Genetic Resources and Crop Evolution **45:** 119–126, 1998.

Genetic variation vis-à-vis molecular polymorphism in groundnut, *Arachis hypogaea* L. *

Polymorphism in groundnut

A.K. Singh[1], J. Smartt[2], C.E. Simpson[3] & S.N. Raina[4]

[1] *Genetic Resources Division, ICRISAT Asia Center, Patancheru 502 324 Andhra Pradesh, India;* [2] *Department of Biology, University of Southampton, Southampton SO16 7PX, UK;* [3] *Texas Agricultural Experiment Station, Texas A & M University, Box 292, Stephenville, Texas 76401, USA;* [4] *Department of Botany, University of Delhi, Delhi 110 007, India*

Received 9 June 1997; accepted 11 September 1997

Key words: Arachis hypogaea, DNA markers, molecular polymorphism, variation, origin, evolution

Abstract

Recent studies on the genus *Arachis* using molecular markers have revealed very little demonstrable polymorphism in the cultivated groundnut, *A. hypogaea*. This has led to the hasty generalization that the groundnut lacks genetic variation. However, this is in complete contradiction to the results of other lines of investigations into the origin and evolution of *A. hypogaea*. Further, a characterization of the world collection for various traits also shows significant levels of variation for almost all genetic traits. The literature review in this article suggests that the lack of genetic variation was inferred because of an inadequacy in the material studied, and the range of techniques used to study molecular polymorphism. A comprehensive and rigorous examination of the material available in the groundnut world collection, either by improving current techniques, or by using such advanced techniques as SSRs and AFLP could well reveal polymorphism at the molecular level.

Introduction

Many recent investigators using such molecular marker techniques as Restriction Fragment Length Polymorphism (RFLP), and such Polymerase Chain Reaction (PCR) based marker analyses as Random Amplified Polymorphic DNA sequences (RAPDs) have concluded that there is little demonstrable polymorphism in cultivated groundnut (Kochert et al., 1991; Halward et al., 1991, 1992; Paik-Ro et al., 1992). They observed significant levels of polymorphism in the genus *Arachis* L. itself. These results led to the generalization that accessions of *A. hypogaea* lack genetic variation. This has restricted the production of polymorphic profiles using DNA molecular marker techniques, and their use in differentiating accessions, and fingerprinting germplasm. However, the morphological variation recorded during characterization and classification of world collections at ICRISAT, Texas Agricultural & Mechanical University, Instituto Nacional de Tecnologia Agropecuaria (INTA) near Cordoba, Argentina, and at Campinas, Brazil, and the fact that these accessions originate from 91 countries, representing distinct agroclimatic conditions ranging between 40° N and 40° S make it hard to agree with such generalizations based on limited DNA molecular marker analyses on the lack of polymorphism in groundnut. This paper attempts to analyze the evidence gathered in the past 3–4 decades from the groundnut world collections on the origin, evolution, phytogeographical distribution, and variation of the crop. The data available from different lines of investigations are critically discussed

* Submitted as Journal Article No. 2062 by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324, Andhra Pradesh, India

and analyzed vis-à-vis molecular marker based observations.

Limitations of molecular DNA marker assessment

There can be several reasons why little polymorphism is detected at the DNA sequence level using the commonest molecular techniques, between accessions of a species even in the presence of a significant level of variation. The major factors are:

1. The number of accessions used,
2. genetic base of the material used,
3. number of enzymes and probes (clones) used,
4. types of clone used and their ability to resolve polymorphism,
5. number of primers used for amplification and identification of polymorphic sequences, and
6. methodology and parameters used for analysis of variation between species, and within a species.

Number of accessions used. The major weakness of most investigations of groundnuts using DNA molecular marker based analysis has been a lack of in-depth, comprehensive, and rigorous examination of the material available in world collections. Most studies (Kochert et al., 1991; Halward et al., 1991, 1992; Paik-Ro et al., 1992) have used very few accessions to detect molecular polymorphism (Table 1). Though the number of accessions has been increased in subsequent studies, it is still too low to represent adequately the total variability of world germplasm collections, and to permit drawing valid conclusions on genetic diversity in the groundnut.

Genetic base of accessions used. Earlier studies were confined to cultigens originating in the USA (Kochert et al., 1991). These do not have a long history, and have their origin in the narrow genetic base introduced since the mid-16th century, predominantly from Africa, and some from central America. Subsequent studies (Halward et al., 1992), however, have attempted to include genotypes originating in a wider range of groundnut growing regions but even they are predominantly from secondary centers of diversity in South America, belonging to only three botanical varieties, *hypogaea, fastigiata*,, and *vulgaris*, of the six botanical varieties now recognized (Krapovickas and Gregory, 1994). Further, these have probably undergone several cycles of stock regeneration. This makes assessment of genetic variability difficult, particularly in a highly autogamous crop like the groundnut. Consequently, these studies have not detected the total variation that has arisen through macroevolution in the differentiation of six botanical varieties and their subtypes, and through microevolution at the agronomic level, to evolve cultivar groups and cultivars (Gibbons et al., 1972) across the secondary centers of diversity and various countries (91) where groundnut is grown.

Number of enzymes, probes (clones) and primers used. Since the plant genome is believed to contain 100,000 or more genes and repetitive sequences, the number of enzyme-probe combinations used in RFLPs; primers used in RAPDs; and clones or DNA sequences used in PCR analysis are very important in diversity studies. This becomes all the more important when one is dealing with a segmental allopolyploid species like the groundnut (which has evolved through amphidiplodization of diploid species with closely related genomes (Singh and Moss, 1982, 1984)). This however can be presumed to have a greater number of loci compared to diploid species, which are probably in the form of repeated sequences many loci, and might actually be largely redundant. However, such variation can be resolved with some of the recently developed DNA molecular marker techniques. Unfortunately, the numbers of enzyme and probe combinations, primers, and clones used in groundnut molecular marker studies have been very limited. It is difficult to visualize how a combination of 21 probes $\times$ 8 enzymes (Kochert et al., 1991); 60 probes $\times$ 13 enzymes; 29 primers and 6 nucleotide groundnut genomic clones (Halward et al., 1992); 2 enzymes $\times$ 30 probes (Paik-Ro et al., 1992) can appropriately cover the total genomic polymorphism, of a segmental allopolyploid like groundnut, unless, its genome organization is similar to crop species where such limited numbers have resolved polymorphism. In any event, failure to detect variation does not prove that it does not exist; it merely reflects the adequacy and appropriateness of the procedures used. This is particularly so in the groundnut where genetically determined morphological variation is abundant.

Types of clones used to generate data. DNA clones used as probes in plants have generally been single or very low copy versions of multilocus probes. Use of multiple restriction enzymes is required to identify most polymorphic restriction enzyme and probe combinations. Each enzyme can reveal variation at different restriction sites at or around the region at which the probe is hybridized.

Table 1. Details of number of genotypes, enzymes, probes, clones, and primers used in molecular marker studies on groundnut

Reference	No. of accessions	No. of botanical varieties	No. of countries	Number of enzymes	clones/ probes	primers
Kochert et al., 1991	8	3	1	8	21 (7)[1]	–
Halward et al., 1995	27	4	10	13 (4)[2]	60 (3)[1]	10
Halward et al., 1992	26	4	11	–	–	10
Paik-Ro et al., 1992	14	3	1	2	16+7	–

[1] Polymorphic probes (clones).
[2] Four cutter enzymes.

Methodology used for analysis of polymorphism. Finally, the general inference of these studies has been that there is significant polymorphism, within the genus *Arachis* but not in the accessions of *A. hypogaea* when the same molecular marker techniques and methods of analysis are used. This is hardly surprising if one uses the same set of techniques and methods of analysis to study variation between species in a genus on the one hand, and between cultivars, landraces, and breeding lines of a single species on the other. Interspecific levels of variation cannot be equated with variation at the level of infraspecific taxa. Clearly, while the diagnostic value of the procedures used is high in making distinctions between species, these procedures, rather unsurprisingly, are insensitive to variation within species. Such inappropriate use of procedures leads to quite unwarranted conclusions.

Evidence of variation from other lines of investigations. Like other crops, groundnut too has produced variation during its evolution (Brassica, Kresovich et al., 1992; Maize, Smith et al., 1991; Soybean, Cregan et al., 1990; Rice, Wang and Tanksley, 1989). Significant levels of genotypic and phenotypic variability are also found in the form of differences in various botanical and agronomic features, and responses to stress factors (Singh and Nigam, 1997). So much so, that the species *A. hypogaea* has been divided into two subspecies, and six botanical varieties and two agronomic types using conventional taxonomic principles (Krapovickas and Gregory, 1994). These botanical varieties have been further broken down into different cultivar groups in different regions/countries (Gibbons et al., 1972). In this context, it is important to appreciate the origin and evolution of this variation relative to time span, space, and environment.

Origin and differentiation at interspecific levels. *Arachis hypogaea* probably originated in the region of southern Bolivia and northeast Argentina (Krapovickas, 1969), about 25° S. The climatic conditions and topography of this region are among the most variable in the world. Though no one is certain when the domestication of the groundnut occurred, there is archeological evidence to suggest groundnut cultivation prior to that of maize at Huaca Prieta. Groundnuts do not appear in pre-ceramic refuse, but appear to have been introduced in association with the first pottery. Carbon dating from this period, and therefore groundnut, ranges from 1200–1500 BC (Hammons, 1994). Archeological evidence indicates much variation in groundnuts found in coastal Peru, and at Supe (Hammons, 1994). The wide range of environmental selection pressures has resulted in the evolution of very distinct types. In southern Bolivia and northern Argentina two subspecies (ssp.), *hypogaea* and *fastigiata* Waldron have been found which have significant genetic differences in their habit, branching pattern, and such botanical features as stem, leaf and fruit; they differ physiologically in relation to their maturity period, water use efficiency and partitioning of photosynthates. These differences suggest that significant levels of variation do in fact occur. Additionally, there is another tetraploid species in this region, *A. monticola* Krapov. & Rigoni, considered to be the groundnut prototype and biosystematically a wild form of *A.*

hypogaea (Singh and Moss, 1982, 1984). Therefore, there are no strong reasons to expect that the groundnut originating in this region will show a lesser degree of polymorphism than any other comparable crop species.

Differentiation and evolution at the intraspecific level. From its origin, groundnut travelled to other parts of South America with human movement, extending between the equator and 35° S. This resulted in further diversification and genetic polymorphism in growth habit, vigor, stem, leaf, seed and pod characteristics; and a physiological polymorphism in maturation period, water use efficiency and in translocation of photosynthate to seeds through partitioning. Various physical and genetic pressures in this part of the continent contributed to further variation, and the evolution of six botanical varieties in seven distinct agroclimatic zones – var. *fastigiata* Krapov. & W.C. Gregory and var. *vulgaris* C. Harz. in the Guarani regions of southern Brazil, Uruguay and Paraguay, and in Goias and Minas Geraes regions of Brazil; type *nambyquarae* Hoehne of var. *hypogaea* in Rondonia and northwestern Mato Grosso of Brazil; predominantly var. *hypogaea* and some var. *fastigiata* in the eastern foothills in the Andes in Bolivia; var. *hypogaea*, var. *hirsuta* Kohler, var. *fastigiata* and var. *peruviana* Krapov. & W.C. Gregory in Peru and Ecuador; var. *fastigiata* and var. *vulgaris* in northeastern Brazil, and var. *aequatoriana* Krapov. & W.C. Gregory in Ecuador. This demonstrates the evolution of specific botanical types associated with specific regions, and presumably that selection responses in the various agroclimatic zones through which groundnut has migrated have produced significant levels of variation in response to those selection pressures.

Diversification and differentiation at varietal levels. From the east coast of South America, the groundnut was taken to Africa, India, and the Far East by the Portuguese; and the Spaniards took it from the west coast of South America to Indonesia, China and up to Madagascar in the sixteenth century (Krapovickas, 1995). Later it was introduced to North America and other parts of the world, and became established as an important crop in most tropical and sub-tropical countries of the world. The variable climate of the secondary centers of diversity, and that of countries to which the crop was introduced, together with human selection pressures for agriculturally adaptive features, ensured further variation in groundnut at the microevolutionary level, and resulted in a very large number of cultivars and cultivar groups in South America and other parts of the world.

Available variation in botanical characters. Growth habit in groundnut varies from the procumbent runner type with a short or long main axis and horizontally running laterals, to the decumbent type where laterals ascend to differing degrees. In ssp. *fastigiata* they are strongly erect, while ssp. *hypogaea* comprises mostly of spreading types with some which are strongly ascending. One of the most consistent differences is that the accessions belonging to ssp. *hypogaea* have no inflorescences on the main axis, while those of ssp. *fastigiata* do. The height of the main axis varies from 15–38 cm, but in var. *hirsuta* it may be greater. There is further variation in the distribution of reproductive and vegetative branches, which can basically be alternate or sequential. Many accessions, however, show a mixture of alternate and sequential growth both within and between plants.

Similarly there is profound variation in depth of color of leaf (light to dark green) and flower (usually orange or yellow to white to brick red). An unstable flower color has also been observed, for which a transposon has been implicated (Dwivedi et al., 1996).

Known variation in agronomic characters. There is much variation between accessions of groundnut in yield, and such yield components as total biomass production, partitioning of photosynthate, thickness of the pod wall, seed size, and the nutritional quality of the seed. Pod yield can vary from 500 kg ha^{-1} in some Peruvian landraces to 11000 kg ha^{-1} in improved cultivars developed in China (Yanhao Sun and Caibin Wang, 1990). Variation in pod and seed characters is enormous – shelling percentage varies from 35–75%, the pods may have deep or almost no constrictions from prominently reticulated to an essentially smooth pod surface, from a distinct beak to no beak, and pod length from 1 to 9 cm. Single seed weight varies from 20–25 mg each, to 1.36 g. More than 20 different basic seed coat colors and combinations occurring in blotches, flecks, hazy suffusions and stripes can be found. Chemically, oil content varies from 31.8–53.1% in *vulgaris*, 32.2–55.0% in *fastigiata*, 32.3–51.5% in *hypogaea* type bunch and 34.4–50.3% in *hypogaea* type runner. Protein content does not vary much between accessions of a botanical variety, but between botanical varieties it ranges from 16.1–34%.

There is also variation in maturation period and seed dormancy; generally accessions belonging to ssp.

Table 2. Range of variation in cultivated groundnut observed at ICRISAT Asia Center, Patancheru, India

Character	Minimum	Maximum	Intermediate(s)
Life form	Annual	–	–
Growth habit	Erect	Procumbent	Decumbent
Branching pattern	Sequential	Alternate	Irregular
Stem pigmentation	Absent	Present	–
Stem hairiness	Glabrous	Woolly	Hairy, very hairy
Reproductive branch length	> 1 cm	10 cm	Continuous
No. of flowers/ inflorescence	1	5	2, 3, 4
Peg color	Absent	Present	–
Standard petal color	Yellow	Garnet orange	Lemon yellow, light orange, dark orange, orange
Standard petal markings	Yellow	Garnet	Lemon yellow, light orange, dark orange, orange
Leaf color	Yellowish	Dark green	Light green, green, bottle green
Leaflet length	17 mm	94 mm	Continuous
Leaflet width	7 mm	52 mm	Continuous
Leaflet L/W ratio	1	6	Continuous
Leaflet shape	Suborbicular	Linear	Elliptic, ovate, lanceolate, obovate, oblong
Leaflet hairiness	Subglabrous	Profuse and long	Scarce and short, scarce and long, profuse and short
No. of seeds/pod	1	5	2, 3, 4
Pod beak	Absent	V. Prominent	Slight, moderate, prominent
Pod constriction	Absent	Very deep	Slight, moderate, deep
Pod reticulation	Smooth	Prominent	Slight, moderate
Pod length	14 mm	65 mm	Continuous
Pod width	7 mm	20 mm	Continuous
Seed color pattern	One	Variegated	
Seed color	White	Dark purple	Yellow, shades of tan, rose, shades of red, grayed orange, shades of purple
Seed length	4 mm	23 mm	Continuous
Seed width	5 mm	13 mm	Continuous
100-seed mass	14 g	140 g	Continuous
Days to emergence	4	18	Continuous
Days to 50% flowering	15	54	Continuous
Days to maturity	75	> 155	Continuous
Fresh seed dormancy	0 days	> 66 days	Continuous
Oil content	31.8%	55.0%	Continuous
Protein content	15.8	34.2	Continuous

hypogaea require a long (140–160 days) to medium (120–140 days) period to mature, while those belonging to ssp. *fastigiata* are of short duration (90–120 days). Table 2 summarizes the range of variability recorded at ICRISAT for various plant, pod and seed characters in the world collection of 14,000 accessions.

Known variation for biotic and abiotic stresses. Three foliar diseases, late leaf spot, early leaf spot, and rust, are the most widely distributed and economically important diseases of groundnut. At ICRISAT over 13,000 accessions have been screened resulting in the identification of 167 lines resistant to rust, 69 to late leaf spot, and 12 for early leaf spot (Singh et al., 1997). Groundnut is attacked by several virus diseases, and variation in resistance to almost all virus diseases exists. This has led to the identification of sources of resistance for groundnut rosette virus in landraces from Burkina Faso (de Berchoux, 1960), for peanut bud necrosis virus in several lines at ICRISAT (Dwivedi et al., 1995), for peanut mottle virus in several lines with low yield losses (ICRISAT, 1983), and for tomato spotted wilt virus in breeding lines from the USA (Culbreath et al., 1994). Variation in reaction to soilborne diseases has also been recorded. For bacterial wilt resistance, lines originating from China and Indonesia have been identified (Mehan and Liao, 1994). Resistance has also been identified to black rot (Green et al., 1983), to *Sclerotium rolfsii* (Smith et al., 1989), and for aflatoxin contamination of pre-harvest seed, *in-vitro* seed colonization and aflatoxin production (Mehan et al., 1991).

The groundnut suffers yield losses due to insects and arachnids feeding on leaves, pegs, pods and seeds. Sources of resistance to most insect pest have been identified both in *A. hypogaea* and wild *Arachis* species (Wightman and Ranga Rao, 1994). Variation for resistance to root-knot nematode has even been found associated with RAPD markers (Burow et al., 1996). Variability for physiological traits, such as resistance to drought occurring at various stages of crop growth, crop growth rate, water use efficiency, and partitioning have also been recorded (Rao et al., 1994).

Intraspecific genome size and DNA variation. Mean 2C DNA amount in groundnut varied by 15–20%, ranging from 10.26 pg to 11.82 pg between accessions. Accessions belonging to ssp. *hypogaea* (mean value 11.27 pg) had significantly higher DNA contents than accessions of ssp. *fastigiata* (mean value 10.97 pg) (Singh et al., 1996). Similarly *A. hypogaea* accessions exhibited considerable variation in their r DNA unit length, which incidently are of same class as that found in other species (Singh et al., 1997). Recently Guahao He and Prakash (personal communication) have recorded significant levels of polymorphism using AFLP and DNA Amplification Fingerprinting (DAF) techniques. In DAF they recorded 3.7% polymorphic bands from 559 primers, while in AFLP 6.7% from 64 primer combinations were recorded.

Conclusions

The analysis of literature on recorded variation in the cultivated groundnut in its botanical and agronomic features, reaction to various biotic and abiotic stresses, and in genome size, r DNA, DAF and AFLP in the light of the crop's origin and evolution indicates significant levels of genetic and physiological variation. On this basis the species has been divided taxonomically into two subspecies, six botanical varieties, two agronomic types, and numerous cultivar groups and varieties distributed around the world. Given this, it would be difficult to accept that the species DNA is devoid of molecular variation. Since most morphological variation is coded in the genes, and polymorphic genes are probably not equally dispersed throughout the genome, it is reasonable to conclude that there is probably variation at the DNA molecular level, but that the DNA molecular techniques used have bot been able to detect this polymorphism because of the complex nature of the groundnut genome. This is probably because of small chromosome size, multiallelic loci, the presence of much repetitive and redundant DNA, and functional sequences being confined to a small region, limited efforts devoted to appropriate development and refinement of the techniques used, and lack of adequate exploration of more recently developed techniques that have been found to be more powerful in detecting such molecular variation.

Suggestions for future research

Since it is highly probable that molecular genetic variation in groundnut does exist, either the RFLP and RAPD techniques used currently require further refinement, or a greater number of accessions representing the total spectrum of variation in an *A. hypogaea* core collection should be used. In RFLP studies more restriction enzymes should be used with

many probes, while in RAPD more primers should be screened to identify an adequate number of polymorphic primers to provide a better coverage of the *A. hypogaea* genome. Greater use of gene specific probes and primers is needed to discern the expected polymorphism at the gene level for certain genes, e.g. those controlling anthocyanin development. If the above attempts fails then advanced techniques such as Amplified Fragment Length Polymorphic Sequences (AFLPs), Simple Sequence Repeats (SSRs), Cleavable Amplified Polymorphic Sequences (CAPs) and Single Nucleotide Polymorphism-Genetic Bit Analysis (SNP-GBAs) should be exploited. Genetic studies in several crop species have shown that variation in relation to different traits in some crops may be accounted for by the variation in a comparatively smaller number of specific genes than in others, in which the number of alleles for specific genes contribute significantly. Therefore, particular techniques may not be equally effective for detecting variation. One has to select appropriate techniques based on the genetic nature of each crop species. Interspersed repetitive DNA sequence elements have been found to be characteristic of eukaryotes. Variable numbers of non-coding DNA sequences and pattern tandem repeats include those loci known as hypervariable regions, i.e., minisatellites and microsatellites. Tandemly repetitive DNA sequences show a range of repeat unit size, number, and pattern. Therefore, these sequences present a rich source of highly polymorphic multiallelic, stable, and widely dispersed markers. In the Variable Number of Tandem Repeat Sequence (VNTRs) loci, DNA sequences flanking the core unit are conserved, allowing the design of PCR primers that will amplify intervening sequences at specific loci in all genotypes of target species. In this way the potential amount of information acquired by use of interspersed repetitive DNA markers is increased. Therefore, simple sequence repeats (microsatellites) may be ideal for distinguishing closely related genotypes in the groundnut, because multiple alleles often occur at numerous loci. If this proves unsatisfactory, AFLP markers are another powerful new type of DNA marker that allows a large number of DNA loci to be screened for polymorphism. This versatile technique can detect the presence of restriction fragments in almost any DNA, regardless of its complexity. Therefore, SSRs and AFLP markers are probably ideal for distinguishing closely related genotypes in the groundnut. SSR marker assays can utilize specific DNA primers that amplify a DNA region containing variable repeats, while AFLP can allow a large number of DNA loci to be screened for polymorphism in DNA fragments. AFLP can complement the information obtained from SSR analysis.

References

Burow, M.D., C.E. Simpson, A.H. Peterson & J.L. Starr, 1996. Identification of peanut (*Arachis hypogaea* L.) RAPD marker diagnostic of root-knot nematode [*Meloidogyne arenaria* (Neal) Chitwood] resistance. Molecular Breeding 2: 369–379.

Cregan, P.B., B.F. Matthews, R.E. Tully & J.M. Weisemann, 1990. DNA finger-printing as a tool to determine genetic distance among soybean genotypes. Am. Soc. Agron. Abstr. p. 84.

Culbreath, A.K., J.W. Todd, D.W. Branch, C.C. Gorbet, W.F. Holbrok & J.W. Demski, 1994. Variation in susceptibility to Tomato Spotted Wilt Virus among peanut genotypes. In: Proceedings of American Peanut Research and Education Society Inc., p. 49.

de Berchoux, C., 1960. La rosette de l'arachide en Haute Volta. Comportement des lignes resistantes. Oleagineux 15: 237–239.

Dwivedi, S.L., S.N. Nigam, D.V.R. Reddy, A.S. Reddy & G.V. Ranga Rao, 1995. Progress in breeding groundnut varieties resistant to peanut bud necrosis virus and its vector. In: Recent studies on peanut bud necrosis disease, pp. 35–40. Proceedings of a meeting, 20 March 1995, ICRISAT Asia Center, India Patancheru 502 324, Andhra Pradesh, India.

Dwivedi, S.L., A.K. Singh & S.N. Nigam, 1996. Unstable white flower color in groundnut (*Arachis hypogaea* L.). J. Heredity 87: 247–248.

Gibbons, R.W., A.H. Bunting & J. Smartt, 1972. The classification of varieties of groundnut (*Arachis hypogaea* L.). Euphytica 21: 78–85.

Green, C.C., M.K. Bute & J.C. Wynne, 1983. A comparison of methods of evaluating resistance to *Cylindrocladium crotalariae* in field tests. Peanut Sci. 10: 66–69.

Hammons, R.O., 1994. The origin and history of groundnut. In: J. Smartt (Ed.) The groundnut crop: A scientific basis for improvement, pp. 24–42. Chapman & Hall, London.

Halward, T., H.T. Stalker, E.A. Larue & G. Kochert, 1991. Genetic variation detectable with molecular markers among unadapted germplasm resources of cultivated peanut and related wild species. Genome 34: 1013–1020.

Halward, T., H.T. Stalker, E. Lerue & G. Kochert, 1992. Use of single-primer DNA amplification in genetic studies of peanut (*Arachis hypogaea* L.). Plant Mol. Biol. 18: 315–325.

ICRISAT (International Crops Research Institute for the Semi-Arid Tropics), 1983. Annual Report 1982. ICRISAT, Patancheru, 502 324, Andhra Pradesh, India.

Kochert, G., T. Halward, W.D. Branch & C.E. Simpson, 1991. RFLP variability in peanut (*Arachis hypogaea* L.) cultivars and wild species. Theor. Appl. Genet. 81: 565–570.

Krapovickas, A. & W.C. Gregory, 1994. Taxonomia del *Arachis* (*Leguminosae*). Bonplandia VIII: 1–187.

Krapovickas, A., 1969. The origin, variability and spread of groundnut (*Arachis hypogaea* L.). In: R.J. Ucko & C.W. Dimbleby (Eds) The domestication and exploitation of plants and animals, pp. 427–440. Duckworth, London.

Krapovickas, A., 1995. Disertacion del Academico Correspondiente Ing. Agr. Antonio Krapovickas. El origin y dispersion de las variedades del mani. Adhesion al 75 Aniversario de la Fundacion de la Facultad de Agricultura, Ganaderia e Industrias Africs – Corruentes. Academia Nacional de Agronomia y Veterinaria Tomo XLIX: 18–26 Buenos Aires, Argentina.

Kresovich, S., J.G.K. Williams, J.R. McFerson, E.J. Routman & B.A. Schaal, 1992. Characterization of genetic identities and relationships of *Brassica oleracea* L. via a random amplified polymorphic DNA assay. Theor. Appl. Genet. 85: 190–196.

Mehan, V.K. & B.S. Liao, 1994. Groundnut bacterial wilt past, present and future. In: Groundnut bacterial wilt in Asia: Proceedings of Third Workshop Group Meeting, 4–5 July 1994, pp. 67–90. Oil Crops Research Institute Wuhan, China. ICRISAT. Patancheru 502 324, Andhra Pradesh, India.

Mehan, V.K., A. Ba, D. McDonald, J.A. Renard, R.C.N. Rao & S. Jayanthi, 1991. Field screening of groundnuts for resistance to seed infection by *Aspergillus flavus*. Oleagineux 46: 109–115.

Paik-Ro, O.G., R.L. Smith & D.A. Knauft, 1992. Restriction fragment length polymorphism evaluation of six peanut species within the *Arachis* section. Theor. Appl. Genet. 84: 201–208.

Rao, R.C.N., A.K. Singh, L.J. Reddy & S.N. Nigam, 1994. Prospects for utilization of genotypic variability for yield improvement in groundnut. J. Oilseeds Res. 11: 259–268.

Singh, A.K. & J.P. Moss, 1982. Utilization of wild relatives in the genetic improvement of *A. hypogaea* L. 2. Chromosome complements of species of section *Arachis*. Theor. Appl. Genet. 61: 305–314.

Singh, A.K. & J.P. Moss, 1984. Utilization of wild relatives in the genetic improvement of *A. hypogaea* L. 5. Genome analysis in section *Arachis* and its implications in gene transfer. Theor. Appl. Genet. 68: 355–364.

Singh, A.K. & S.N. Nigam, 1997. Groundnut. In: Fuccillo, Stapleton & Sears (Eds) Biodiversity in trust: Conservation and use of plant genetic resources in CGIAR centers, pp. 113–135. Cambridge University Press, U.K.

Singh, A.K., V.K. Mehan & S.N. Nigam, 1997. Sources of resistance to groundnut fungal and bacterial diseases, an update and appraisal. Information Bulletin no. 50. ICRISAT, Patancheru 502 324, Andhra Pradesh, India.

Singh, K.P., S.N. Raina & A.K. Singh, 1996. Variation in chromosomal DNA associated with the evolution of *Arachis* species. Genome 39: 890–897.

Singh, K.P., S.N. Raina, A.K. Singh & V. Ogihara, 1997. Characterization and heritability of the nuclear ribosomal DNA units and phylogeny of *Arachis* L. Wild forms and cultivated peanuts. Theor. Appl. Genet. (communicated).

Smith, J.S.C. & O.S. Smith, 1991. Restriction fragment length polymorphism can differentiate among U.S. maize hybrids. Crop Sci. 31: 893–899.

Smith, O.D., T.E. Bosewell, W.J. Gricher & C.E. Simpson, 1989. Reaction of selected peanut (*Arachis hypogaea* L.) lines to southern stem rot and Pythium pod rot under varied disease pressure. Peanut Sci. 16: 9–13.

Wangs, Z.Y. & S.D. Tanksley, 1991. Restriction fragment length polymorphism in *Oryza sativa* L. Genome 32: 1113–1118.

Wightman, J.A. & G.V. Ranga Rao, 1994. Groundnut pests. In: J. Smartt (Ed.) The groundnut crop: A scientific basis for improvement, pp. 396–469. Chapman & Hall, London.

Yanhao Sun & Caibin Wang, 1990. Factors contributing to high yields of groundnut in Shandong, China. International *Arachis* Newsletter 8: 7–8.