis* species through interspecific hybridization has resulted in the development of many elite germplasm lines and several cultivars with improved levels of resistance to diseases and insect pests. The utilization of synthetic amphiploids such as "TxAG-6" (Simpson et al., 1993) has made possible the transfer of resistance genes from wild species into cultivated peanut. TxAG-6 is a synthetic amphiploid derived from crossing an AA genome donor hybrid (*A. cardenasii* × *A. diogoi*) with a BB genome species (*A. batizocoi)* followed by colchicine treatment of the sterile diploid to produce the semifertile tetraploid line TxAG-6 (Simpson et al., 1993). This amphiploid has been synthesized using species that are not in the direct lineage of the cultivated peanut. However, it is crossable with the cultivated peanut and produced semi-fertile progenies, thus proving useful for introducing genetic variability into the cultigen. Using this amphiploid in crossing programs with cultivated groundnut has resulted in the release of two cultivars, "COAN" and

"NemaTAM," carrying genes for root-knot nematode (*M. arenaria*) resistance from *A. cardenasii* (Simpson and Starr, 2001; Simpson et al., 2003). At ICRISAT, several elite lines have been developed with desirable characters transferred from wild *Arachis* species such as ICGV 86699 (Reddy et al., 1996), ICGV 87165 (Moss et al., 1998), ICGV 99001 and 99004 with resistance to LLS, and ICGV 99003 and 99005 resistant to rust (Singh et al., 2003). 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 (Nigam et al., 1998), ICGV-SM 86715 (Moss et al., 1998), and Bailey (Isleib et al., 2011) all have a genetic base from wild *Arachis* species and were released for cultivation.

Other than resistant sources, studies also indicated the possibility of improving agronomic traits, including yield, through introgression of genes from the wild species into the cultigens. Similarly in peanut, by using an amphiploid "TxAG-6" with very low 100-seed weight (~12 g) and poor pod yield (2–5 g plant^{-1}) in hybridization with "TMV 2" (100-seed weight, 32 g), breeding lines with cryptic introgression have been developed with much higher 100-seed weight (130 g) and from 23–68% higher pod yield than TMV 2 (3, 343 kg ha^{-1}) (Upadhyaya et al., 2014b). This demonstrates that the novel alleles of wild relatives that were considered to be lost in evolution to cultivated types could still be used to enhance the important agronomic and nutrition-related traits in cultivars. The availability of molecular markers will greatly assist in reducing linkage drag and increasing the efficiency of introgression in prebreeding programs.

High yield potential has been one of the most important objectives in peanut breeding programs worldwide. Moreover, breeding for other objectives such as quality traits and resistance to biotic and abiotic stresses is normally based on high yielding genetic background. Traditional breeding approaches have been widely used in enhancing yield in peanut. In most cases, peanut cultivars with the highest yield potential have large pods and seeds. Identification or creation of large seeded germplasm lines is crucial for high yield.

High oil content of peanut cultivars is a crucial trait for oil processing industry, especially in developing countries where most peanuts are produced for a major source of cooking oil. Limited attention has been paid to breeding for oil content in developed countries where peanut has been rarely used for oil. As peanut oil has been relatively less competitive with other plant oils such as rapeseed and soybean oils because of its relatively higher market price, breeding for cultivars with higher oil content could not only increase oil production, but also enhance the market competitiveness of peanut oil. Most peanut accessions with relatively high oil content (over 55%) belong to Spanish type with early maturity. Liao et al. (2008, 2010) reported several high oil lines from recombinant inbred populations derived from the cross of Yuanza 9102 × Zhonghua 5. Jiang et al. (2010c) reported that the oil content of 87 wild *Arachis* accessions ranged from 51.4 to 62.9% with an average of 55.8%, and 12 of the 87 accessions

possess oil content higher than 58%, indicating the potential of enhancing oil content in *A. hypogaea* by introgressing genes from the wild *Arachis* species to the cultivated types. Liao et al. (2010) reported several high oil content lines from a recombinant inbred line population.

Quality of peanut oil is largely determined by its fatty acid components. Oleic and linoleic acids comprise over 80% of the oil content in peanut while linoleic acid is less saturated and less stable than oleic acid. The oxidative stability and shelf-life of peanut and peanut products can be enhanced by increasing the O/L ratio. The Dragon type (var. *hirsuta*) has the highest oleic acid content at 53.6% and Valencia types have the lowest content at 43.4% (Jiang and Ren, 2006). Norden et al. (1987) identified two lines with 80% oleic acid and very low linoleic acid (2%). Since this finding, breeding for high oleic acid has been attracting more research efforts in many countries. Markers have been developed to detect all possible genotypes for the selection of the high oleic trait greatly streamlining the process of cultivar development (Barkley et al., 2010, 2011). These markers were further utilized to link fatty acid phenotypes with each genotype (Barkley et al., 2013).

Germplasm Utilization and Impact of Germplasm

Peanut is an important crop in tropics and subtropical regions worldwide. The largest producers of peanut are China, India, the USA, and certain African countries. There are currently four different market types of peanut production in the United States including runner, Virginia, Spanish, and Valencia. Overall, in the United States, the runner type peanuts are more predominately grown than any of the other three market types with almost 82.5% of the production area, whereas Virginia peanuts are produced on 15.1%, Spanish 1.1%, and Valencias 1.4% of the production area (www.aosca.org; compiled by T.G. Isleib). Only a handful of cultivars of each market type are grown to produce the majority of peanuts which puts the crop at risk to new diseases that may emerge. The modern practice of large-scale cultivation and the genetic uniformity of cultivars has increased the vulnerability of many agricultural crops often with disastrous consequences (Rao and Hodgkin, 2002). At times, certain production areas were close to near monoculture such as from 1972 to 1993 when anywhere from 60 to 95% of the acreage was dominated by "Florunner" (Isleib et al., 2001). The danger of monoculture in peanut can be best demonstrated with the first outbreak of tomato spotted wilt virus (TSWV) in 1987. The disease increased during the following years (Culbreath et al., 1992) and significantly affected commercial production because cultivars produced at the time all were highly susceptible.

Cultivated peanut has a fairly narrow genetic base likely due to not being able to exchange alleles with the wild species. Many of the cultivars today can trace their ancestry back to a handful of lines from which they are derived. The USDA germplasm collection has been utilized to introduce additional genetic variability

into breeding populations in order to improve and develop new cultivars with new allele combinations. Most improved cultivars have 12.5–25% plant introduction (PI) ancestry (Isleib et al., 2001). Genetic resources have been particularly useful in the development of disease-resistant cultivars especially Sclerotinia blight, root-knot nematode, and TSWV which has helped impact peanut farmers favorably (Isleib et al., 2001). Therefore, preservation of genetic diversity can be seen as a defense or an insurance policy against the biotic and abiotic problems that arise from intense monoculture production which is highly vulnerable to new stress in the environment (Rao and Hodgkin, 2002). In peanut, a single line in the US germplasm collection, PI 203396, was found to have resistance to TSWV which was subsequently utilized in breeding programs to select resistant runner cultivars. If this germplasm had not been collected in 1952 at a Brazilian market prior to some of the restrictions that are now imposed in collecting germplasm then the peanut industry may have suffered devastating losses. The economic impact of this single germplasm line is estimated at $200 million annually (Isleib et al., 2001).

The general purpose of characterization and enhancement of peanut germplasm is to develop improved cultivars with high yield, improved quality, and resistance to biotic and abiotic stresses. In China, many of the elite peanut genotypes have been identified from landraces, improved cultivars, and special germplasm materials introduced from foreign countries. The identified elite genotypes have been extensively utilized in production and/or in breeding programs. In the pedigree of the peanut cultivars released (more than 200) in the past five decades in China, about 40 landraces were used as direct or indirect parents in breeding, among which, more elite germplasm lines of Virginia and Spanish types have been involved as parents. The genotype named “Fuhuasheng” was a direct or indirect parent in 161 released cultivars while “Shitouqi” was a direct or indirect parent in 52 released cultivars. The bacterial wilt-resistant germplasm lines identified and development of resistant cultivars have also contributed greatly to the increased productivity of peanut in the regions naturally infested with *Ralstonia solanacearum* in China and other southeast Asian countries.

Many peanut germplasm accessions from ICRISAT have been used to develop cultivars in various countries. In addition, many germplasm lines when evaluated by National Agricultural Research System (NARS) produced higher grain yield and have been directly released as cultivars. Globally, 16 germplasm lines have been directly released as 19 cultivars in 16 countries, from the peanut germplasm distributed to users from ICRISAT gene bank. These cultivars have greatly benefited those countries by increasing both production and productivity.

CONCLUSIONS

Collecting and preserving genetic resources is critical in order to improve agricultural production around the world. Ensuring enough food to provide adequate nutrition for the global population is going to be a huge challenge for

plant breeders going forward as the human population steadily increases. Famines due to new diseases, drought, and wars have been part of human history; however, they could be mitigated or limited by sharing research findings, new varieties, diversity in the diet, and technology transfer to those in need. As the human population grows more natural environments are lost to urban development often destroying areas in which valuable plant populations are adapted. If these plants are not already preserved then they will be forever lost. The value of a particular plant is often unknown initially, but can turn up to be the sole source of resistance to an important disease such as TSWV. Even though maintaining these collections tend to be costly and labor intensive, they have proven to be valuable for research, breeding programs, and have a direct economic impact to the farmers.

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