

holdings in Kalahasti malady endemic areas of Chittoor, Nellore and Prakasam districts varied from 3.6 to 4.1 t ha<sup>-1</sup>, with a mean of 3.7 t ha<sup>-1</sup>, an increase of 22% over JL 24. The mean pod yield in JL 24 was 3.1 t ha<sup>-1</sup>. In north-coastal and northern Telangana districts where excess vegetative growth due to high rainfall is a problem in the rainy season, the performance of Kalahasti was encouraging. It produced a pod yield of 1.4 t ha<sup>-1</sup> which was 28% higher than that of JL 24 or the local variety.

Kalahasti is a short-duration (105–110 days), high-yielding, Kalahasti malady resistant, spanish bunch variety. Its distinguishing morphological features are: plant height 22–25 cm, sequential branching pattern, short internodes, and short, broad obovate dark green leaflets. Pods are medium in size (100-pod mass ranges between 108 and 142 g, 100-seed mass ranges between 42 and 46 g) with shallow constriction, slight reticulation, and moderate beak. Shelling outturn is 74 to 76%. Seeds have red testa and contain 52% oil.

Kalahasti is recommended for postrainy season cultivation especially in Kalahasti malady endemic areas. It is suitable for rainy season cultivation in high rainfall areas of north-coastal and northern Telangana districts of Andhra Pradesh. For better pod-filling in this variety, gypsum application is essential at full bloom stage. A post-sowing irrigation is also needed to ensure uniform germination because of the high moisture requirement of this variety for germination.

## Biotechnology

### Genetic Relationship Among *Arachis* Species Based on Molecular Data

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The origins of modern *Arachis* can be traced to the valleys of South America, in the Brazil-Paraguay region (Simpson et al. 2001) where it is distributed even today. Cultivated groundnut (*Arachis hypogaea*) shows great morphological variability but limited molecular polymorphism (Dwivedi et al. 2001). Based on morphological characters and cross compatibility relationships, Krapovickas and Gregory (1994) classified the genus *Arachis* into nine sections.

Traditionally morphological and agronomic traits have been used to measure genetic diversity but most of the vegetative characteristics are influenced by environmental factors, show continuous variation and have high degree of plasticity. In an attempt to overcome these problems, biochemical and molecular techniques have been used to assess genetic and taxonomic relationships. For such studies a flexible and reliable marker system to detect high levels of polymorphism is required. Galgaro et al. (1998) based on restricted fragment length polymorphism (RFLP) data have shown that sections *Arachis* and *Extranervosae* form two clearly defined groups and sections *Heteranthae*, *Caulorrhizae* and *Triseminatae* form the third group. Gimenes et al. (2002) used amplified fragment length polymorphism (AFLP) to study genetic relationships among *Arachis* species. Their study grouped section *Arachis* species together with *A. glandulifera* showing distant relationship between *A. hypogaea* and the A and B genome species. Species from section *Erectoides* grouped with *A. glabrata* (section *Rhizomatosae*) and *A. rigoni* (section *Procumbentes*) showed close relationship with *A. dardani* (section *Heteranthae*).

Amongst the different types of markers, randomly amplified polymorphic DNA (RAPD) markers are easy to use and do not need sequence data. These are also economical and do not need expensive kits or equipment. The RAPDs can produce multiple bands using a single primer; thus a relatively small number of primers can be used to generate a very large number of fragments. These fragments are usually generated from different regions of the genome and hence multiple loci may be examined very quickly. The sequence changes in genomic DNA may result in a change in the pattern of amplification products following agarose gel electrophoresis. This makes RAPD a very powerful technique for screening populations for sequence diversity as well as plant diversity analysis. RAPD markers have been used in evolutionary studies of wild species from section *Arachis* (Halward et al. 1992) and in the creation of genetic linkage map (Halward et al. 1993). These have also been used to distinguish seventeen wild species from five sections of *Arachis* and cultivated groundnut *A. hypogaea* and introgression of alien genes in wide crosses (Fennell 1994, Mallikarjuna 2002).

Thirty-two accessions of wild species of *Arachis*, belonging to twenty-five species and grouped under six sections, including *A. hypogaea* were used to study their genetic relationship using RAPDs. Twenty-nine primers belonging to OPH 1-20 and OPM 1-9 were used in this study. All the primers showed polymorphic bands, with

the number of bands per locus varying from 5 to 33. Pair-wise similarities ( $S_{ij}$ ) between accessions (i and j) were estimated using Jaccard similarity coefficient (Jaccard 1908). A dendrogram was constructed (Fig. 1) based on the  $S_{ij}$  values using clustering technique of unweighted pair group method of arithmetic means (UPGMA) (Sneath and Sokal 1973). Similarity values ( $S_{ij}$ ) for 464 pair-wise comparisons among 32 accessions ranged from 0 to 49%, with an average of 15%.

*Arachis hypogaea* grouped with *A. monticola*, a tetraploid wild species from section *Arachis*. The A genome was represented by many diploid species including *A. stenosperma*, B genome by *A. batizocoi* (Singh and Moss 1982), *A. ipaensis*, *A. hoehnei*, *A. valida* and *A. magna* (Milla 2003), and D genome by *A. glandulifera* (Stalker and Moss 1987). *Arachis stenosperma* accessions grouped together. Wild species from section *Arachis* with the B genome formed two

clusters, with one cluster having *A. batizocoi* showing distant relationship and the other cluster with *A. hoehnei* showing close relationship. The D genome accession *A. glandulifera* remained apart. Most of the wild species grouped according to their expected relationship with each other, based on crossability (Nalini Mallikarjuna and Bramel 2001) and morphological characters (Krapovickas and Gregory 1994). But accessions of *A. cardenasii* (ICGs 11558 and 11559) from section *Arachis* did not group with any of the A, B or D genome species from section *Arachis* and with each other.

The RAPDs were used to distinguish species belonging to different sections of *Arachis*. Although more than 200 simple sequence repeat (SSR) markers have been developed for *Arachis* (ME Ferguson, ICRISAT, Kenya, personal communication), there is no information that they would identify different species belonging to different sections.

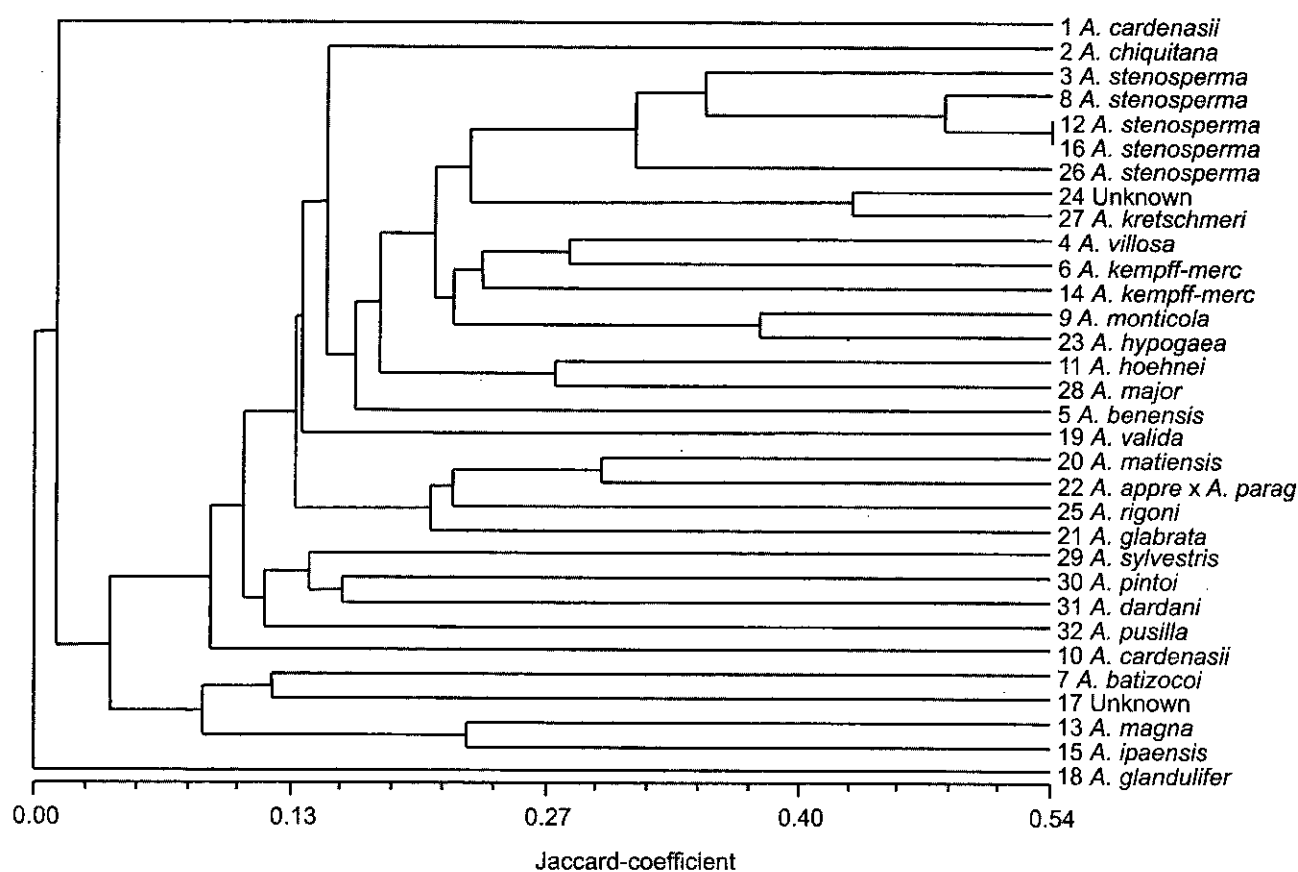


Figure 1. UPGMA-based dendrogram of *Arachis* species prepared from RAPD data. (Note: 22 refers to *A. appressipila* × *A. paraguariensis*.)

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## AFLP Diversity Among Selected Rosette Resistant Groundnut Germplasm

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Groundnut rosette is the most destructive disease of groundnut (*Arachis hypogaea*) in Africa. It is sporadic and unpredictable but causes significant loss in groundnut in years of epidemics (Naidu et al. 1999). Three synergistic agents cause rosette disease: groundnut rosette virus (GRV), a satellite RNA of GRV and groundnut rosette assistor virus (GRAV) (Bock et al. 1990). All three agents need to be present in the plants for aphid (*Aphis craccivora*) transmission. Resistance to groundnut rosette has been detected in 116 accessions of *A. hypogaea*. These accessions possess resistance to GRV but are susceptible to GRAV (Subrahmanyam et al. 1998). A few rosette resistant accessions are also resistant to *Aphis craccivora* (Padagham et al. 1990, Minja et al. 1999). These represent a wide range of biotypes and landraces from Latin America, Africa, and Asia, but their genetic relationships are not known.

Molecular marker-based diversity estimates are useful to select diverse lines for developing populations that may be used for mapping studies to identify DNA markers linked with resistance to rosette in groundnut. Nine amplified fragment length polymorphism (AFLP) assays (Vos et al. 1995), using primer pairs E-ACA + M-CAA, E-ACA + M-CAG, E-AGC + M-CTG, E-AGC + M-CTA, E-ACT + M-CAG, E-ACC + M-CAG, E-ACC + M-CAA, E-AAC + M-CTG and E-AAC + M-CAG, were performed on nine rosette resistant (ICGs 3436, 6323, 6466, 9558, 9723, 10347, 11044, 11968 and 12876) and one susceptible (ICG 7827) groundnut accessions. Young leaves from 2-week old plants were bulk harvested for each accession and immediately placed in liquid nitrogen for DNA extraction. DNA was extracted using the CTAB method (Saghai-Maroo et al. 1984). The concentration of DNA was assessed by spectrophotometer analyses, and the quality by gel electrophoresis using 0.8% agarose with a known concentration of uncut lambda DNA. 500 ng of genomic DNA was double digested with *EcoR* 1 and *Mse* 1 in a restriction buffer in a total volume of 15 µl. *Mse* 1 and *EcoR* 1 adapters were subsequently ligated to digested