Synthetics for Groundnut Improvement

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Abstract

Productivity of groundnut (Arachis hypogaea L), an important legume cultivated in over 110 countries, is constrained due to various biotic and abiotic stresses. Levels of resistance to important diseases and insect pests are low in cultivated species. Wild Arachis species are diverse and harbor genes for important traits including insect pests and disease resistance which were lost in cultivated groundnut during the course of domestication. Transfer of genes from wild Arachis into cultivated groundnut is valuable, however it is challenging due to ploidy differences and genomic incompatibility between species. Synthetic groundnuts have been developed using various pathways to overcome barriers to gene transfer and used mostly to transfer genes for resistance into cultivated groundnut. At ICRISAT, we have developed cryptic introgression lines, using synthetic groundnut that have high pod yields, high 100-seed weight and drought tolerance traits. Research is in progress to identify chromosome segment from wild diploid species that cause enhancement of agronomic traits in the introgressions lines using genomic tools.

Keywords: Synthetic groundnut; amphidiploid, wild *Arachis*; gene transfer; introgression; germplasm

Introduction

Groundnut (*Arachis hypogaea L.*), also called as peanut, is one of the most important legume crop, grown globally over 110 countries. Groundnut is rich in oil and protein, and plays an important role in human diet. The seeds are eaten raw, boiled or roasted, made into peanut butter, confectioneries etc. and its foliage is an important fodder. The major groundnut growing regions are Africa (54%) and Asia (41%), together contributing about 90% of global groundnut production during 2014 (FAOSTAT 2017). Large yield differences exist within and among regions, and it ranges from 965 kg/ha in Africa to 3333 kg/ha in Americas. Yield of groundnut in India is 1399 kg/ha. The top five groundnut growing countries in the world are India (4.68 m ha), China (4.60 m ha), Nigeria (2.77 m ha), Sudan (2.10 m ha) and Tanzania (1.62 m ha) contributing about 68% of total groundnut production.

Cultivated groundnut is an allotetraploid (2n = 4x = 40) originated from a single hybridization event between two wild diploids with A and B genome followed by a spontaneous duplication of chromosomes or fusion of unreduced gametes (Halward et al 1991). The wild species *A. duranensis* and *A. ipaënsis* are considered as the A and B genome donors,

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respectively (Kochert et al 1996; Milla et al 2005; Fávero et al 2006; Seijo et al 2007).

Cultivated groundnut has experienced domestication bottleneck that resulted in low genetic diversity. The low level of DNA polymorphism among cultivated groundnuts has been reported earlier by many researchers (Kochert et al1991; Halward et al 1992; He and Prakash 1997; Hopkins et al 1999; Huang et al 2012). This results in lack of diversity for important traits particularly for insect pests and disease resistance. However the wild diploid species of *Arachis* harbor genes which were lost in cultivated groundnuts during the course of domestication, and therefore they are considered as the potential sources to enhance stress tolerance and to broaden the genetic base of the crop.

Gene introgression from wild species is therefore essential to explore the largely untapped reservoir of useful alleles of interest that remain in the wild species. However many wild *Arachis* are not cross compatible with cultivated groundnut, mainly due to differences in ploidy and genomes between cultivated and wild *Arachis* species.

The alternate way to achieve gene introgressions is to follow different introgression pathways and induce chromosome doubling as suggested by Simpson (2001) which results in production of synthetic tetraploids, also called synthetic groundnut. This paper focuses on *Arachis* wild species and their significance, and development and utilization of synthetic tetraploids in groundnut improvement.

Taxonomy and Genepool

Arachis species

The genus *Arachis* consists of 81 species, including diploids and tetraploids, and according to morphology and crossability species are categorized into nine sections: *Trierectoides, Erectoides, Extranervosae, Triseminatae, Heteranthae, Procumbentes, Caulorrhizae, Rhizomatosae* and *Arachis* (Krapovickas and Gregory 1994; Valls and Simpson 2005; Valls et al 2013). Genomic groups have evolved in the genus which mostly follow sectional designations (*A, B, D, F* and *K, Arachis; C, Caulorrhizae; E, Erectoides; EX, Extranervosae; H, Heteranthae; PR, Procumbentes; R, Rhizomatosae; TE, Trierectoides;* and *T, Triseminatae* (Smartt and Stalker 1982; Stalker 1991; Seijo et al 2004; Robledo and Seijo 2010; Stalker 2017) (Table 1).

Based on the presence or absence of flowers on the main axis and spreading or erect growth habit, *A. hypogaea* L. is classified into two subspecies; *hypogaea* - characterized by absence of flowers on main axis, regular alternation of vegetative and reproductive branches on the laterals, and long life cycle; and *fastigiata* - characterized by presence of flowers on main axis, absence of any specific order of vegetative and reproductive branches on the laterals, and shorter life cycle.

These two subspecies can be further classified into six botanical varieties based on morphology. The subsp. *fastigiata* contains four botanical varieties namely *vulgaris, fastigiata, peruviana* and *aequatoriana,* while the subsp. *hypogaea* contains two botanical varieties namely *hypogaea* and *hirsute* (Krapovickas and Gregory 1994; Valls and Simpson 2005).

Table 1: *Arachis* species, chromosome number and genome information (from Krapovickas and Gregory 1994; Valls and Simpson 2005; Valls et al 2013; Santana and Valls 2015)

Section ***Arachis***	
2n =2x= 20, A genome	*Arachis cardenasii* Krapov. & W.C. Gregory
	Arachis correntina (Burkart) Krapov. & W.C. Gregory
	Arachis diogoi Hoehne
	Arachisduranensis Krapov. & W.C. Gregory
	Arachis helodes Martius ex Krapov. & Rigoni
	Arachis herzogii Krapov., W.C. Gregory and C.E. Simpson
	Arachiskempff-mercadoi Krapov., W.C. Gregory & C.E. Simpso;
	Arachis kuhlmannii Krapov. & W.C. Gregor
	Arachis linearifolia Valls, Krapov, & C.E. Simpson
	Arachis microsperma Krapov., W.C. Gregory & Valls
	Arachisschininii Valls & C.E. Simpson
	Arachis simpsonii Krapov. & W.C. Gregory
	Arachis stenosperma Krapov. & W.C. Gregory
	Arachis villosa Benth.
2n=2x=20, B genome	*Arachis gregoryi* C.E. Simpson, Krapov, & Valls
	Arachis ipaënsis Krapov., W.C. Gregory
	Arachishoehnei Krapov. & W.C. Gregory
	Arachismagna Krapov., W.C. Gregory & C.E. Simpson
	Arachis valida Krapov. & W.C. Gregory
	Arachis williamsii Krapov. & W.C. Gregory
2n=2x=20, D genome	*Arachis glandulifera* Stalker
2n=2x=20, F genome	*Arachis benensis* Krapov., W.C. Gregory & C.E. Simpson
	Arachistrinitensis Krapov. & W.C. Gregory
2n=2x=20, K genome	*Arachis batizocoi* Krapov. & W.C. Gregory
	Arachiscruziana Krapov., W.C. Gregory & C.E. Simpson
	Arachiskrapovickasii C.E. Simpson, D.E. Williams, Valls & I.G. Vargas
2n=4x=40; AB genome	*Arachis hypogaea* L.
	Arachismonticola Krapov. & Rigoni
Others 2n=2x=18	*Arachis decora* Krapov., W.C. Gregory & Valls
	Arachispalustris Krapov., W.C. Gregory & Valls
	Arachis praecox Krapov., W.C. Gregory & Valls
Section ***Caulorrhizae***	
2n=2x=20, C genome	*Arachis pintoi* Krapov. & W.C Gregory
	Arachis repens Handro
Section ***Erectoides***	
	Arachis archeri Krapov. & W.C. Gregory
	Arachisbenthamii Handro
	Arachisbrevipetiolata Krapov. & W.C. Gregory
	Arachis cryptopotamica Krapov. & W.C. Gregory
	Arachisdouradiana Krapov. & W.C. Gregory
	Arachisgracilis Krapov. & W.C. Gregory
	Arachishatschbachii Krapov. & W.C. Gregory
	Arachis hermanni Krapov. & W.C. Gregory
	Arachis major Krapov. & W.C. Gregory
	Arachismartii Handro
	Arachis oteroi Krapov. & W.C. Gregory

Section ***Erectoides*** **(continued)**	*Arachisparaguariensis* ssp. paraguariensis Chodat & Hassl.
	Arachis paraguariensis ssp. *capibarensis* Krapov. & W.C. Gregory
	Arachis stenophylla Krapov. & W.C. Gregory
2n=2x=18, E genome	*Arachis porphyrocalyx* Valls & C.E. Simpson
Section ***Extranervosae***	
2n=2x=20; EX genome	*Arachis burchellii* Krapov. & W.C. Gregory
	Arachis lutescens Krapov. & Rigoni
	Arachis macedoi Krapov. & W.C. Gregory
	Arachismarginata Gradner
	Arachis pietrarellii Krapov. & W.C. Gregory
	Arachis prostrata Benth.
	Arachisretusa Krapov., W.C. Gregory & Valls
	Arachis setinervosa Krapov. & W.C. Gregory
	Arachis submarginata Valls, Krapov. & C.E. Simpson
	Arachisvillosoulicarpa Hoehne
Section ***Heteranthae***	
2n=2x=20, H genome	*Arachis dardani* Krapov. & W.C. Gregory
	Arachisgiacomettii Krapov., W.C. Gregory, Valls & C.E. Simpson
	Arachisinterrupta Valls & C.E. Simpson
	Arachispusilla Benth.
	Arachisseridoensis Valls, C.E. Simpson, Krapov, & R. Veiga
	Arachis veigae S.H. Santana & Valls
Section ***Procumbentes***	
2n=2x=20, PR genome	*Arachis appressipila* Krapov. & W.C. Gregory
	Arachis *chiquitana* Krapov., W.C. Gregory & C.E. Simpson
	Arachishassleri Valls & C.E. Simpson
	Arachis kretschmeri Krapov. & W.C. Gregory
	Arachislignose (Choda and Hassl.) Krapov. & W.C. Gregory
	Arachis matiensis Krapov., W.C. Gregory & C.E. Simpson
	Arachis pflugeae C.E. Simpson, Krapov, & Valls
	Arachis rigonii Krapov. & W.C. Gregory
	Arachis subcoriacea Krapov. & W.C. Gregory
	Arachis vallsii Krapov. & W.C. Gregory
Section ***Rhizomatosae***	
Ser. Prorhizomatosae	
2n=2x=20, R_1	*Arachis burkartii* Handro
Ser. Rhizomatosae	
2n=40, R_2 genome	*Arachis glabrata* var. glabrata Benth.
	Arachis glabrata var. hagenbeckii Benth. (Harms ex. Kuntze) F.J. Herm.
	Arachis nitida Valls, Krapov, & C.E. Simpson
	Arachis pseudovillosa (Cheda & Hassl.) Krapov. & W.C. Gregory
Section ***Trierectoides***	
2n=20; TE genome	*Arachis guaranitica* Choda & Hassl.
	Arachis tuberosa Bong. ex Benth
	Arachis sesquijuga Valls, L.C. Costa & Custodio
Section ***Triseminatae***	
2n=2x=20, T genome	*Arachis triseminatae* Krapov. & W.C. Gregory

Genepool

Species of the genus *Arachis* can be grouped into four gene pools based on crossability with cultivated groundnut, *A. hypogaea* (Smartt 1990; Singh and Simpson 1994). The primary gene pool consists of landraces and traditional cultivars of groundnut and wild *A. monticola* having free crossability with *A. hypogaea* producing normal segregants. The secondary gene pool consists of diploid species from section *Arachis* which are cross-compatible with *A. hypogaea*, despite ploidy differences and produce sterile to partially fertile hybrids. The tertiary gene pool includes species of section *Procumbentes*, *Erectoides* and *Rhizomatosae*. The quaternary gene pool of the remaining *Arachis* species that are cross-incompatible or very weakly cross-compatible to species of section *Arachis*, are classified into five other sections.

Origin, Evolution and Diversity

Origin and evolution

The genus *Arachis* has its origin in South America where the species of this genus are widespread (Krapovickas and Gregory 1994). Cultivated groundnut (*A. hypogaea*, AABB genome) originated from a single hybridization event between two wild diploids with A genome of *A. duranensis* and B genome of *A. ipaënsis* and was followed by a spontaneous duplication of chromosomes or fusion of unreduced gametes (Halward et al 1991; Kochert et al 1996; Milla et al 2005; Fávero et al 2006; Seijo et al 2007). This hypothesis was further supported by cytogenetic, phylogeographic and molecular evidence that indicate A. *duranensis* and *A. ipaënsis* as the donors of the A and B subgenome, respectively (Kochert et al 1996; Moretzsohn et al 2013; Seijo et al 2007; Robledo amd Seijo 2010; Robledo et al 2009; Grabiele et al 2012; Koppolu et al 2010, Bertioli et al 2016). The occurrence of two progenitor species, *A. duranensis* and *A. ipaënsis*, when considered with archaeological evidence suggest southern Bolivia to northwestern Argentina as the center of origin for the cultivated species *A. hypogaea* (Hammons 1982; Stalker and Simpson 1995).

Diversity of *Arachis* species

Polyploidy creates severe genetic bottlenecks resulting in the genetic vulnerability of important crops (Burow et al 2001). Cultivated groundnut has relatively low genetic diversity; though a considerable amount of variability exists for different morphological, physiological and agronomic traits. For example, Upadhyaya (2003) assessed diversity in the groundnut core collection (Upadhyaya et al 2003) consisting of 1704 accessions including 910 belong to subsp. *fastigiata* and 794 to subsp. *hypogaea* for 16 morphological descriptors and for 15 agronomic characteristics during a rainy season, and for 17 descriptors during a post-rainy season at Patancheru, India. The core collection represented diversity of groundnut collection (14,310 accessions) conserved at ICRISAT genebank, India. Results revealed that the two subspecies differed significantly for traits investigated except leaflet surface and oil content. The subsp. *hypogaea* group showed significantly greater mean pod length, pod width, seed length, seed width, yield per plant, and 100-seed weight than the subsp. *fastigiata* group whereas it was opposite for plant height, leaflet length, leaflet width and shelling percentage.

Upadhyaya et al (2011a) evaluated 269 accessions of 20 wild *Arachis* species belonging to six sections for 41 morpho-agronomic traits, and 89 accessions for oil, protein and total sugar content. A large range of variations were found and most of the traits differed

significantly between species. Accessions were clustered into four groups, cluster 1 and 2 represented with mostly annuals, while cluster 3 and 4 with perennials. Koppolu et al (2010) assessed genetic relationships among seven sections of genus *Arachis* (96 accessions belonging to 36 species) using SSR markers. A total of 109 species specific alleles were detected in different wild species, and *A. pusilla* exhibited maximum of 15 species specific alleles. Huang et al (2012) investigated genetic diversity of 72 wild *Arachis* accessions representing 19 species along with three cultivated groundnut accessions using 136 genome-wide SSR markers, and reported abundant diversity across the 19 wild species. *A. duranensis* exhibited the highest diversity (Shannon index of 0.35) with a total of 12 unique alleles, while *A. rigonii* exhibited the maximum of 75 unique alleles. Khera et al (2013) used a set of 96 informative single nucleotide polymorphisms and developed groundnut Kompetitive Allele Specific Polymerase Assays Markers (GKAMs). Initially GKAMs were screened on a validation set consisting 94 genotypes that included parental lines of 27 mapping populations, seven synthetic autotetraploid and amphidiploids, and 19 wild species accessions.

The 73 polymorphic GKAMs were screened on 280 diverse accessions of the reference set and cluster analysis of marker allelic data grouped accessions according to their genome type, subspecies and botanical variety. The diploid wild species grouped separately. Average PIC ranged from 0.21 (AA genome) to 0.33 (EE genome), while BB and AABB genomes had PIC values of 0.31 and 0.32, respectively (Khera et al. 2013). Chopra et al (2016) sequenced (RNAseq) a diverse panel of 22 *Arachis* accessions representing *A. hypogaea* botanical types (12 genotypes), eight diploid wild species having A-, B-, and K-genome, a synthetic amphidiploid, and a tetraploid wild species. They found the presence of substantial genetic variability among wild species, and significant but lesser variability at the molecular level among accessions of the cultivated species. Molecular marker based cluster analysis grouped *Arachis* genotypes according to their genome type, subspecies and botanical variety (Koppolu et al 2010; Khera et al 2013; Chopra et al 2016).

Draft genome sequence of the two parental species of groundnut are available (Bertioli et al 2016; Chen et al 2016) that will accelerate groundnut genomics for candidate gene identification and marker assisted improvement. With DNA identity of the *A. ipaënsis* genome and the B subgenome of cultivated peanut and biogeographic evidence, Bertioli et al (2016) concluded that *A. ipaënsis* may be a direct descendant of the same population which contributed the B subgenome to cultivated peanut.

Constraints in Groundnut Production

Groundnut crop is adversely affected by both biotic and abiotic stresses. Drought, salinity and nutrients deficiencies such as low availability of phosphorus under acidic soils and non-availability of iron in calcareous soils in many parts in the world are important abiotic stresses yield-reducing factors (Upadhyaya et al 2011b). The major foliar fungal diseases worldwide are early leaf spot (*Cercospora arachidicola*), late leaf spot (*Phaeoisariopsis personata*) and rust (*Puccinia arachidis*). Stem and pod rot caused by *Sclerotium rolfsii* is a potential threat to groundnut production in many warm, humid areas, especially where irrigated groundnut

cultivation is expanding. Aflatoxins are potent carcinogen produced by *Aspergillus* spp. infection in seed forcing several countries to have strict regimes in place on permissible levels of aflatoxins in their imports. Bacterial wilt is predominant among bacterial disease, while nematodes such as root-knot nematodes and lesion nematodes are important in groundnut. Aphids (*Aphis craccivora*), several species of thrips (*Frankliniella schultzei, Thrips palmi*, and *F. fusca)*, leaf miner (*Aproaerema modicella*), red hairy caterpillar (*Amsacta albistriga*), jassids (*Empoasca kerri* and *E. fabae*) and *Spodoptera* are the major insect pests in groundnut. In addition, several insect pests act as vector for important viral diseases, for example *T. palmi* for peanut bud necrosis, *F. occidentalis* and *F. fusca* for tomato spotted wild virus and *Aphids crassivora* for groundnut rosette virus. Termites, white grubs, and some storage pests such as groundnut borer or weevil (*Caryedon serratus*) and rust-red flour beetle (*Tribolium castaneum*) are also important pests in groundnut. Diseases like rust, early and late leaf spot are the most common widely distributed foliar disease of groundnut worldwide. It is common to find leaf miner in South Asia; army worm and bacterial wilt in South-east Asia; groundnut rosette disease and termite in Africa; and nematode, corn earworm, lesser corn stock borer and southern corn rootworm in North America (Upadhyaya et al 2011b).

Wild *Arachis*: Global Status and Significance

Ex *situ* collections

Globally over 120,884 accessions (4480 wild and 116,404 cultivated) of *Arachis* species have been conserved in > 70 genebanks in 46 countries. ICRISAT, India and National Bureau of Plant genetic Resources (NBPGR, India) are the two major genebanks that conserve about 25% of total global groundnut collections (12.78% in ICRISAT and 12.07% at NBPGR). Embrapa Recursos Genéticos e Biotecnologia, Brazil (2042 accessions) conserves the largest greenhouse collection of groundnut wild species and the next is ICRISAT, India (447 accession) (Table 2).

Wild *Arachis* as sources for Important Traits

Wild species of *Arachis* were screened and several genotypes have been reported as resistant to major diseases and insect pests that damage cultivated groundnut (Table 3). Some genotypes of different wild species show very high levels of resistance to rust, early leaf spot, late leaf spot, nematodes, groundnut rosette disease, peanut bud necrosis virus, thrips, leaf miner, *Spodoptera*, aphids etc. (Table 3). For example, Pande and Rao (2001) identified one wild accession each of *A. hoehnei* (ICG 8190) and *A. duranensis* (ICG 13199) that remained asymptomatic to late leaf spot, and ICG 8954 of *A. kuhlmannii* that remained asymptomatic to rust. Kalyani et al (2007) reported *A. duranensis* (ICG 8139, ICG 8195, ICG 8200, ICG 8203, ICG 8205, ICG 11550), *A. villosa* (ICG 8144) and *A. stenosperma* (ICG 13210) accessions as sources for tobacco streak virus.

Michelotto et al (2015) reported *Arachis* wild accessions such as V 15076 (*A. stenosperma*), V 6413 (*A. kuhlmannii*), V 13250 (*A. kempff-mercadoi*), Sv 3712 (A. *stenosperma*), KG 30006 (*A. hoehnei*), V 6325 (*A. helodes*) and GKP 10017 (*A. cardenasii*) as most promising accessions with multiple resistance to late leaf spot, early leaf spot, rust and scab. Michelotto et al (2017) reported V 7635 (*A. vallsii*), V 13250 (*A. kempff-mercadoi*), K 9484 (*A. batizocoi*), V 1118 (*A. williamsii*), V 14167 (*A. duranensis*) and V 13751 (*A. magna*) as promising sources for resistance to thrips.

Table 2: The major genebanks conserving groundnut wild and cultivated germplasms (source: http://www.fao.org/wiews-archive/germplasm_query.htm?i_l=EN)

Institute	Wild	Cultivated	Total
All India Co-ordinated Project on Groundnut, India	45	6229	6274
Australian Tropical Crops & Forages Genetic Resources Centre, Australia	113	1083	1196
Banco Base de Germoplasma, Instituto de Recursos Biológicos, Instituto Nacional de Tecnología, Argentina	355	7992	8347
Centro de Investigaciones de Nataima, Instituto Colombiano Agropecuario, Colombia	225	-	225
Centro Internacional de Agricultura Tropical, Colombia	171	-	171
Crop Science Department, North Carolina State University, USA	264	3524	3788
Estación Experimental Agropecuaria Manfredi, Argentina	113	2045	2158
Greenhouse Collection, Embrapa Recursos Genéticos e Biotecnologia, Brazil	2042	-	2042
Institute of Crop Germplasm Resources, Chinese Academy of Agricultural Sciences, China	-	6565	6565
Institute of Oil Crops Research, Chinese Academy of Agricultural Sciences, China	-	5688	5688
Instituto de Botánica del Nordeste, Universidad Nacional de Nordeste, Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina	113	29	142
International Crop Research Institute for the Semi-Arid Tropics, India	477	14968	15445
National Bureau of Plant Genetic Resources, India	-	14593	14593
National Research Centre for Groundnut, India	-	8934	8934
Plant Genetic Resources Conservation Unit, Southern Regional Plant Introduction Station, University of Georgia, USA	226	9738	9964
Texas Agricultural Experiment Station, Texas A and M University, USA	307	1360	1667

Sharma et al (1999) had reported seven accessions, ICG 8952 (*A. helodes*), ICG 13211 (A. *sylvestris*), ICG 13224 (*A. kretscmeri*), ICG 13231 (*Arachis sp.*), ICG 14862 (*A. kuhlmannii*), ICG 14868 (*A. stenosperma*), and ICG 14915 (*A. sylvestris*) as highly resistant to *Meloidogyne javanica* nematode reproduction and root damage. Sharma et al (2003) reported wild *Arachis* species resistant to multiple insects and diseases (Table 3). Plant morphological characteristics such as main stem thickness, hypanthium length, leaflet shape and length, leaf hairiness, standard petal length and petal markings, basal leaflet width, main stem thickness and hairiness, stipule adnation length and width, and peg length showed

Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad 500 030

significant correlation with damage by *Spodoptera* and leafhoppers, and these traits can possibly be used as markers to select initially for resistance to insect pests.

Upadhyaya et al (2011a) identified the best 20 wild accessions having superior agronomic traits for use in introgression of new diversity into *A. hypogaea.* The traits considered were days to flowering, primary branches, main stem thickness, pod length, pod width, seed length, seed width and 100-seed weight, nutritional quality (oil, protein and sugar content) and drought tolerance as indicated by SCMR (soil plant analytical development - chlorophyll meter reading) and SLA (specific leaf area), both at 60 and 80 days after sowing. Accessions of *A. duranensis* exhibited the maximum intra-specific variation for important traits including days to flowering, primary branches, plant width, pod length, pod width, SCMR and SLA. An accession, ICG 8144 of *A. villosa* had high SCMR, low SCA and high sugar content; ICG 13223, ICG 13244, ICG 14868, ICG 14872, ICG 14874 and ICG 14884 of *A. stenosperma* accessions were superior in pod length and width and/or seed length and width; ICG 13211 of *A. pusilla* was the earliest to flower; ICG 13178 of *A. monticola* and ICG 13189 of *A. duranensis* accessions were high in sugar content; and ICG 15142 of *A. pusilla* and ICG 13227 of *A. dardani* were high in protein content. Upadhyaya and Liao (2016) reported 41 to 66% oil content in 304 accessions of 41 *Arachis* species. Huang et al (2012) found significant oil content in the 72 accessions of 20 wild species from five sections.

Table 3: Wild *Arachis* species as sources of resistance to diseases and insect pests

Early leaf spot	*A. hagenbeckii, A. glabrata, A. repens, A. diogoi, A. apressipila, A. triseminatae, A. magna, A. sylvestris, A. pusilla, A. valida, A. dardani*	Gibbons and Bailey (1967); Abdou et al (1974); ICRISAT (2000)
Late leaf spot	*A. batizocoi, A. benensis, A. cardenasii, A. decora, A. diogoi, A. correntina, A. duranensis, A. hoehnei, A. ipaënsis, A. kempff-mercadoi, A. kuhlmannii, A. stenosperma, A. valida, A. villosa*	Subramanyam et al (1983); Pande and Rao (2001); Abdou et al (1974)
Rust	*A. batizocoi, A. duranensis, A. spegazzinii, A. correntina, A. stenosperma, A. cardenasii, A. chacoense, A. villosa, A. apressipila, A. paraguariensis, A. pusilla, A. villosulicarpa. A. hagenbeckii, A. glabrata, A. hoehnei, A. kuhlmannii, A. benensis, A. chiquitana*	Subrahmanyam et al (1983); Pande and Rao (2001)
Cylindrocladium black rot (*Cylindrocladium* parasiticum)	*A. valida, A. cruziana, A. microsperma, A. williamsii, A. kempff-mercadoi, A. kuhlmannii, A. helodes, A. cardenasii and A. correntina*	Tallury et al (2014)
Sclerotinia blight	*A. glandulifera*	Tallury et al (2014)
Aflatoxin – seed colonization and production	*A. cardenasii and A. duranensis*	Nigam et al (1991)
Groundnut rosette disease	*A. diogoi, A. hoehnei, A. kretschmeri, A. cardenasii, A. villosa, A. pintoi, A. kuhlmannii, A. appressipila, A. stenosperma, A. decora, and A. triseminata*	Subrahmanyam et al (2001)

Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad 500 030

Peanut bud necrosis	*A. benensis, A. cardenasii, A. villosa, A. apressipila, A. triseminatae*	Reddy et al (2000)
Tobacco streak virus	*A. duranensis, A. villosa, A. stenosperma*	Kalyani et al (2007)
Peanut stunt virus	*A. duranensis, A. villosa*	Herbert and Stalker (1981)
Peanut Stripe, Peanut Mottle and Tomato Spotted Wilt Viruses	*A. diogoi, A. helodes, A. glabrata*	Rao et al (1993)
Thrips	*A. williamsii, A. vallsii, A. kempff-mercadoi, A. batizocoi, A. duranensis, A. magna*	Michelotto et al (2017)
Leaf miner, *Helicoverpa*, leaf hopper	*A. cardenasii, A. duranensis, A. kempff-mercadoi, A. monticola, A. stenosperma, A. paraguariensis, A pusilla, A. triseminatae*	Sharma et al (2003)
Nematode resistance ***Meloidogyne javanica***	*A. helodes, A. sylvestris, A. kretscmeri, A. kuhlmannii, A. stenosperma*	Sharma et al (1999)
Meloidogyne arenaria:	*A. batizocoi, A. cardenasii*	Nelson et al (1989); Holbrook and Noe (1990)
Multiple resistance to late leaf spot, early leaf spot, rust and scab	*A. stenosperma, A. kuhlmannii, A. kempff-mercadoi, A. hoehnei, A. helodes, A. cardenasii*	Michelotto et al (2015)
Multiple insects and disease: Leaf miner, *Helicoverpa*, rust and late leaf spot	*A. cardenasii, A. duranensis, A. kempff-mercadoi, A. monticola, A. stenosperma, A. paraguariensis, A pusilla, A. triseminatae*	Sharma et al (2003)

Most accessions had oil contents ranging from 55 to 58%, and one *A. rigonii* accession (WH10026) had the highest oil contents (61 to 63%); and seven other accessions (WH4347, WH4377, WH10034, WH4330, WH10025, WH4376, and WH4367) had oil contents of more than 57%. The accessions from section *Procumbentes* had an average oil content of 57.65% (ranging from 54.31% to 62.26%), higher than that in other sections. Seven wild species had higher oil content than the average oil content of 72 wild *Arachis* accessions (56.69%): they were *A. chacoense* (56.70%), *A. monticola* (57.57%), *A. villosa* (57.75%), *A. cryptopotamica* (56.69%), *A. oteroi* (57.18%), *A. chiquitana* (56.70%), and *A. rigonii* (58.62%). Huang et al (2012) also identified nine alleles of five SSR markers associated with oil content based on association analysis. Three alleles were associated with higher oil content but were absent in the cultivated peanut, indicating the great potential to increase the oil content in *A. hypogaea* by using the wild *Arachis* germplasm.

Utilization of Wild *Arachis* in Groundnut Improvement

The first groundnut cultivar released through inter-specific hybridization was 'Spancross' by Hammons (1970)followed by 'Tamnut 74' by Simpson and Smith (1975), both cultivars from cross between *A. hypogaea* and *A. monticola* .

At ICRISAT, inter-specific hybrid lines that were selected for diseases and insect resistance at North Carolina State University were utilized to develop groundnut cultivars such as ICGV 86699 and ICGV 87165. ICGV 86699 is a high yielding inter-specific derivative of cross between (*A. batizocoi* × *A. duranensis*) × *A. hypogaea* (NC2) (Reddy et al 1996), and ICGV 87165, an inter-specific derivative of a cross

between PI 261942 (*A. hypogaea* sup *fastigiata* var *fastigiata*) and *A. cardenasii* (Moss et al 1997). In 2002, GPBD 4 derived from cross between Indian cultivar KGR 1 and CS 16 ICGV 86855) was released in India (Gowda et al 2002) which was highly resistant to rust and late leaf spot. CS 16 (ICGV 86855) was a disease resistant inter-specific line from the *A. hypogaea* x *A. cardenasii* population.

Inter-specific lines produced at ICRISAT were utilized in hybridization with cultivated groundnut resulted in release of several groundnut cultivars. For example, ICGV-SM 86715, an improved Virginia peanut germplasm line developed by the SADC (Southern African Development Community) groundnut project in Malawi and ICRISAT, was released in 1992 as cv Veronica for cultivation in Mauritius (Moss et al 1998). ICGV-SM 86715 was derived from a cross between *A. hypogeae* subsp. *hypogaea* var *hypogaea* cv. Makulu Red and a tetraploid backcross derivative (Samaru 38 x *A. diogoi* GKP 10602) x Samaru 61), and resistant to rust, late leaf spot and pepper spot. Four groundnut germplasm lines (ICGV 99001, ICGV 99003, ICGV 99004 and ICGV 99005) were released in 2001 by the Plant Material Identification Committee of ICRISAT for their resistances to foliar fungal diseases, all of these were derived from *A. hypogaea* and wild *Arachis* species crosses (Singh et al 2003). ICGV 99001 derived from *A. hypogaea* x *A. villosa*, ICGV 99003 from *A. hypogaea* x (*A. duranensis* x *A. stenosperma*), ICGV 99004 from *A. hypogaea* x *A. cardenasii* and ICGV 99005 from *A. hypogaea* x (*A. batizocoi* x *A. duranensis*). Indian Institute of Groundnut Research (IIGR), Junagadh has developed large number of inter-specific breeding lines over a period of time to introgress desirable genes from wild *Arachis* species using cultivated groundnut as female parent and wild *Arachis* species such as *A. diogoi, A. correntina, A. helodes, A. pusilla, A. cardenasii, A. duranensis, A. batizocoi, A. stenosperma, A. monticola, A. villosa, A. kempff-mercadoi, A. pintoi, A. kretschmeri, A. oteroi* and A. *villosulicarpa* as male parents (Bera et al 2014). These inter-specific breeding lines were screened for peanut bud necrosis disease (PBND) and 42 lines were identified as highly resistant (0 to 1% PBND incidence) and 73 resistant (1.1 to 5% PBND incidence) by Kamdar et al (2014). Bera et al (2014) identified nine SSR markers, RNOX536, PM15, PM36, PM65, PM145, PM188, PM201, PM204 and PM322 associated with major quantitative trait loci (QTL) for resistance to PBND.

Synthetics for Cultivated Groundnut Improvement

Pathways for obtain synthetics

The gene transfer from wild *Arachis* species into cultivated peanut was first attempted by W.C. Gregory and A Krapovickas in 1940s, while their first attempt was unsuccessful, continued efforts resulted in improvement of success rate in next five decades (Gregory and Gregory 1979; Krapovickas and Gregory 1994). Main challenges in use of wild species in groundnut improvement are: (i) majority of wild species are diploid, while the cultivated species is allotetraploid and (ii) genome differences between wild *Arachis* and cultivated tetraploid *A. hypogaea*. Gregory and Gregory (1979) conducted an extensive hybridization program and reported cross-compatibility relationships in *Arachis*. Hybridization between species within the same section was more successful than crosses between species among sections, and F_1s of inter sectional crosses were highly sterile. To overcome crossing barriers, complex hybrids were attempted (Gregory and Gregory 1979; Stalker 1981), but fertility was not restored. Thus, introgression from wild *Arachis* species

to *A. hypogaea* by conventional hybridization is believed to be restricted to members of section *Arachis*. Even within section *Arachis*, hybrids may be difficult to obtain because of genomic and/or ploidy differences.

Simpson (2001) suggested three different pathways for successful utilization of wild *Arachis* species in the genetic enhancement of the cultivated groundnut: hexaploid route; diploid/tetraploid pathway with a two-way cross; and diploid/tetraploid pathway with three-way cross using bridge species for gene introgressions from wild *Arachis* to cultivated groundnut, and induce chromosome doubling to obtain fertile tetraploids - also called synthetic groundnut.

Hexaploid route

In this pathway, *A. hypogaea* (2n = 4x = 40) is hybridized with a diploid wild *Arachis* species (2n = 2x = 20) to produce a sterile triploid (3x = 30), which is then chromosome-doubled using colchicine to produce a hexaploid (2n = 6x = 60). This amphiploid is first crossed and then selfed or backcrossed with *A. hypogaea* until the tetraploid hybrid is obtained after eliminating the excess chromosomes during segregation. Sterility is a major issue during the backcrossing cycles though some combinations are much easier to work with than others. For example, hexaploids produced from crosses between most Virginia market-type cultivars and *A. diogoi* or *A. cardenasii* will be highly sterile, while if a Spanish or Valencia market-type cultivar is used, the hexaploid will be usually somewhat fertile. This method has been used with some success in North Carolina State University, USA and ICRISAT, India and numerous sets of disease and insect resistant germplasm lines have been distributed. This pathway has limitations as it is time consuming and unpredictable; however the advantage is through selfing, as selfing the amphiploid increases recombination between the chromosomes of different genomes.

Diploid/tetraploid pathway with a two-way cross

In this pathway, two wild *Arachis* species are first doubled with colchicine followed by hybridization of these two amphiploids to form a tetraploid. Another variant of this pathway is to first crossing two diploid wild *Arachis* species followed by doubling the chromosome number of the hybrid to obtain amphiploid. This pathway was attempted in Texas (Simpson 1991), but without both A and B genome in crossing program, the success is severely limited because of high sterility.

Diploid/tetraploid pathway with three-way cross using bridge species

This pathway was proposed by Smartt et al (1978) as a solution to overcoming the sterility barrier between *A. hypogaea* and diploid species crosses, by using B genome in crossing scheme as a bride species. Use of the B genome parent might make the complex amphiploids more cross-compatible with *A. hypogaea*, and is the most successful introgression pathway. For example, in the Taxas program, *A. cardenasii* (2n=2x=20) was initially crossed with *A. diogoi* (2n=2x=20) and the resulting hybrid was crossed as the male parent onto *A. batizocoi*. The resulting diploid three-way hybrid was sterile and was chromosome doubled with colchicine treatment to obtain fertile amphiploid (Simpson 1991).

Development of Synthetic Groundnuts

Among three pathways, hybrids between A and B genome *Arachis* parents appear to be useful for introgressing characters into *A. hypogaea* from wild species. The B-genome can serve as an effective bridge species between A-

genome wild *Arachis* and *A. hypogaea*. Following different synthetic groundnut development pathways, several synthetic tetraploids have been developed. Simpson (1991) developed the first amphidiploid by crossing *A. cardenasii GKP10017* (2n = 2x = 20) with *A. diogoi* GKP 10602 (2n = 2x = 20), and the resulting hybrid (52% pollen stained) was used as the male parent and crossed with *A. batizocoi* K9484. Thus derived diploid three-way hybrid was sterile (pollen stained <1%) and was chromosome doubled with colchicine to obtain amphiploid. This amphiploid had above 90% pollen stained and was easily crossed with *A. hypogaea* cv Florunner. Progenies that were highly fertile were selected and backcrossed to *A. hypogaea*. This method has successfully transferred high levels of early and late spot resistance and root-knot nematode resistance into *A. hypogaea* (Simpson et al 1993).

Further several synthetic groundnuts have been developed (Table 4). Fávero et al (2006) developed an amphiploid utilizing *A. ipaënsis* and *A. duranensis*, and used it to develop backcross populations and then constructed genetic and QTL maps (Foncéka et al 2009, 2012). Fávero et al (2015) developed 16 synthetic groundnuts (13 amphidiploid and 3 autotetraploid) by involving A-, B-, and K-genome wild species that were previously identified for fungal disease resistant (Table 4).

Table 4: Examples of synthetic groundnut developed from various wild *Arachis* species

Amphidiploid: *A. batizocoi* K9484 × (*A. cardenasii* GKP10017 × *A. diogoi* GKP10602)	Simpson et al (1993)
Amphidiploid : *A. ipaënsis* KG30076 and *A. duranensis* V14167	Fávero et al (2006)
Amphidiploid:*A. duranensis* × *A. ipaënsis* (ISATGR 1212); *A. batizocoi* × *A. cardenasii* (ISATGR 9A); *A. ipaënsis* × *A. duranensis* (ISATGR 40A); *A. valida* × *A duranensis* (ISATGR 168B); *A. duranensis* × *A. batizocoi* (ISATGR 278-18); *A. kempff-mercadoi* × *A. hoehnei* (ISATGR 265-5); *A. batizocoi* × *A. cardenasii* (ISATGR 268-5); *A. batizocoi* × *A. duranensis* (ISATGR 35A); *A. kempff-mercadoi* × A. hoehnei (ISATGR 80A); *A. duranensis* × *A. valida* (ISATGR 206) **Autotetraploid:***A. magna* × *valida* (ISATGR 1, ISATGR 11A, ISATGR 10B); *A. magna* × *A. batizocoi* (ISATGR 5B); *A. Kempff-mercadoi* × *A. stenosperma* (ISATGR 90B); *A. diogoi* × *A. cardenasii* (ISATGR 99B & ISATGR 160)	Mallikarjuna et al (2011)
Amphidiploid: *A. batizocoi* 9498 × *A. cardenasii* 10007; *A. batizocoi* 9498 × *A. kempff-mercadoi* 13250; *A. batizocoi* 9498 × *A. helodes* 6325; *A. gregoryi* 6389 × *A. duranensis* 14167; *A. batizocoi* 9498 × *A. duranensis* 14167; *A. ipaënsis* 30076 × *A. villosa* 12812; *A. magna* 13751 × *A. stenosperma* 3; *A. gregoryi* 6389 × *A. linearifolia* 9401;*A. magna*13751×*A. linearifolia* 9401; *A. magna* 13751 × *A cardenasii* 10017; *A. gregoryi* 6389 × A. stenosperma 12488; *A. gregoryi* 6389 × *A. villosa* 12812; *A. gregoryi* 6389 × *A. kuhlmannii* 13721 **Autotetraploid**: *A. hoehnei* 30006 × *A. helodes* 6325; *A. hoehnei* 3006 × *A. simpsonii* 13710; *A. hoehnei* 3006 × A. cardenasii 10017	Fávero et al (2015)
Amphidiploid: *A. batizocoi* K9484 × A. *duranensis* V14167 (BatDur1); A. *batizocoi* K9484 × *A. duranensis* SeSn2848 (BatDur2) ***Autotetraploid:****A. batizocoi* K9484 × A. *stenosperma* V10309(BatSten1)	Leal-Bertioli et al (2015)
Amphidiploid: *A. magna* V13751 and *A. kempff-mercadoi* V13250	de Paula et al (2017)

Mallikarjuna et al (2011) also assessed genetic relatedness of the synthetics and cultivated lines using DArT markers and reported high level of diversity among the synthetics (genetic distance range 0.020 to 0.725) while low diversity was observed among cultivated lines (genetic distance range 0.041 to 0.073); and synthetics were clustered into four groups although cultivated lines formed a single group. Leal-Bertioli et al (2015) developed three amphidiploids by crossing *A. batizocoi* with *A. duranensis* and *A. stenosperma* (Table 4). These induced allotetraploids were vigorous and fertile, and hybridized easily with *A. hypogaea*, however fertility of F_1 varied according to cultivated groundnut × allotetraploid combinations, i.e hybrids with *A. hypogaea* were significantly more fertile than those with the subspecies *fastigiata*, suggesting the influence of stochastic genetic or epigenetic events. de Paula et al (2017) developed an amphidiploid named An13 by crossing *A. magna* V 13751 and *A. kempff-mercadoi* V 13250.

Synthetics as Sources of Variation for Important Traits

Synthetic groundnuts have been successfully used in diversifying cultivated gene pool through backcrossing synthetic tetraploids with cultivated *A. hypogaea*. Simpson et al (1993) developed two groundnut germplasm lines, TxAG-6 and TxAG-7, by inter-specific hybridization. TxAG-6 was an amphidiploid derived from a cross involving *A. batizocoi* K9484 x (*A. cardenasii* GKP10017 × *A. diogoi* GKP10602), while TxAG-7 was derived by crossing TxAG-6 with the Florida line UF-439-16-10-3-2 (a component line of A. *hypogaea* L. cv. Florunner) as female. These two lines were resistant to nematode and leaf spot, and have been crossed and backcrossed extensively with a wide range of *A. hypogaea* genotypes (Simpson et al 1993). Evaluation of synthetic groundnuts developed by Mallikarjuna et al (2011) has led to the identification of several lines as resistant to groundnut bud necrosis, late leaf spots and rust (Shilpa et al 2013). Apart from disease resistance, Shilpa et al (2013) also observed considerable variation in synthetic groundnuts for oil and protein contents.

Kumari et al (2014) utilized the two synthetic amphidiploids namely ISATGR 278-18and ISATGR 5B developed at ICRISAT (Malligarjuna et al. 2011), and generated backcross progenies using five cultivated genotypes as recurrent parents (ICGV 9114, ICGS 76, ICGV 91278, JL 24 and Dh 86). Both the synthetics showed high levels of resistance to rust and late leaf spot (disease score 2 to 3 of 1, on 9 scale where 1 means no disease and 9 represents 81 to 100% severity) while cultivated lines were susceptible (disease score 6 to 7). Among BC_2F_4 introgressions lines, a total of 120 lines were identified as resistant to rust and late leaf spot, and high frequency of resistant lines (90 lines) obtained were from the cross ICGS 76 × ISATGR 278-18 in addition to 18 lines from Dh 86 × ISATGR 278-18, while no resistant plants were found in JL 24 × ISATGR 5B and ICGV 91114 × ISATGR 5B. This showed ISATGR 278-18 as a potential source of disease resistance for diversifying the groundnut cultivated gene pool. Apart from resistance to rust and late leaf spot, backcross population also showed a large variation for morpho-agronomic traits (Kumari et al 2014). Further, using synthetic groundnut developed by Mallikarjuna et al (2011), two pre-breeding populations were developed involving cross between ICGV 91114 (cultivated) and ISATGR 1212 (synthetic amphiploid), and between ICGV 87846 (cultivated) and ISATGR265-5A (synthetic amphiploid) at ICRISAT, India (Sharma et al 2017), and obtained introgression lines (ILs) with high levels of resistance to late

leaf spot and rust, and significant variability for morpho-agronomic traits.

de Paula et al (2017) reported resistance of hybrids derived from cross between amphidiploid (An13, Table 4) with cultivated groundnut cultivar IAC OL4 to mites. Michelotto et al (2017) reported thrips (*Enneothrips flavens*) resistance of amphidiploids - An 12 (*A. batizocoi* x *A. kempff-mercadoi*), An 9 (*A. gregoryi*×*A. stenosperma*), and An 8 (*A. magna*×*A. cardenasii*). Michelotto et al (2016) have identified three amphidiploids (*A. magna* V 13751 × *A. cardenasii* GKP 10017; *A. magna* K 30097 × A. *stenosperma* V 15076; and *A. vallsii* V 7635 × A. *stenosperma* V 10229) with the high levels of resistance to early and late leaf spot and rust. All these studies indicated synthetic groundnuts as important genetic resources to broaden genetic base of cultivated groundnut, and to induct new diversity for insect pests and disease resistance beside agronomic traits.

Utilization of Synthetics in Groundnut Improvement

Several synthetic amphiploids have been developed following different pathways as suggested by Simpson (2001), and hybridization of synthetics with cultivated groundnuts has opened the avenue for gene transfer from wild species into cultivated groundnut. Use of TxAG-6, an amphidiploid derived from a backcross introgression pathway (Simpson 1991) in crossing programs with cultivated groundnut has resulted in the release of three cultivars namely, COAN (Simpson and Starr 2001), NemaTAM (Simpson et al 2003) and Webb (Simpson et al 2013), carrying genes for root-knot nematode resistance from *A. cardenasii*. NemaTAM and COAN are a runner market-type groundnut cultivar with high level of resistant to root-knot nematode (*Meloidogyne arenaria* and *M. javanica*). Webb is a high-yielding with high-oleic fatty acid content, nematode-resistant groundnut cultivar and a moderate level of resistance to Sclerotinia blight (caused by *Sclerotinia minor*).

North Carolina Agricultural Research Service (NCARS) has released several inter-specific groundnut germplasm lines of *A. hypogaea* (PI 261942) and *A. cardenasii* (GPK 10017) by following triploid-hexaploid route. The first generation hybrids obtained were colchicine treated to restore fertility at the hexaploid (2n = 6x = 60) level (C1 generation), and was self-pollinated to increase to the C5 generation. Chromosome number was determined from numerous heterogeneous population and all individuals were at the tetraploid chromosome level. Seeds of this population were distributed to institutions both in the USA and overseas and also evaluated for insect pest and disease resistance. The most promising resistant lines were released by the NCARS: GP-NC WS 1, GP-NC WS 2, GP-NC WS 3 and GP-NC WS 4 in 1992 with resistance to early and late leaf spot (Stalker and Beute 1993); GP-NC WS 5 and GP-NC WS 6 in 1997 with resistance to two root-knot nematodes (Stalker et al 2002a); and GP-NC WS 7, GP-NC WS 8, GP-NC WS 9 and GP-NC WS 10 in 1997 with resistance to corn earworm, potato leafhopper, southern corn rootworm (Stalker and Lynch 2002). Leaf spot resistant amphiploid hybrid derivatives (Stalker and Beute 1993) were further crossed with large-seeded Virginia-type leaf spot resistant cultivars and released five leaf spot resistant germplasm lines (GP-NC WS 11, GP-NC WS 12, GP-NC WS 13, GP-NC WS 14 and GP-NC WS 15) by NCARS in 1997 (Stalker et al 2002b). Further, GP-NC WS 12 (Stalker et al 2002b) was used in crossing program with cultivated groundnut (C-99R and DP-1) and

obtained GP-NC WS 16 and GP-NC WS 17 germplasm lines resistant to multiple diseases such as early leaf spot, *Cylindrocladium* black rot, *Sclerotinia* blight and tomato spotted wild; the line GP-NC-WS 17 also exhibited drought tolerance (Tallury et al 2014). Another line N97076L derived from the *A. hypogaea* x *A. cardenasii* GP-NC WS 13 population was released by NCARS in 2006 as resistant to multiple diseases including early leaf spot, *Cylindrocladium* black rot, *Sclerotinia blight* and tomato spotted wilt virus (Isleib et al 2006). Further, N96076L was successively used as the source of multi-disease resistance and released cv Bailey (Isleib et al 2011), the most widely grown Virginia type cultivar in Virginia-Carolina production region. At ICRISAT, Upadhyaya (2016) used a synthetic amphidiploid TxAG-6 (Simpson et al 1993) which is poor yielding and with a low 100-seed weight (about 8 g), with cultivated groundnut cultivar TMV 2, and developed 60 cryptic introgression lines (ICGV 15434 to ICGV 15493), some having exceptional 100-seed weight (Figure 1a), and with specific adaptation to either rainy season or post-rainy season or both. ICGV 15439, ICGV 15442 and ICGV 15446 are adapted to rainy (June to October), ICGV 15436, ICGV 15464 and ICGV 15466 to irrigated post-rainy (November to March), and ICGV 15443, ICGV 15449, and ICGV 15452 to both rainy and post-rainy seasons. The lines on average produced 20.1 to 23.7% more pod yields in the three rainy seasons, 34.4 to 63.1% more pod yields in the four post-rainy seasons, and 16 to 26% more pod yields in the seven rainy and post-rainy seasons (Table 5) than the cultivated parent TMV 2 (1.53 to 2.27 t/ha).

Table 5: Pod yield and 100-seed weight of cryptic introgression lines from TMV 2 x TxAG6 and cultivated parent TMV 2 during three rainy (2013, 2014, 2015) and four postrainy seasons, ICRISAT Patancheru, India

Identity	Pod yield (kg/ha)	Increase over TMV2 (%)	100-seed weight (g)	Increase over TMV2 (%)
Three rainy seasons (2013, 2014, 2015)				
Trial 1				
ICGV15434	2647	16.9	63	70.3
ICGV15439	2801	23.7	68	83.8
ICGV15440	2717	20.0	71	91.9
ICGV15442	2721	20.1	73	97.3
ICGV15446	2790	23.2	68	83.8
TMV2	2265		37	
Trial 2				
ICGV15477	2529	10.0	80	105.1
TMV2	2299		39	
Four postrainy (2012-13 to15-16) seasons				
Trial 1				
ICGV15436	2308	34.4	62	40.9
ICGV15444	2041	18.9	93	111.4
ICGV15445	2244	30.7	81	84.1
ICGV15450	2212	28.8	88	100
ICGV15451	2184	27.2	81	84.1
TMV2	1717		44	

Identity	Pod yield (kg/ha)	Increase over TMV2 (%)	100-seed weight (g)	Increase over TMV2 (%)
Trial 2				
ICGV15454	1842	20.6	80	63.3
ICGV15457	1835	20.2	83	69.4
ICGV15464	2462	61.2	84	71.4
ICGV15466	2490	63.1	88	79.6
TMV2	1527		49	
Seven (three rainy and four post-rainy) seasons combined				
Trial 1				
ICGV15443	2310	16.0	77	87.8
ICGV15449	2381	19.6	86	109.8
ICGV15452	2509	26.0	87	112.2
TMV2	1991		41	
Trial2				
ICGV15465	2437	25.8	70	62.8
ICGV15469	2389	23.3	70	62.8
ICGV15470	2454	26.7	67	55.8
TMV2	1937		43	

A few cryptic introgression lines also had high SCMR and low SLA (ICGV 15439, ICGV 15441, ICGV 15444, ICGV 15445 and ICGV 15447). Upadhyaya (2016) also generated BC_2F_1 populations using an amphiploid derived from *A. duranensis* x *A. ipaënsis*, with cultivars such as TMV 2, JL 24, ICGV 91114, GG2 and JUG 26, and reported a large variation for flowering, maturity, pod and seed size and shape, and for root traits (Figure 1b).

Researchers have developed several synthetic tetraploid groundnuts and reported high genetic diversity and as sources for important traits. Synthetic groundnuts resistant sources can be used in breeding program to introduce resistance genes into cultivated genotypes; however it is necessary to identify the best backcross-derived inter-specific hybrids due to the loss of resistance alleles among the resulting progenies.

Conclusion

Domestication bottleneck during the course of evolution has resulted in low genetic diversity of *A. hypogaea* and susceptibility to numerous diseases and insect pests as evidenced from low to moderate levels of resistance identified in the cultivated germplasm collections. Wild *Arachis* species harbor genes or alleles which were lost in cultivated groundnut, and are reported to have a high level of resistance to important insect pests and diseases. Gene introgression from wild *Arachis* to cultivated groundnut is therefore essential to utilize the untapped potential of wild *Arachis* species.

Utilizing synthetic groundnuts in hybridization program with cultivated groundnut resulted in development of breeding lines with improved levels of resistance to biotic stresses and release as cultivar. Cryptic introgression lines developed at ICRISAT using synthetic groundnut have high pod yield, exceptionally high 100-seed weight and traits related to drought tolerance. Research is in progress to identify chromosomal segments of wild *Arachis* species responsible for enhancing these agronomic traits.

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Figure 1a. Enhanced seed size using amphidiploid T x AG 6

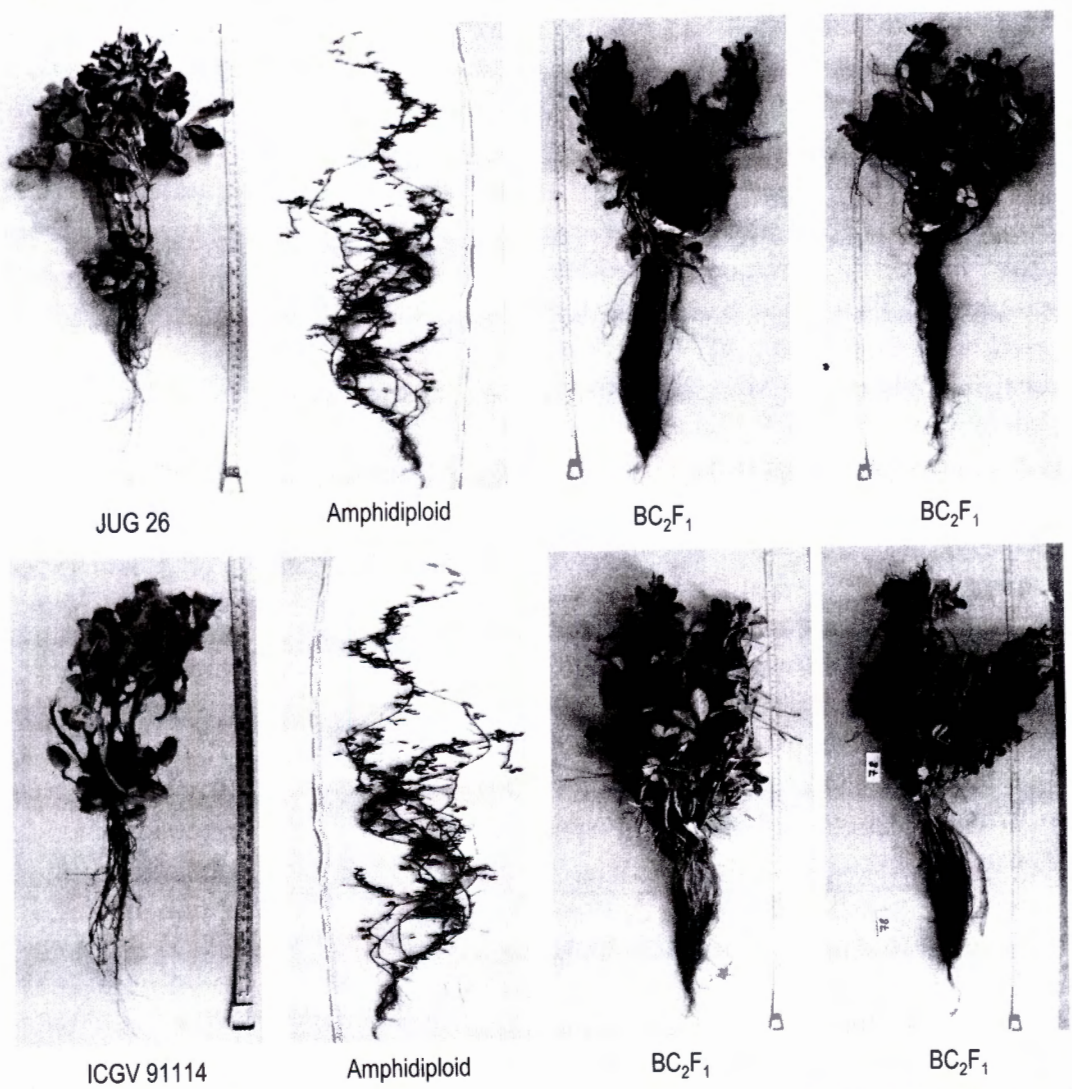

Figure 1b. Increased root length and volume using amphidiploid derived from *Arachis duranensis* x *A. ipaënsis* with cultigens

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