Training Manual On Hybridization Techniques in Groundnut

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Botany, systematics, characterization and utilization of groundnut germplasm

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Introduction

Groundnut (*Arachis hypogaea* L.) is an annual legume which is also known as peanut, earthnut, monkeynut and goobers. It is the 13th most important food crop and 4th most important oilseed crop of the world. Groundnut seeds (kernels) contain 40-50% fat, 12-36 % protein and 10-20 % carbohydrate. Groundnut seeds are a nutritional source of vitamin E, niacin, falacin, calcium, phosphorus, magnesium, zinc, iron, riboflavin, thiamine and potassium. Groundnut kernels are consumed directly as raw, roasted or boiled kernels or oil extracted from the kernel is used as culinary oil. It is also used as animal feed (oil pressings, green and dry haulms) and industrial raw material (for oil cakes and fertilizer). These multiple uses of groundnut plant make it an excellent cash crop for domestic markets as well as for foreign trade in several developing and developed countries. Groundnut is one of the most popular and universal crops cultivated in more than 100 countries in six continents (Nwokoto 1996). It is grown in 25.2 million hectares with a total production of 35.9 million metric tons (FAO, 2006). Major groundnut growing countries are India (26%), China (19%) and Nigeria (11%). Its cultivation is mostly confined to the tropical countries ranging from 40º N to 40º S. Major groundnut producing countries are: China (40.1%), India (16.4%), Nigeria (8.2%), U.S.A (5.9%) and Indonesia (4.1%).

Origin and distribution

The groundnut plant is believed to have originated in the South American continent, primarily in the tropical areas of Peru. But no evidence has been found out regarding the confirmation of the above assumption. But the domestication of this plant was done in the valleys of Paraguay only. Groundnut was being cultivated in the new world countries since 2500BC and this was the place where a diversity of the species of groundnut was cultivated.

When Columbus found America, groundnut came in to the contact of the rest of the world. The Spaniards who explored the southern America encountered with this nut like seed and soon after, the different varieties of groundnut started to get spread around the world. A type of variety named ‘Virginia’ was taken to Mexico first and then to west Africa. Then it moved to the North America courtesy the West Indies and West Africa in the 17th century. The Peruvian variety was taken to the southeastern Asian regions of China and Philippines by the Spanish ships and ultimately these foreign beans spread all over Asia. The Spanish variety was taken into Africa directly by the Portuguese explorers and there it got mixed with the Virginia variety and was finally introduced into Spain in the 18th century. The latest groundnut variety i.e. Valencia was taken to the country of Spain from Argentina in the 19th century and then it spread over all the Europe later.

i) Taxonomy

Groundnut is a species in the legume family (Fabaceae). It is an annual herbaceous plant, growing to 30 to 50 cm (1 to 1.5 ft) tall. The leaves are opposite, pinnate with four leaflets (two opposite pairs; no terminal leaflet), each leaflet 1 to 7 cm long and 1 to 3 cm broad. The flowers are a typical pea flower in shape, 2 to 4 cm across, yellow with reddish veining. After pollination, the fruit develops into a legume 3 to 7 cm (1 to 2 in) long, containing 1 to 4 seeds, which forces its way underground to mature. The taxonomical treatment of this species is as follows

Kingdom	*: Plantae*
Division	*: Tracheophyta*
Class	*: Magnoliophyta*
Order	*: Fabales*
Family	*: Fabaceae*
Subfamily	*: Faboideae*
Tribe	*: Aeschynomeneae*
Genus	*: Arachis*
Species	***: hypogaea***

Classification of varieties

Arachis hypogaea L. is the only species cultivated worldwide for its seed and oil and this species is not known to occur in wild state. However, at least two other species have been cultivated for their seed. They are *A.villosulicarpa* (cultivated in northwestern Brazil) and *A.stenosperma* (cultivated in central and southwestern Brazil). Two other species, *A. glabrata and A. pintoi* also have been cultivated as forage plants in South and North America and Australia, while the species *A.repens* has been used as a ground cover in S. America

The cultivated groundnut *(Arachis hypogaea* L.) has been divided into 4 different varieties namely 'Virginia', 'Peruviana', 'Valencia', and 'Spanish'. These different types are believed to have originated in different locations. Virginia variety may have been developed in Amazonia. The Peruvian variety is the common type found in archaeological sites in the oases of Peru and is believed to have been developed in Peru. The Spanish variety was grown by the peoples of northeastern Brazil. The Valencia variety may have been developed by the Guarani peoples of the Paraguay-Praraná basin.

The subspecies *hypogaea* and *hirsuta* share similar morphological features as they don't have floral axes on main axis (Weiss 2000, Bunting et al 1985). Pairs of vegetative branches and floral axes alternate along lateral branches. The Virginia type is less hairy , with short branches, whereas Peruvian type is more hairy with long branches. The Virginia and Peruvian varieties are prostrate, possess seed dormancy, and require 4 to 6 months growing season. The prostrate varieties are commonly called as ‘runners’ or ‘spreading types’ as lateral branches remain close to the ground, giving a spreading appearance. The accessions of Peruvian runner (var. *hirsuta* types), however, are very few in world germplasm collections

While, the subspecies *fastigiata* and *vulgaris* share similar morphological features where floral axes are found on the main axis. There is a continuous run of multifloral axes along lateral branches. Valencia type is little branched whereas Spanish type is more branched. The Valencia and Spanish varieties are erect, have non-dormant seeds, and mature in 3 to 5 months. Erect types are also called as 'bunch types' as the upright growth of branches give mature plant a tightly bunched, bushy appearance. Erect types often have lower individual pod yields per plant than the prostrate types. However, erect types tend to have slightly higher seed oil and seed protein contents. Spanish variety is particularly rich in oil. In 1994, Krapovickas and Gregory proposed two new varieties of subsp. *fastigiata* in addition to the existing ones, namely var. *peruviana* and var. *aequatoriana*.

Bases for classification

The groundnut is morphologically variable and there are many recognizably distinct varieties. Variants of *A. hypogaea* have frequently been described as distinct species, subspecies and botanical varieties. The general taxonomic position was reviewed by Gregory *et al*. (1951), who also proposed a classification based on the important distinction in branching pattern between two groups. In the notation used by them, the main axis is denoted n, and first, second and higher order branches are n + 1, n + 2 etc.

In the first group, Virginia, alternating pairs of vegetative and reproductive branches (inflorescences) are borne on the cotyledonary and other n + 1 branches. **The first two branches on n + 1 laterals are always vegetative** and the main axis produces vegetative branches only. The alternating branching pattern is repeated in the higher orders of branching. In their second group, Spanish-Valencia, reproductive branches are borne in a continuous series on successive nodes on the cotyledonary and other n + 1 branches, on which the first branch is always reproductive. Reproductive branches are also borne directly on the main axis at higher nodes. Most n + 2 and all n + 3 branches are reproductive. The Spanish and Valencia sub-groups differ in the pattern of production of n + 2 vegetative branches. Spanish varieties produce such (n+2) branches irregularly, but Valencias frequently have none; if any are produced they are formed in sequence distal to the 5- 8th node of n + 1 branches. The differences in morphology are provided in Table 1.

Inflorescence

Groundnut inflorescence are borne on axils of leaves on primary or secondary branches, are spike-like, simple or compound monopodia and each node has up to five flowers. However, three flowers per inflorescence is most common. Only one flowers per inflorescence opens at any given time.

The flowers are modified sessile papillonaceous flowers that appear to be stalked due to presence of tubular hyphanthium or "calyx lobe". The flower is subtended by a bract, with a second bract on the inflorescence branch. There are two calyx lobes, an awn-like one opposite the keel (includes one sepal) and a broad one opposite to the back of the standard (includes four sepals-

fused). The corolla consists of 5 petals (1standard, 2 wings, 2 keel) and the calyx has 5 sepals both are borne at distal end of the calyx tube. The standard petal is light yellow to deep orange or rarely is white. A central crescent area exists on the face of the standard petal which can be deeper in colour as that of standard or even express a different colour. The wing is usually the same colour as standard. But it may vary according to the variety.

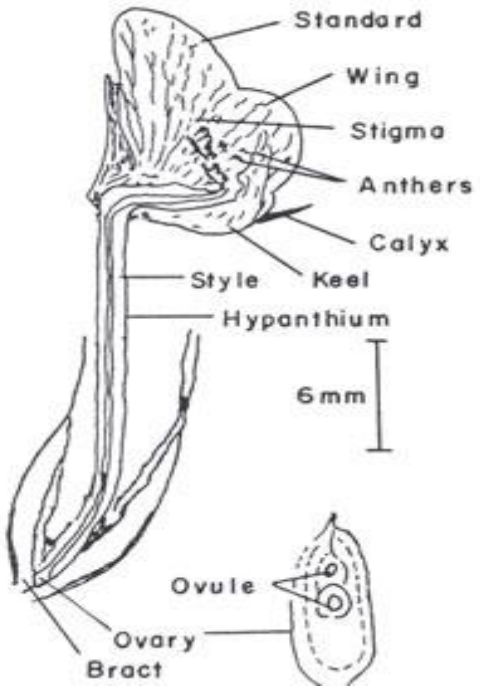

Figure 1. Parts of a groundnut flower

The style is contained within the calyx tube and both calyx tube and style elongate rapidly up to 5-7 cm in the 24 hours prior to anthesis. The androecium is a monoadelphous structure with a staminal tube bearing five oblong and five globular anthers. The filaments are fused for two thirds of their length. Among the globular anthers, two are sterile. This number usually varies in different varieties. In erect types, the sterile anthers are more common while it may be absent in spreading types.

The pollen matures 6 - 8 hours before anthesis. At the time of anthesis the pollen has two generative nucleus. The self pollination occurs because the stigma and anthers are enclosed by the keel. Cross pollination (ranging from 0 to 6.16%) occurs through bees.

The stigma is at the same level or protrudes beyond anthers; papillate type without surrounding hairs (surrounded by many papillae), elongated, and strongly curved. Stigma is receptive before anthesis. Pollination takes place at or near the time of anthesis (flower opening). Enzymes associated with pollen germination are produced on the stigmatic surface from 48 hours before to 8 hours after anthesis.

The ovary is unilocular, and has 1 -3 ovules, superior with the calyx tube attached to the base of the ovary. Fertilization is complete within 6 hours after pollination or before midday. After fertilization, the flower drops, the corolla closes, the calyx tube bends. The hypanthium and the style may remain attached to the base of the ovary for 4-5 days.

The ovary at the base of the calyx tube starts growing actively within a week by the activation of the intercalary meristem located below the ovary. The green ovary becomes purplish from tip downwards. The developing ovary pierces through the floral parts to reveal an elongating peg, a green stalk like structure which is botanically a 'carophore' or 'gynophore' and starts growing (elongating) geotropically. The peg carries the fertilized the ovule at its tip. The numerous plastids that develop after fertilization in the epidermal wall of the peg were found responsible for its positive geotropic movement. The peg becomes diageotrpic after penetrating the soil and ceases to elongate due to the formation of auxins formed in its distal potion. The diageotropism is such that the ovules are always located on the upper wall of the pod with the pod tip pointing away from the plant. Differences between cultivars in the depth at which pod are formed have also been reported. The normal poding zone is 4-7 cm below soil surface. The optimum temperature in podin zone is about 31-33°c. Lower soil temperature around 23°c increases number of pods and pod weight but increases filling duration thus increasing maturity. It takes about 60 days from the time of fertilization to full maturity.

Flowering

Depending on photoperiod, temperature and genotype flowering starts at about 25 days after emergence. The number of days required to first flowering reduces to 38 to 24 days when the daily mean temperature rises to 20-30°C in spreading-semi-spreading types while it drops from 35 to 24 days in Spanish-Valencias'. The most prolific flowering occurs between 5 and 11 weeks after planting depending on the duration of cultivar, and the season with a high degree of first formed flowers producing mature fruits.

Usually four to five stages of flowering can be observed. Very few flowers are produced in stage I, followed by rapid flowering in stage II. A peak is reached at stage III, followed by a decline in the number of flowers at stage IV and V. In some genotypes there can be two peaks of flowering. Very few flowers that are formed after the first and or second peaks of flowering produce mature fruits, unless early flowers are prevented from functioning normally due to some environmental stress.

Groundnut produces more flowers than the plant can sustain to develop in to pods. About 40% of flowers fail to develop from the outset, while another 40% produce only pegs. Less than 20% of flowers only produce mature fruits. Genotypes which flower early and produce most of the flowers during first two weeks of the flowering period produce greater number of pods.

A day before anthesis, the flower bud is 6-10 mm long. During day time the hypanthium elongates slowly and the buds grow to 10-20 mm long. The elongation is faster during the night

and at the time of anthesis the buds are about 50-70 mm long. Flower buds generally open at the beginning of the light period; it may be delayed in cold or wet weather.

The dehiscence of anthers takes place 7-8 hours before the flower opens or some times much later. Generally it takes place 1 hour before opening. Similarly the stigma is reported to be receptive from 24 hours before to 12 hours after the opening of the flower, but generally it is receptive only a few hours before anthesis. The pollen tube grows at the rate of about 1 cm per hour resulting in fertilization 5-6 hours after pollination.

Morphological Characterization

Any gerplasm includes the following materials:

1. Accessions: World collections assembled from different agencies with an accession number. This may include both cultivated and wild.

2. Land races: Un-selected or non-uniform material collected from framers' fields or purchased in local markets

3. Breeding line: Material developed by breeders but not released as a variety

4. Genetic stock: Genotypes identified by special features or sources of resistance to biotic and/or abiotic stresses.

The procedure for morphological characterization in groundnut as laid out by the National Test Guidelines for testing the Distinctiveness, Uniformity and Stability along with their descriptor states is provided below:

Grouping of varieties

1. The collection to be grown should be divided into groups to facilitate the assessment of distinctness. Characteristics that are suitable for grouping purposes are those which are known from experience not to vary, or to vary only slightly, within a variety and which in their various states are fairly evenly distributed within the collection.
2. It is recommended that the competent authorities use the following characteristics for grouping varieties.
    i. Commercial grouping
    ii. Time of maturity (for curing) (Characteristics 9)
    iii. Flowering: pattern on side branches (Characteristics 7)
    iv. Kernel: weight of 100 kernel (Characteristics 18)

VI. Characteristics and symbols

1. To assess distinctness, uniformity and stability, the characteristics and their states as given in the Table of Characteristics should be used.
2. Notes (1 to 9), for the purposes of electronic data processing, are given opposite the states of the different characteristics.
3. Legend:

(*) Characteristics that should be used every growing period for the examinations of all varieties and should always be included in the description of the variety, except when the

state of expression of a preceding characteristic or regional environmental conditions render this impossible.

(+) See Explanations on the table of characteristics in Chapter VIII.

4. The optimum stage of plant growth for assessment of each characteristic is indicated in the sixth column of table of characteristics.

5. Type of assessment of characteristics indicated in column 7 of table of characteristics is as follows:

MG: Measurement by a single observation of a group of plants or parts of plants

MS: Measurement of a number of individual plants or parts of plants

VG: Visual assessment by a single observation of a group of plants or parts of plants

VS: Visual assessment by observations of individual plants or parts of plants

Table 1. Botanical classification of *Arachis hypogaea* as proposed by Krapovickas and Gregory, 1994

Varieties	**Market type**	**South American location where it is abundant**	**Characteristics**
Sub-species ***hypogaea***			
			No floral axes on main stem; alternating pairs of floral and reproductive axes on branches; branches short; less hairy
hypogaea	Virginia runner	Bolivia,Amazon	Less hairy; large seeded
hirsuta	Peruvian runner	Peru	More hairy, small seeded
			Floral axes on main stem; alternating pairs of floral and vegetative axes on branches
Sub-species ***fastigiata***			
fastigiata	Valencia	Brazil Guranian Goias Minas gerais Paraguay Peru Uruguay	Less branches, long upright branches, hairy leaf
peruviana	Peruvian forms	Peru, N.W. Bolivia	Less hairy; deep pod reticulation
aequatoriana	Peruvian forms	Ecuador	Very hairy; deep pod reticulation; purple stems; more branched, erect
vulgaris	Spanish bunch	Brazil, Guranian Goias, Minas, Gerais, Paraguay Uruguay	More branched; upright branches

Table 2. Most widely adopted classification of groundnut (*Arachis hypogaea* L.)

Subspecies	Varieties	Botanical type	Branching pattern	Growth habit	Seed/pod
hypogaea	*hypogaea*	Virginia	alternate	prostrate to semi-erect	2-3
	hirsuta	Peruvian runner	alternate	prostrate	2-4
fastigiata	*fastigiata*	Valencia	sequential	erect	3-5
	vulgaris	Spanish	sequential	erect	2

Breeding methods and important achievements

The area under groundnut in India which was only 0.23 million ha during 1900-01 to 1909-10 has grown nearly 8.0 million ha during the past century. The decennial annual compound growth rate during different periods depicts that the growth in area (17.2%) was the major factor for the increase in groundnut production during 1951-1960. After 1970's, the productivity growth (2.5% during 1971-80; 8.2% during 1981-90 and 6.64% during 1991-2000) contributed more towards augmenting groundnut production than the area increase (Basu and Singh, 2004).

The major reasons for the increase in production and productivity even under the rainfed cultivation of groundnut are attributed to the release of niche specific varieties coupled with development of suitable improved production technologies especially under the umbrella of All India Coordinated Research Project on Oilseeds (AICORPO). Prior to 1970s, only 25 (9 Spanish Bunch; 14 Virginia; 2 Valencia**)** groundnut varieties were released and notified as against 182 varieties released so far in the country**.** The new generation improved varieties were bred for high yield and in-built resistance/tolerance to various biotic and abiotic stresses.

Groundnut growing regions have been demarcated in to five specific agro-climatic zones (Table 12 and 13) separately for *kharif* and *rabi*-summer seasons. The promising breeding material are tested in these zones before identifying a cultivar suited to a specific zone in addition to the varieties released at state level (Basu and Rathnakumar, 2004).

Since groundnut is not native to India, the breeding efforts were initiated by introducing the popular varieties under cultivation from other countries. Purposeful introduction of improved groundnut varieties in India was made by the then Madras state government towards the end of nineteenth century. In 1884, the 'Mauritius' variety was introduced to Pondichery and Madras from Mauritius; 'Spanish' and 'Virginia' from the United States of America and 'Small Japan' and 'Large Japan' from Japan in 1901-02. Three of these varieties gradually spread over the various parts of the country with variant names viz., 'Groundnuts', 'Spanish Groundnuts' (variants of Spanish); 'Coromandal', 'Mauritius', 'Mozambique' (variants of 'Mauritius'); and 'Bold' (variant of Big Japan) and thus formed the basis of groundnut improvement programmes and research in India (Seshadri, 1962). Since then the methods of breeding common to the self-pollinated crops like introduction, mass and pure line selection hybridization and selection (pedigree, modified and bulk pedigree, single seed descent, back cross methods), mutation

breeding and interspecific hybridization were employed in developing the cultivars. Few novel breeding approaches like multiline (Norden, 1973) synthetics (Emery, 1965), modified composite cross (Hammons, 1976), convergent crosses (Norden, 1980; Wynne and Isleib, 1980; Branch, 1981), selective diallel mating system (Wynne and Isleib, 1980; 1983) have been attempted but with limited success. A comprehensive list of breeding methods employed and the varieties developed through different breeding methods (Table 14) in groundnut are discussed briefly.

Table 3. Breeding methods employed in developing groundnut varieties in India

Breeding method	**No. of varieties released**
Introduction	3
Mass selection	29
Pure line selection	23
Pedigree	107
Bulk pedigree	17
Modified Bulk Pedigree	13
Single seed decent	1
Mutation	7
Total	**200**

Future thrust areas for groundnut improvement

Most groundnut cultivation is confined to rainfed areas under low input conditions. Significant gains in yield have been achieved but they are not as spectacular as in the case of wheat, rice or maize. The following research areas could lead to further progress in yield improvement in the near future.

Number of germplasm in the world collections have increased in the recent years (>14,000), which includes all the three gene pools. Several species in different sections have been found to possess desirable agronomical, pest and disease resistance and for quality traits. Major attempts have also been made to utilize them for groundnut improvement. However, the pace of utilization and the economic end product, 'the cultivar' does not commensurate with the available resources. Although several advanced and stable breeding lines have been developed their performance when compared to the local varieties remain at par or even below. In spite of difficulties, looking in to the narrow genetic base, lack of stable and high level of resistance for diseases and pests in the cultivars, land races and cultivated germplasm of groundnut, the wild *Arachis* gene source offers unparalleled mine to harvest. At least in the near future introgression of useful genes from species of section *Arachis* should certainly find a priority in the era of high cropping intensity, growing use of pesticides and declining trend of agricultural lands. For which the suitable pre-breeding pathways integrated with available different parasexual techniques have

to be exploited. Similar to sugarcane, hotspots for enhanced natural seed set in interspecific hybrids can be identified and crosses effected. In addition choice of parents, suitable bridging species, which combine better fertility and seed set, should be identified to introgress more and more wild genes sources to the cultivated back ground. Conventional breeding and molecular marker based selection should go hand in hand for priority-specific and directed exploitation of large gene pool cutting the time and resources. Collaborative long term and multidisciplinary approach in utilizing wild gene source would be crucial for the success.

Understanding the aflatoxin biosynthesis pathway and suitable genetic modifications to check the aflatoxin production is another priority area of research. Current research at the USDA Southern Regional Research Centre in New Orleans, USA, is focused on understanding the aflatoxin biosynthesis pathway. Scientists have identified 0-methyl- sterigmatocystin as the last known precursor, and the enzyme oxidoreductase, that catalyses the conversion of this precursor to aflatoxin B1. When the gene responsible for the enzyme is located, it could be removed or altered to stop production of aflatoxin.

Other areas where recourse to bioengineering may help are resistance to *Spodoptera* and leaf miner among insect pests while bud and stem necrosis virus in case of diseases. Identification of novel resistant genes and stable integration in to appropriate agronomically superior genetic backgrounds should find a focus in the national programme.

Groundnut fodder is a valuable by product especially to the resource poor groundnut farmer with one or two draft or milch animals. Improvement of fodder quality traits is a virgin area as far as groundnut is concerned. A slight improvement in the fodder quality trait(s) will have a major impact on the animal health. Identification of traits associated with fodder quality, their inheritance and integration in to the breeding programme are to be attempted.

Groundnut crop faces stiff challenge from other cheaper sources of oils like soybean, oilpalm etc. But a wide array of products for direct consumption and diversification can be made from groundnut. Hence, confectionery quality is yet another grey area which needs to be addressed. Understanding the inheritance of nutritional traits and early selection strategies should be worked out.

Suggested Readings

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Utilization of wild *Arachis* Species for groundnut improvement

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Introduction

Groundnut *(Arachis hypogaea* L) is the most widely cultivated grain legume in the world and is one of the five most important oilseeds in India. It has important nutritional qualities, containing approximately 50% high quality unsaturated fats and 30% digestible proteins. Besides, a few wild *Arachis* species (*A. glabrata, A. kempff-marcadoi, A. pintoi and A. repens*) are being cultivated extensively as tropical forages and ground covers, mostly in South America (Valls *et al*.1994).

The species has been cultivated for nearly four centuries in Western Africa, the East Indies, India, China and Japan, however, not known to occur in wild state in these regions. Archeological evidences proved pre-Columbian (before 600 AD) cultivation of groundnut in Peru, South America and distributed in eastward of Andes Mountains from the Amazon River to the La Plata River. The center of origin of cultivated groundnut thought to lie in northern Argentina and southern Bolivia (Stalker and Simpson 1995). Besides, evidence indicates northwest Peru may be another possible site for the origin of the cultivated groundnut (Simpson *et al*. 2002).

II. Taxonomy of *Arachis Species*

The genus *Arachis* is morphologically well defined and clearly delineated from its closest relatives by the presence of geocarpic peg. *Arachis* is placed with its relatives *Stylosanthes, Chapmannia, Arthrocarpum and Pachecoa* in the subtribe *Stylosanthinae* of the tribe Aeschynumeneae on the basis of the shared morphological characters of a staminal tube with alternately attached basal and dorsal anthers, flowers in terminal or axillary spikes or small heads (which are sometimes raceme-like), pinnate leaves, and leaflets without stipules.

The only member of the genus known to science till 16th century was *A. hypogaea* described by Linnaeus (1753). There are more than 80 individual species within the genus. However, so far, 80 species have been formally described and classified into 9 sections namely, *Trierectoides, Erectoides, Extranervosae, Triseminatae, Heteranthae, Caulorrhizae, Procumbentes, Rhizomatosae* and *Arachis* (Barkley et al., 2016). Extensive collection and research have also been conducted for the wild relative species (Upadhyaya et al., 2011). The largest collections of *Arachis* germplasm are housed in International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) in India, the United States, China, and Brazil, although smaller collections do exist in many countries around the world (Barkley et al., 2016). However, taxonomic revision of the genus is one of the highest priorities for documentation and utilization since new accessions of wild species are adding up.

Based on the cross compatibility relationships, the *Arachis* gene pool has been classified into primary, secondary, and tertiary gene pools. *Arachis hypogaea* and *A. monticola* are two tetraploid species of section *Arachis* grouped under primary, while all other diploid species of section *Arachis* fall in secondary gene pool. Species that belong to section other than *Arachis* are grouped under tertiary gene pool. The gene flow among different gene pools and between different sections within tertiary gene pool is generally limited.