EVALUATION OF MINI CORE SET OF GERMPLASM IN GROUNDNUT (*Arachis hyposgaea* L.)

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I. INTRODUCTION

Groundnut is one of the most important food crops of the world. It is the world's fourth most important source of edible oil and third most important source of vegetable protein (Anonymous, 2000). It is cultivated in 100 countries located between 40^0 N to 40^0 S with a world production of 36.1 million tons from an area of 26.4 m. ha with productivity of 1.4 metric tons/ha (FAO, 2004). Of the total world production, 67 per cent is in Asia, 25.13 per cent in Africa, 5.93 per cent in North America, 2.02 per cent in South America and 0.3 per cent in Europe. In India groundnut is grown on 5.7 million ha with a production of 4.7 million tons, with an average productivity of 0.8 tons / ha during the rainy season and in the post-rainy season it is grown on 0.9 m. ha with a production of 1.5 million tons and an average productivity of 1.6 t / ha. Within India, the principal groundnut growing states are Gujarat, Andhra pradesh, Karnataka, Tamilnadu and Maharastra which accounts for more than 80 per cent of the all India production as well as area.

Arachis belongs to the family Fabaceae, tribe Aeschynomeneae and sub tribe Stylosanthinae. It is an allotetraploid (2n=40) with two genomes, A and B. Linnaeous described the cultivated species in 1753 as *Arachis* (derived from the Greek " arachos" meaning a weed) *hypogaea* (meaning an underground chamber) or in botanical terms, a weed with fruits produced below the soil. It is believed to have originated in the Bolivian region of South America where the greatest diversity is found (Krapovickas 1969; Gregory and Gregory 1976). The gene pool of cultivated groundnut is divided into two subspecies *fastigiata* and *hypogaea*. The subsp. *fastigiata* is subdivided into four botanical varieties, *fastigiata, peruviana, aequatoriana* and *vulgaris* whereas, subsp. *hypogaea* includes varieties *hypogaea* and *hirsuta* (Krapovickas and Gregory, 1994).

For a crop improvement program, it is a pre-requisite to maintain the genetic variability that allows identification of promising genes in the germplasm collection that can be incorporated in the breeding programs to develop promising cultivars. Germplasm collection contains a vast reservoir of genetic variability, which would help to broaden the genetic base of the cultivars. The utilization of exotic germplasm resources in the breeding programs also enhances the diversity of cultivars. The characterization of diversity in germplasm collection is important to plant breeders to utilize and to the gene bank curators to manage the collection efficiently and effectively. Though germplasm collection contains a great amount of genetic diversity, it has not been extensively used in cultivar development (Knauft and Gorbet 1989). Ironically, the size of the collection has limited its use because of the costs associated with screening all the accessions for traits that could be used in cultivars development and nonavailability of descriptive characters and uncertainty about the best evaluation methods for tapping germplasm resources. Utilization of the collection could be enhanced by the development of more efficient evaluation techniques.

To overcome this problem Frankel (1984) designed the strategy of core collection. A core collection is a subset of accessions from the entire collection that captures most of available genetic diversity of the species (Brown, 1989a). Core collections are becoming important tools to enhance utilization of genetic resources in crop improvement programs. Frankel and Brown (1984) described the method to select core collection using information on the origin and characters of the accessions. In setting a core collection the first issue is its size, Brown (1989a) using sampling theory of selectively neutral alleles argued that the entries in a core collection should be about ten per cent of the total collection which retains nearly 70 per cent of the alleles of the entire collection. The second issue is the degree of genetic similarity among the accessions and determining groups within the entire collection, hierarchy of grouping within groups as suggested by taxonomy (species, sub species, races) followed by assigning accessions to major geographical groups (country, state), climate or agro ecological regions.

There are several ways a peanut core collection may be useful. A core collection can serve as a logical and efficient starting point for projects screening the germplasm collection for source of desirable alleles. This information would then be used to determine which clusters should be more extensively examined. A core collection also provides a logical subset of germplasm to examine when it is not feasible to examine to entire collection. The development of peanut core collection will also result in a subset of accessions that is

representitative of the entire collection and for which larger amount of seed is readily available. The peanut core collection has been very effective in enhancing the utilization of peanut genetic resources for oligogenic-governed characters. However, an even smaller subset of germplasm is needed for traits that are difficult and/or expensive to measure. These traits also display genotype x environment interaction and multi location testing should be considered to identify useful parents. The problem is how to reduce the size of core subset further without loosing the spectrum of species diversity. Upadhyaya and Ortiz (2001) suggested a strategy for sampling entire and core collections for developing a mini core subset, that contains about 1 per cent of total accessions but is representative of the diversity of the collection. The mini core collection, because of its drastically reduced size, can be evaluated extensively to select useful parents. Mini core collection can be used to improve the efficiency of identifying desirable traits in the core collection.

Knowledge of genetic diversity in a crop species is fundamental to its improvement. A variety of molecular and morphological descriptors are used to characterize the genetic diversity among and with in crop species. Substantial diversity exists among cultivated peanut genotypes for various morphological, physiological and agronomic traits (Stalker, 1992). Molecular marker techniques including random amplified polymorphic DNA (RAPD) and restriction fragment length polymorphism (RFLP) have found no or very low polymorphism in cultivated types to abundant polymorphism in wild Arachis (Kochert *et al*., 1991; Halward *et al*., 1992; Paik-Ro *et al*., 1992). However, recent studies revealed polymorphism in the cultivated groundnut using amplified fragment length polymorphism (AFLP) (He and Prakash, 1997), RAPD (Bhagwat *et al*., 1997, Subrahmanian *et al*., 2000) and simple sequence repeat (SSR) (Hopkins *et al*., 1999) assays. The low level of polymorphic variation in groundnut is attributed to its recent origin from a single polyploidization event in the evolutionary time scale (Young *et al*., 1996). This urges the need for studying more germplasm to assess diversity. The present investigation was under taken with an aim to characterize and evaluate mini core subset of groundnut with the following objectives.

1. To evaluate mini core for productivity and confectionery traits besides reaction to *Phaeoisariopsis personata* (late leaf spot) and *Aspergillus flavus* infection and

2. To assess phenotypic diversity using morphological traits and molecular diversity using RAPD markers.

II. REVIEW OF LITERATURE

Germplasm resource is a very wide term that covers all the allelic resources spread in types ranging from most primitive wild progenitors to the highly bred cultivated varieties and strains. Genetic variability is the gift of nature and its fruitful utilization in any crop species requires systematic collection, evaluation, description and grouping based on economic descriptors. Groundnut (*Arachis hypogaea* L.) breeding programmes with a goal of rapid cultivar development have used mainly elite breeding lines and cultivars which has resulted in the development of breeding material with narrow genetic base. Utilization of exotic germplasm resources in breeding programmes is needed to enhance the diversity of cultivars, but lack of proper characterization and evaluation of germplasm was one of the bottlenecks in its utilization. (Knauft and Gorbet, 1989). To fulfill these constraints it is necessary to identify a smaller subset or 'core' collection that likely represent most of the genetic variation in entire collection. A thorough understanding of the genetic diversity, extent of variation and genetic architecture of the plant among these genotypes would help in developing sound plant improvement programme.

The literature relevant to objectives of the present study is reviewed under the following headings.

2.1 CENTERS OF ORIGIN AND DIVERSITY

The center of origin of the genus *Arachis* was most probably, central Brazil (Gregory *et al.,* 1980). The ancient species are found in higher elevations, their immediate descendent relatives occupy the next lower erosion surface and their distantly evolved descendants occupy a still lower and more recent eroded surface (Gregory *et al.* 1973, 1980). The cultivated groundnut (*A. hypogaea*) most probably originated in the region of southern Bolivia and Northern Argentina, since *A. monticola*, the only wild tetraploid species that crosses with *A. hypogaea*, is found in this area. Hence, this region was presumed as center of origin and primary center of diversity for *A. hyprogaea*.

Krapovickas (1969) and Gregory and Gregory (1976) recognized six gene centers for cultivated groundnut in South America where the greatest amount of genetic diversity is found. The Guarani region, the center of variation for the variety *vulgaris,* and Spanish types were probably also disseminated from this region (Krapovickas, 1969; Gregory and Gregory, 1976). Where as, Brazil was presumed as center of origin for *A. hyprogaea fastigiata* var. *fastigiata*; north-west Mato Grosso as the center of variation for the *A. hypogaea* var. *hypogaea*; the eastern foothills of the Andes in Bolivia as a great center of variability of subsp. *hypogaea* var. *hypogaea* with a few races of var. *fastigiata* and Peru as the center of variation for the *A. hypogaea hypogaea* var *hypogaea* and var. *hirsuta, A. hypogaea fastigiata* var. *fastigiata.*

The Spaniards carried the domesticated peanut from the east coast of South America 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). The peanuts were likely introduced into North America from Brazil by way of slave ships that were resupplied in northeastern Brazil to complete the voyage (Stalker and Simpson 1995).

2.2 GENETIC RESOURCES

Systematic collection expeditions in South America were started by Archer in 1936 (Valls *et al.,* 1985). The world collection and assembly of groundnut genetic resource was enhanced through specific collection expeditions in various groundnut-growing regions of the world and in centers of diversity in South America. In these expeditions various international agencies such as IBPGR, and national programmes, such as those of countries of exploration and the United States Department of Agriculture (USDA) have collaborated very closely (Simpson 1982, 1984) leading to the establishment of the world's largest repository of groundnut germplasm at ICRISAT and in the USA under the USDA with 14,000 and 7545 accessions, respectively. These collections provide basic genetic stock to the international scientific community for further improvement of the groundnut. Besides the collection of land races of cultivated groundnut, efforts were also made in these expeditions to collect distinct *Arachis* species (Simpson, 1982). Williams (2001) discussed use of the geographic information system (GIS) for more effective study, locate and conserve *Arachis* genetic resources based on existing germplasm collections and geographical distribution of genetic diversity within primary and secondary centers of diversity or origin.

2.3 CORE COLLECTION

Very large collections of germplasm from around the world are being assembled in efforts to conserve the genetic variation of numerous species for further to evaluation and utilization. But in these collections it is necessary to identify a smaller subset or core collection that likely represents most of the genetic variation in the entire collection and to evaluate the relationship among the traits studied (Skinner *et al*., 1999). Frankel (1984) proposed that a germplasm collection could be reduced to minimize repetitiveness within the collection and should represent the genetic diversity of a crop species. The concept of core collection was given by Brown (1989b) to represent genetic diversity of a species with minimum repetitiveness and it represents a subset of collection which includes all useful characters so that identification of useful entry becomes easy and accessible for special attention. Brown (1989a) using sampling theory of selectively neutral alleles argued that the size of a core subset should be about 10 per cent of total accessions with a ceiling of 3000 per species and this sample size seems to be effective in retaining 70 per cent alleles present in the entire collection. Frankel and Brown (1984) and Brown (1988,1989a), described methods to select a core collection using information on the origin and characteristics of the accessions. This core collection could serve as a working collection that could be extensively examined and the accessions excluded from the core collection would be retained as the reserved collection.

The development of a core collection in peanut would provide a working collection, which could be extensively evaluated to generate the information that would allow estimation of genetic variation for economically important traits, and these data would also provide an indication of clusters in the collection that should be extensively examined for particular traits (Holbrook *et al* 1993). Holbrook *et al* (1993) developed a peanut core collection of 831 accessions on the basis of six morphological variables (plant type, pod type, seed size, testa color, seed per pod and seed weight) of 7432 US peanut accession and data that regards to six phenotypic traits indicated that the genetic variation expressed for each trait in the entire collection has been preserved in this core collection.

A core subset of 1704 accession was developed by Upadhyaya *et al* (2002) based on taxonomic, geographic and morphologic description of 14,310 peanut accession from ICRISAT gene bank and this core subset includes 11.9 per cent of accessions and represents the genetic variation available in the entire collection and also preserve the co-adapted gene complexes represented in the entire collection. Upadhyaya and Ortiz (2001) suggested a strategy for sampling the entire and core collection for developing a mini core subset, which contains about 1 per cent of total accessions in the entire collection but captures most of the useful variation of the crop.

2.4 MORPHOLOGICAL DESCRIPTION

Upadhyaya *et al.* (2001b) compared the entire collection of 14310 accessions comprising 7668 of subsp. *fastigiata* and 6642 of subsp. *hypogaea* with the core collection of 1704 accession comprising of 916 of subsp. *fastigiata* and 788 of subsp. *hypogaea,* no difference was found between two sets with respect to the growth habit and flower colour. Both had the mean value of 4.6 and 4.95 for the growth habit and flower colour respectively.

Upadhyaya (2003) evaluated core collection of 1704 accessions and reported, sequential branching, which is characteristic of subsp. *fastigiata*, was observed in 910 accessions followed by alternate pattern of *hypogaea* in 794 accessions and also observed pigmentation on stem of mature plants in 284 accessions and it was absent in 1,420 accessions. He reported, 15 classes of seed color in the core subset, with tan as the most common (1,225 accessions) followed by red in 265 accessions. Pale tan was seen in only one accession. Dark tan, salmon, purplish red/reddish purple and dark purple were observed in none of the accessions. In core collection, pod beak was absent in 105 accessions, slight in 727, moderate in 740, prominent in 124, and very prominent in only 8 accessions. He studied pod constriction and found that slight constriction was present in mature pods of 1,041 accessions, moderate in 592 accessions and also revealed moderate pod reticulation in maximum accessions (1,256), followed by slight in 214 accessions, prominent in 185 accessions, very prominent in 44 accessions and absent in only 5 accessions.

Upadhyaya *et al*., (2002) evaluated core collection of 1704 accession and mini core subset of 184 accessions and found that there was no significant difference between mean values of the core and mini core subsets for the stem hairiness, pod beak, pod constriction and pod reticulation. The variances of the core and mini core subsets were homogeneous for the above morphological traits.

Upadhyaya *et al*., (2003) studied phenotypic variation in the core collection consisting of 1704 accession from the six groundnut botanical varieties for 16 morphological descriptors to estimate phenotypic diversity and determine importance of different descriptor traits. The two groups differed significantly for all the traits except leaflet surface. The mean values for stem colour, stem hairs, leaf hairs, pod colour and seeds per pod were significant between the core collection and entire collection. However, individual botanical variety wise, the differences were significant for stem colour, stem hairs, branching pattern and leaf colour in *hypogaea*, for leaf colour and pod reticulation in *fastigiata*, pod reticulation and seeds per pod in *peruviana* and flower colour, streak colour and pod colour in *vulgaris*.

2.5 PRODUCTIVITY TRAITS

Initiation of flowering varied between 21-26 DAS in bunch type, 21-30 DAS in semi spreading type, 24-29 DAS in spreading and 26-30 DAS in erect type (Varisai Muhammad *et al*., 1973a). In groundnut flowering commences from 20-30 days after emergence depending on the environmental conditions, at 30^0 C they flowered at 25 DAS in subsp. *hypogaea* and 24 DAS in subsp. *fastigiata*. While, a report of flowering between 24-30 DAS in subsp. *hypogaea* var. *hypogaea* was made by Omaran *et al.* (1980).

Bhagat *et al.* (1984) found that among 51 accessions of different botanical varieties pod yield per plant ranged from 2.2 to 8.1 g per plant in all bunch types with a mean of 5.7, 4.1 and 4.1 in var. *vulgaris*, var. *fastigiata* and var. *hypogaea*, respectively. On the other hand in runner type belonging to subsp. *hypogaea* var. *hypogaea* it varied from 1.5 to 11.6 with mean pod yield of 3.6 g per plant. Upadhyaya *et al* (2002) evaluated core collection of 1704 accession and mini core subset of 184 accessions to determine the degree of genetic diversity among them. There was no difference between mean values of the core and mini core subset and was non significant for yield per plant in two seasons.

In an evaluation of 672 genotypes comprising 234 of bunch type, 170 of semi spreading type and 268 of spreading type, Natarajan *et al.* (1978) reported that shelling percentage ranged from 61.6 to 62.3 per cent in bunch type, 60.0 to 81.5 per cent in semi spreading and 62.1 to 83.6 per cent in spreading type respectively. Rajgopal *et al.* (2004) evaluated 551 accessions consisting of 115 Virginia bunch, 69 Virginia runner, 172 Spanish bunch and 195 accessions of Valencia. The shelling percent ranged from 31.8 per cent to

87.6 %. High shelling percentage ranging from 78.0 per cent to 80.3 per cent was observed in Spanish bunch types. Upadhyaya (2003) evaluated the groundnut core collection consisting of 1704 accessions of which 910 belonged to subsp. *fastigiata* and 794 to subsp. *hypogaea*. The shelling percent mean values of *fastigiata* and *hypogaea* were significantly different from each other and showed that *fastigiata* had higher shelling percentage than *hypogaea* type in both season.

Holbrook *et al.* (1993) compared the germplasm collection of 7432 accessions with its core collection of 831 accessions for 100 seed weight. Non-significant difference was observed with the mean values of 50.81 and 51.46 g, respectively. The differences between mean values for 100-seed weight (g) of *fastigiata* and *hypogaea* groups were significant in both the seasons and locations among the Asian core collection of 504 accessions (Mallikarjuna swamy *et al*., 2003).

2.6 CHEMICAL TRAITS

2.6.1 Lectin activity

Groundnuts contain a galactose specific lectin. Substantial amounts of active dietary peanut lectin appear in human systemic circulation, through internalization after ingestion of peanuts (Qiang Wang *et al*., 1998). Because of specific binding and mitogenic properties, it could be responsible for coronary heart diseases (Kritchevsky *et al.,* 1998) and colorectal tumors (Ryder *et al*., 1992) in humans. Qualitative and quantitative differences exist for lectins among groundnut genotypes (Pueppke, 1981).

Vishwanath *et al*., (2004) evaluated hundred genotypes representing varieties, induced mutants, disease resistant germplasm and observed that the mutants (15 fold) and germplasm (9 fold) showed high variability for lectin activity compared to varieties (4 fold). Among the induced mutants, Spanish bunch and Virginia mutants had higher mean activity than Valencia mutants. But Valencia mutants followed by Virginia mutants showed very high variability for lectin activity compared to Spanish bunch mutants.

2.6.2 Protein

“Nuts in the past, were considered unhealthy food because of their high fat content,” (Harvard Researcher Dr. Frank Hu). Conventional wisdom had it that fat food increases obesity and type 2 diabetes. But research in the past decade has shown that nuts generally contain good kinds of fat as well as other nutrients that can help keep cholesterol healthy levels. The peanut also known as groundnut is the world’s fourth most important source of edible oil and third most important source of vegetable protein (Lusas, 1979). The legume dried bean family; peanuts are terrific protein source and also contain high quality plant protein. This is especially important for children, vegetarian and people eating more meatless meals (http:// www.fao.org).

The development of nutritionally balanced foods to feed the growing population of dietary deficiencies of proteins is receiving increasing attention of the food scientist and nutritionists. To overcome this problem to large extent, oil seeds are utilized to manufacture and market high protein foods at reasonably low prices (Bookwalter *et al*., 1979) although peanuts are more popular for their oil content, processing for oil consumption (Lusas, 1979)

Dwivedi *et al*., (1993) analyzed 8000 germplasm accessions at ICRISAT, a range of 16 to 34 per cent for protein was observed. However, these ranges of variation were not maintained when selected genotypes with such variations were tested over seasons and locations. Of the 188 bold-seeded accessions evaluated for two years, 12 accessions were further evaluated for protein content at NRCG, Junagadh, India (Rajgopal *et al*, 2000). The protein content ranged from 19.3 per cent (NRCG 912) to 23.5 per cent (NRCG 2863). Manivel *et al*., 2000 reported the protein content ranging from 15.5 per cent to 32.9 per cent and PBS 11039 had higher protein content than the best control, B 95. In twelve advance breeding lines, wide variability has been noticed for protein content, large seeded groundnut genotypes, TGLPS-7 exhibited stability for protein content (Mohan Bentur, 2002).

2.7 DISEASE RESISTANCE

2.7.1 *Aspergillus* SEED COLONIZATION

Environmental factors such as soil moisture, soil temperature in the pod zone and soil type influence the degree of groundnut seed infection by *Aspergillus flavus* and other fungi which complicates resistance screening, as level of infection vary considerably within a genotype over seasons and locations (Murari Singh *et al*., 1989).

Mehan *et al*., 1991; Mehan *et al*., 1995 reported that irrespective of soil types, resistant genotypes showed lower level of seed infection than susceptible ones and seed infection in the storage is more in wet seasons than in dry seasons. Incidence of storage fungi ranged from 4-34 per cent depending on the moisture content of fresh pods (22-36 percent) and drying conditions. Immature and shriveled pods were 2-3 times more heavily contaminated than mature pods (Palanisami *et al*., 1990). Harvesting the crop beyond optimum maturity was correlated with increased pod damage by many soil borne insects, which in turn enhance the invasion by *A. flavus* (Lynch and Wilson, 1991; Bowen and Mack 1993; Eeden *et al*., 1994).

Mixon and Rogers (1973) of U.S.A. developed a laboratory inoculation method for screening groundnut genotypes for resistance to *Aspergillus flavus* invasion and colonization of re-hydrated seeds. Artificial inoculation helps to ensure uniform testing condition, which reduces variation due to escapes that often masks genetic differences (Will *et al.* 1994). *Aspergillus flavus* and *A. parasiticus* Speare can colonize seed of several agricultural crops including peanut (Cast 1989) this can result in the contamination of the edible yield from these crops with the toxic fungal metabolite aflatoxin. Mehan and Mc Donald (1980) at ICRISAT screened 850 germplasm accessions for resistance to *in vitro* seed colonization by *Aspergillus flavus* strain *AF*-8-3-2A (IVSCAF). Genotypes with 15 per cent or fewer seeds colonization were regarded as resistant. Sporulation and growth of *A. flavus* was dense on the seeds of susceptible cultivars but was sparse on resistant lines (Naguib *et al*., 1989).

Thakur *et al.* (2000) evaluated 35 wild *Arachis* germplasm belonging to 24 species in 6 sections for *in vitro* seed colonization with a highly aggressive and toxigenic strain of *A. flavus* (isolate *Af* 11-4) and for aflatoxin production. Large variation existed for seed colonization severity (1.0 to 4.0) and aflatoxin production. Peanut genotypes with some resistance to invasion by *A. flavus* have been reported (Mehan *et al.,* 1991; Cole *et al.,* 1995) and were identified by screening germplasm for *in vitro* colonization by *A. flavus* of rehydrated sound mature kernels and got promising correlations between field resistance and *in vitro* resistance in Africa (Zambettakis *et al.* 1981; Waliyar *et al.* 1994) and India (Mehan *et al.* 1986,1987). However, *in vitro* screening has produced inconsistent results when compared to a natural field situation in the United States where Anderson *et al* (1995) and Blankenship *et al* (1985) did not observe significant levels of preharvest aflatoxin resistance in genotypes previously reported with *in vitro* resistance. Kisyombe *et al* (1985) observed significant field resistance in only one of 14 *in vitro* resistant selections.

Holbrook *et al.* (1997) reported that development of resistant cultivars could be accelerated if an effective trait for indirect selection can be identified. However, the mechanisms of resistance to late leaf spot and mold, these two fungi did not affect colonization by *A. flavus* or aflatoxin products.

2.7.2 Late leaf spot

The *Cercospora* or tikka leaf spots (early and late leaf spot) are the most important fungal diseases of groundnut. In India, late leaf spot is more predominant compared to early leaf spot because of its fast spreading nature. The late leaf spot caused by *Cercospora personatum* Berk and Curt. Was first described in USA in 1875. The perfect stage *Mycosphaerella berkeleyii* was described by Jenkins (1938). But recently, it was renamed as *Phaeoisariopsis personata* (Berk and Curt) V. Arx.

Holbrook and Anderson (1995) used data on resistance to late leaf spot (Berk and M.A. Curtis) that was available for the entire peanut germplasm collection to determine how effective the use of this core collection for identifying the source of resistance in the entire

collection. The use of a two stage screening approach on peanut core collection had resulted in identification of 61 leaf spot resistant accessions by examining the data, first to determine how many leaf spot resistant accession would have been identified by examining the core collection and later, data were also examined to determine how many leaf spot resistant accessions would have been identified by examining all accessions from cluster having a resistant indicator value.

2.8 PHENOTYPIC DIVERSITY

Upadhyaya *et al*., (2002) calculated Shannon-Weaver diversity index for 13342 accessions of groundnut contained in ICRISAT gene bank and compared phenotypic diversity among 38 morphological characters and regions. South America region had highest H' for all the morphological descriptor traits except growth habit, branching pattern, leaflet shape and pod beak. Primary seed colour among morphological traits and leaflet length in rainy season and shelling percentage in post rainy season among agronomical traits had highest pooled H'. South America had the highest H' indicating that the diversity for different traits from region consisting of primary and secondary centers of diversity has been conserved in the ICRISAT collection. Upadhyaya *et al.,* (2002) also subjected the data to principal component analysis. This revealed three regional clusters on first seven principal component scores. North America, Middle East and East Asia in the first cluster. South America in the second cluster and West Africa, Europe, Central Africa, South Asia, Oceania, Southern Africa, Eastern Africa, South East Asia, Central Asia and Caribbean in the third cluster.

Upadhyaya (2003) examined a core collection of 1704 accessions for genetic divergence using principal component analysis. This analysis showed that 15 agronomical traits were important in explaining multivariate polymorphism. However, seed width and protein content did not significantly account for variation in the first five principal components of *fastigiata* and *hypogaea* types as well as for the core collection. Evaluating the core collection for response to drought, Upadhyaya (2005) reported, five *vulgaris* and 13 *hypogaea* accessions selected on the basis of specific leaf area, specific chlorophyll meter reading values compared with the control cultivars. These accessions and four control cultivars were grouped in different clusters by scores of the first 15 principal components (PCs). The selected 11 *hypogaea* entries and control cultivar M 13 (*hypogaea*) grouped in cluster II with ICG 6766 and ICG 14523 forming two separate clusters, indicating that they were diverse from the control cultivars. The groundnut core collection of 1704 accessions was subjected to Shannon-Weaver diversity index (Upadhyaya, 2003). Among the morphological descriptors primary seed colour in *fastigiata* group and pod beak in *hypogaea* group showed highest H'. Among the agronomic traits in *fastigiata* group, 100 seed weight in rainy season and plot yield in post rainy season, and in *hypogaea* group pod width in rainy season and seed length in post rainy season had the highest H'. The average H' values across traits were similar between rainy and post rainy seasons in both groups as well as in entire core collection.

The Shannon-Weaver diversity index (H') was calculated to compare phenotypic characters in the core and a mini core subset. The average H' for the 13 morphological descriptors and agronomic traits in the mini core subset was similar to the core sub set indicating that the diversity of the core was represented indicating that the diversity of the core was represented in the mini core subset. (Upadhyaya *et al*., 2002).

Mallikarjuna Swamy *et al*., (2003) took Asia core collection of 504 accessions to understand the nature of genetic divergence using principal component analysis. This showed that 20 agronomical traits were important in explaining multivariate polymorphism but pod yield per plant did not significantly account for variation in the first five principal components of *fastigiata* and *hypogaea* types as well as for the entire regional collection. Evaluation of Asian core collection for Shannon diversity index, showed that in *fastigiata* group leaf let length in rainy, pod width in post rainy at Raichur, seed length in rainy and plant height in post-rainy at Kawadimatti, seed length in rainy, number of total pods in post rainy at Raichrur, number of primary branches in rainy and plant height in post rainy at Kawadimatti had the highest H'. The average H' value across seasons and locations was highest for leaflet length in the *fastigiata* group and for seed length in the *hypogaea* group. The average H' across traits were similar between rainy post rainy seasons in both locations and groups.

2.9 GENETIC DIVERSITY

Markers based on DNA sequence variations are increasingly being utilized in crops for construction of genetic maps and marker-assisted selection. Application of molecular markers in plant breeding has established the need for information on variation in DNA sequence even in those crops in which little classical genetic and cytogenetic information is available. DNA-based markers provide a reliable means of estimating the genetic relationships between genotypes and taxonomic groups as compared to morphological markers (Gepts, 1993).

Molecular tools such as DNA markers are increasingly becoming important as effective tools in crop breeding programs and their application in groundnut enhancement is lagging behind because of limited knowledge of the genome. Extent of variation for morphological and physiological characteristics exists in both wild and cultivated groundnut (Halward *et al*., 1992) but abundant DNA polymorphism has been observed only in wild diploid *Arachis* species (Kochert *et al.* 1991; Halward *et al.* 1993). Alleles differed from each (Radhakrishnan *et al.* 2004) screened eight primers for their ability to distinguish cultivars by detecting polymorphisms in selected twelve released cultivars. The RAPD analyses indicated that genetic diversity exists in the released cultivars within and across habit groups of the genus *Arachis.*

Halward *et al* (1991) observed very low level of DNA polymorphism in cultivated groundnut germplasm using different techniques such as restriction fragment length polymorphism (RFLP) and polymerase chain reaction (PCR) analysis, though a diverse variation for phenotypic characteristics in cultivated species did not show significant variability at the DNA level. Because of the lack of polymorphism at DNA level, this crop lagged behind in genetic mapping, marker-assisted selection, resistance gene cloning, and crop evolutionary study compared to other crops. This problem has hindered the improvement of cultivated groundnut by molecular techniques (He *et al*., 2003). Two peanut cultivars, 25 unadopted germplasm lines of *A. hypogaea*, the wild allotetraploid progenitor of cultivated peanut (*A. monticola*), *A. glabrata* (a tetraploid species from section *Rhizomatosae)* and 29 diploid wild species of *Arachis* were evaluated for variability using random primers. No variation in banding pattern was observed among the cultivars and germplasm lines of *A. hypogaea* using RAPD techniques (Halward *et al.* 1992), where as the wild *Arachis* species were uniquely identified with most primers tested.

Paik-Ro *et al*. (1992) reported that DNA polymorphism could not be detected within or between *A. hypogaea*, *A. monticola* and the lines of interspecific origin involving *A. hypogaea* and *A. cardenasii* with the 32 endonuclease probe combination of RFLP. Although significant polymorphism has been observed in diploid *Arachis* species (Garcia *et al.,* 1995), these markers could not be utilized in the improvement of *A. hypogaea* because of the incompatibility problem between species.

Bhagwat *et al*., (1997) studied the difference in the banding pattern between Spanish Improved and its x-ray induced mutants, using 12 random primers. The large seed mutant and dwarf mutant showed greater difference with Spanish Improved. He and Prakash (1997) reported the presence of DNA polymorphism in cultivated groundnut using the amplified fragment length polymorphism (AFLP) technique and low level of DNA polymorphism observed (Hopkins *et al.* 1999) in wild and cultivated groundnut using simple sequence repeat (SSRs). Subramanian *et al.* (2000) reported, seventy selected genotypes representing variability for several morphological, physiological and other characters and studied for polymorphism employing RAPD assay using 48 oligonucleotide primers, out of which 7 yielded the polymorphism. Out of total 408 bands 27 (6.6%) bands were polymorphic.

Raina *et al.* (2001) reported random and simple sequence repeat primers revealing 42.7 and 54.4 per cent polymorphism, respectively among the 13 accessions. Dendrogram based on RAPD, ISSR and RAPD + ISSR data precisely organized the five botanical varieties of the two subspecies into five clusters. These results strongly supported that, *A. monticola* and *A. hy*pogaea are very closely related and *A. villosa* (AA) and *A. ipaensis* (BB) are the diploid wild progenitors of these tetraploid species.

Accessions possessing varying levels of resistance to early leaf spot could show polymorphism ranging from 11.7 to 55 per cent using RAPD assay, with an average of 27.4 per cent per primer (Dwivedi and Gurtu, 2002).

Micro satellites as DNA markers are advantageous over many other markers, as they are highly polymorphic, highly abundant, co-dominant inheritance, analytically simple, readily transferable (Weber, 1990). Microsatellites are reported to be more variable than RFLPs or RAPDs and have been widely utilized in plant genomic studies (Burow *et al*., 1996). Micro satellites have become one of the most widely used molecular markers for genetic studies in recent years.

A diverse array of plant accessions from cultivated peanut and wild species screened for SSRs could reveal high level polymorphism compared to RAPD's or RFLPs (Hopkins *et al*., 1999; Dwivedi and Varma, 2002; Ferguson *et al*., 2002a,b; He *et al*., 2003; Ferguson *et al*., 2004; Moretzsohn *et al*., 2004). He *et al*., (2005) evaluated forty-eight cultivated accessions representing six botanical varieties to identify botanical variety specific markers. Eight SSR markers were found to classify botanical varieties *fastigiata* and *vulgaris*, one to botanical varieties *hypogaea* and *hirsuta* and one to botanical varieties *peruviana*, and *aequatoriana* also three of them derived from peanut expressed sequence tags (ESTs) were associated with putative genes as botanical varieties have different morphological traits and belong to different subspecies in *A. hypogaea* these markers might be associated with genes involved in the expression of morphological traits.

Fourty four accessions of cultivated peanut representing six botanical varieties of two subspecies and three accessions of the wild relative *A. monticola* were subjected to AFLP analysis. Fifteen AFLP primer pairs (EcoRI/MseI) generated 28 distinct polymorphic markers, which could give unique profiles of all accessions. The botanical varieties *aequatoriana* and *peruviana* were found to be closer to ssp. *hypogaea* than ssp. *fastigiata* to which they belong and the wild *A. monticola* was not distinct from the cultivated *A. hypogaea* (He and Prakash, 2001)

Genetic relationship was studied among 60 accessions of *A. hypogaea* and 36 wild accessions representative of the section *Arachis* using allelic variation of SSR loci. Brazilian peanut germplasm collection showed considerable level of genetic diversity. Micro satellite marker transferability was up to 76 per cent for species of the section *Arachis*, but only 45 per cent of species from the other eight *Arachis* sections tested. A new marker (Ah-041) presented 100 per cent transferability and could be used to classify the peanut accessions as AA and non-AA genome carriers (Maretzsohn *et al*., 2004)

Singh *et al*., (1998) reported, the utilization of molecular markers on the genus *Arachis* and revealed very little demonstrable polymorphism in the cultivated groundnut, *A. hypogaea*. This has led to the hasty generalization that the groundnut lacks genetic variation. 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.

Dwivedi *et al*., (2001) selected twenty-six accessions and eight primers for random amplified polymorphic DNA assay to determine the genetic diversity. The genetic similarity (S_{ij}) ranged from 59.0 per cent to 98.8 per cent with an average of 86.2%. Both multidimensional scaling and unweighted pair group method with arithmetic averages (UPGMA) dendrogram revealed the existence of five distinct clusters. However, this classification could not be related to known biological information about the accessions falling into different clusters. Some accessions with diverse DNA profile (ICG 1448, 7101 and 1471 and ICGV 99006 and 99014) were identified for mapping and genetic enhancement in groundnut.

III. MATERIAL AND METHODS

The information on materials and methods adopted are presented in this chapter.

3.1 DESCRIPTION OF THE MATERIAL

The experimental material comprised 188 accessions of groundnut mini core set, representing *fastigiata* (33), to *vulgaris* (71), to *peruviana* (2), one *aequatoriana* (1), *hypogea* runner (33) and *hypogea* bunch (48) obtained from ICRISAT, Patancheru. Besides these accessions, three breeding lines, (R 9227, MN 1-28, MN 1-35) and four cultivars (GPBD-4, TAG 24, JL 24, M 28-2) were also included (Appendix I).

3.2 EXPERIMENTAL SITE

The research work was conducted at Botanical garden of University of Agriculture Sciences, Dharwad for one season, rainy 2004. This location is situated in the transitional tract of Karnataka at $15^{0}13$, at latitude $75^{0}07^{1}$ E longitudes at an altitude of 678 m above mean sea level.

3.3 CULTIVATION PRACTICES

The seedbed was prepared to fine tilth before taking up sowing. The recommended package of practices for cultivation of groundnut was adopted.

3.4 ENVIRONMENTAL SEASONS

In rainy season, the experimental material was sown on 27th June 2004, in a Lattice Square Design (14 x 14) with two replications. Each genotype was grown in one row with spacing of 30 cm between rows and 10 cm between plants in a row, respectively. The crop was harvested from 20th to 26th October 2004. The monthly metrological data obtained from meteorological department from main Agricultural Research Station, U.A.S., Dharwad, with regard to temperature, relative humidity, rainfall and number of rainy days during the course of investigations is presented in (Appendix 2).

3.5 DESCRIPTION OF THE OBSERVATION

3.5.1 Phonological traits: Morphological characters (Growth habit, branching pattern, stem pigmentation, stem hairiness, leaf let shape, flower colour, pod beak, pod constriction, pod reticulation and seed colour) were recorded according to a descriptor list (Anonymous, 1992) on whole plot basis. (Table 1). Variation for stem hairiness, pigmentation, and leaf let shape and seed colour is presented in Plate 1, 2 and 3.

3.5.2 Productivity traits

3.5.2.1 Days to 50 % flowering

The days taken to 50 per cent flowering of the plants in the net plot from date of sowing was calculated for each genotype.

3.5.2.2 Pod yield (g) per plant (PYP)

Yield per plant was calculated by dividing total pod yield per plot by number of plants in the plot expressed as g / plant.

Pod yield per plant (g) = Pod yield per plot (g) / Number of plants per plot

3.5.2.3 Shelling per cent (SPP)

Hundred grams of pods were taken and shelled. The weight of kernels gave shelling per cent.

Table 1: Morphological descriptor list for Groundnut

Sl. No.	Traits	Recording of observation	Classified as
1.	Growth habit	Recorded at poding stage	1. Procumbent-1 2. Procumbent-2 3. Decumbent-1 4. Decumbent-2 5. Decumbent-3 6. Erect 7. Others
2.	Branching pattern	Determined based on the (n+1) cotyledonary branches at poding stage	1. Alternate 2. Sequential 3. Irregular with flowers on main stem 4. Irregular without flowers on main stem 5. Others
3.	Stem pigmentation	It was recorded on mature plants	0 Absent + Present
4.	Stem hairiness	Recorded on the main axis	3 Scarce 7 Abundant
5.	Leaflet shape	Shape of fully expanded leaflet on the main axis and apical leaflet	1. Cuneate 2. Obcuneate 3. Elliptic 4. Oblong-elliptic 5. Narrow-elliptic 6. Wide-elliptic 7. Suborbicular 8. Orbicular 9. Ovate 10. Obovate 11. Oblong 12. Oblong-lanceolate 13. Lanceolate 14. Linear-lanceolate
6.	Flower colour	Recorded from fresh, fully opened flowers	1. White 2. Lemon yellow 3. Yellow 4. Orange-yellow 5. Orange 6. Dark orange 7. Garnet/brick red 8. Others
7.	Pod beak	Recorded from the well developed and well cleaned pods	1 Absent 3 Slight 5 Moderate 7 Prominent 9 Very prominent
8.	Pod reticulation	Recorded from the well developed and well cleaned pods	0 None 3 Slight 5 Moderate 7 Prominent 9 Very prominent
9.	Pod constriction	Recorded from the well developed and well cleaned pods	0 None 3 Slight 5 Moderate 7 Prominent 9 Very prominent
10.	Primary seed colour	Primary or major colour of the seeds recorded.	1. Tan 2. Off white 3. Rose 4. Dark red 5. Red 6. Light red 7. Brown 8. Dark purple 9. Salmon with purple flecks 10. Red with white flecks

Plate 1. Variation for stem hairiness and Pigmentation

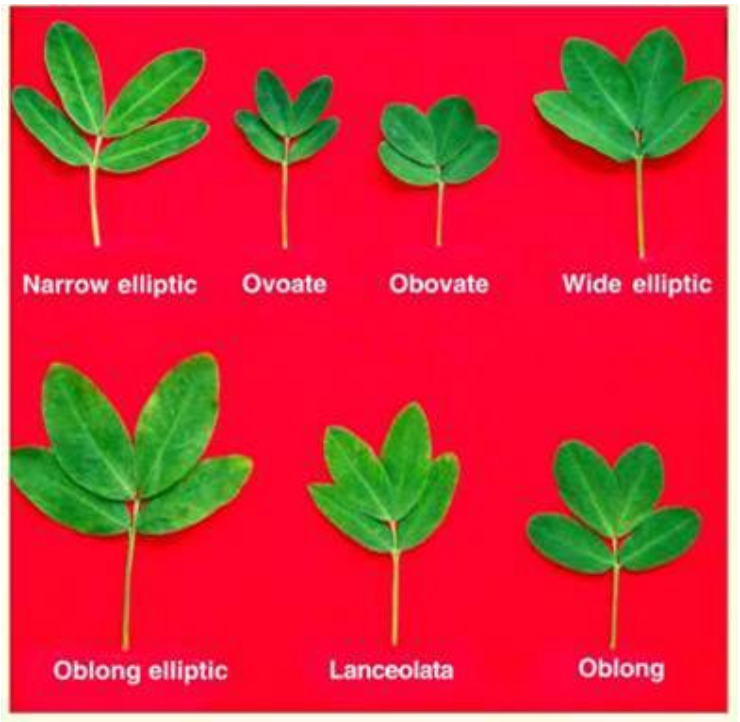
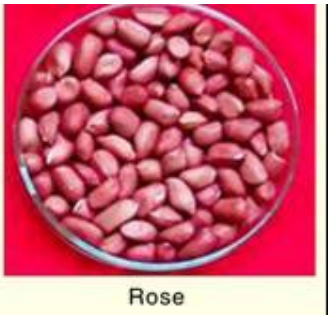


Plate 2. Variability for leaflet shape


Red


Off white


Rose


Dark purple


Brown


Salmon with purple flecks


Light red


Tan


Red with white flecks


Dark red

Plate 3. Variability for seed colour

3.5.2.4 Sound mature kernels (SMK)

After shelling the known quantity of pods, the kernels obtained were sorted out into well-matured and shriveled kernels and the number of good kernels was counted and expressed as percent of total number of kernels to obtain SMK.

3.5.2.5 Test weight (TWT)

The well-dried and cleaned pods from each genotype were shelled and 100 kernels at random were counted and weight was recorded in grams.

SMK (%) = Number of well-matured kernels / total number of kernels x 100

3.5.2.6 Disease resistance

3.5.2.6.1 *Aspergillus* seed colonization

Biological material of *Aspergillus flavus*

Pure culture of *Aspergillus flavus* strain *Af* 11-4, was procured from ICRISAT (International Crop Research Institute for semi Arid Tropics), Patancheru, Hyderabad, Andhra Pradesh, India and is considered to be highly aggressive and toxigenic strain (Thakur *et al*., 2000).

Chemicals and Glasswares

Mercuric chloride	- 0.1 % (w/v) aqueous solution was used for surface sterilization of seeds.
Ethyl alcohol	- 70% (v/V) was used for surface sterilization of inoculation chamber and Inoculation loop.
Tween – 80	- One or two drops are added to the inoculum (spore suspension) for uniform distribution of spores in the suspension.

Screening germplasm under *in vitro* condition for seed colonization by *Aspergillus flavus*

Maintenance of the fungus culture

The pure culture of the fungus, *Aspergillus flavus* (*Af* 11-4) was sub cultured on the potato Dextrose Agar (PDA) slants and allowed to grow at 25 ± 1^0 C temperature. The culture so obtained was stored in refrigerator at 4^0 C for further use. The sub culturing was done regularly at one-month interval. Standard procedure was followed for preparation of PDA.

Preparation of conidial suspension (Inoculum)

Aspergillus flavus was grown on PDA slants. The culture was incubated at room temperature for seven days from the time of inoculation to ensure maximum sporulation. The test tubes having thick/heavy sporulation were washed with 5 ml of distilled sterile water and the conidia were harvested into suspension by gentle brushing using an inoculation loop without disturbing the agar. This suspension was added to a test tube containing 5 ml of distilled sterile water to make up 10 ml of spore suspension. To this, about one to two drops of Tween-80 were added for uniform dispersal of conidia in the suspension. Serial dilution and pour plate method was used for enumeration of spores or conidia per ml of spore suspension. Accordingly the original spore suspension was diluted using distilled sterile water to get final concentration of 1×10^6 spores /ml and was used for inoculation of groundnut seeds.

Inoculation of groundnut seeds

Fifty seeds (weighing 4-10 g depending on the seed size) which are sound matured with intact seed coat and free from any damage were selected from each genotype for *in vitro* inoculation by *A. flavus*. Seeds were surface sterilized with 0.1% (w/v) aqueous solution of

mercuric chloride for two minutes and subsequently washed in two changes of distilled sterilized water to remove traces of mercuric chloride.

Each seed was uniformly wounded by pricking with a sterile needle to facilitate the invasion by *A. flavus* spores. Seeds were placed in a sterilized petridish (90 mm diameter) and spray inoculated with *A. flavus* spore suspension (1x 10^6 spores/ml) using an atomizer under strict aseptic conditions. The petridishes were shaken vigorously to roll the seeds allowing uniform distribution of inoculum on the seeds. The experiment was conducted in two replication with 25 seeds per replication.

Incubation

The petridishes were placed at high humidity (>95% RH) in semi-rigid plastic boxes lined with cotton wool and blotting paper with closely fitting lids and incubated at 25 ± 1^0 C in dark for 10 days.

Scoring for colonization severity

Individual seeds were scored for surface colonization by *A. flavus* and for colonization severity following rating scale given by Thakur *et al*. (2000) (Table.2)

3.5.2.6.2 Late leaf spot

The modified 9-point scale for late leaf spot as given by Subbarao *et al*. (1990) was used for screening germplasm. (Table.3) The visual scores (1-9) and the extent of leaf area destroyed (0-100%) are linearly related. The field disease scores are mainly based on the extent of leaf area damage.

3.5.2.7 Chemical traits

3.5.2.7.1 Lectin activity

Hemaglutination assay of the lectin was assayed using trypsinized, desialylated human erythrocytes, by the serial two-fold dilution technique of Liener and Hill (1953) in microtitre assay plate having U- bottom with some modifications. To all the wells of a row, saline (50µl) was added and only to the first well, the lectin sample (50µl) was added the content of the first well (100µl) were mixed and 50µlof it was transferred to the second well and the process was repeated serially for the remaining wells. Finally to this, 50µl of trypsinised, neuraminidase treated erythrocyte suspension was added. After incubation for one hour at 37°C, the plates were visually examined for hemagglutination. The highest dilution of the extract causing visible hemagglutination was regarded as the titre (HAU).

3.5.2.7.2 Protein

Estimation of protein by Lowry's method:

Soluble protein was determined by Lowry's method in alkaline medium, the peptide bonds in polypeptide chain-reacts with copper sulphate. This form a blue coloured product and has absorption maximum at 660 nm.

Reagents

1 Alkaline copper sulphate reagent: Add 1 ml of Reagent 3 to 50 ml of Reagent 2. This mixture is unstable and should be prepared fresh

2 Folin's reagent: Dilute the reagent appropriately so that it is 1 N in respect of its acid content

3 20 % (*w/v*) TCA: Dissolve 20 g of trichloroacetic acid in water and make the volume to 100 ml

4 Bovine serum albumin (BSA): 100 mg/ml solution in distilled water

Sample extraction: Weigh 1 g sample (seeds), macerate the sample in pestle mortar in 5 ml of phosphate buffer and transfer the material to centrifuge tubes. Centrifuge the homogenate at 8000 rpm for 20 min. collect the supernatant and repeat the extraction 4-5 times. Combine

Table 2: Seed surface colonization and severity scale (Thakur *et. al.,* 2000)

Scale	Description
1.	< 5 per cent seed surface colonized with scanty mycelial growth and scanty sporulation.
2.	5-26 per cent seed surface colonized with good mycelial growth and scanty sporulation.
3.	26 – 50 per cent seed surface colonized with good mycelial growth and good sporulation.
4.	> 50 per cent seed surface colonized with heavy sporulation

Table 3: Modified 9-point scale used for field-screening of groundnut genotypes for resistance to late leaf spot

Disease Score	Descrition	Disease severity (per cent)
1.	No disease	0
2.	Lesions present largely on lower leaves; no defoliation	1-5
3.	Lesions present largely on lower leaves, very few on middle leaves; defoliation of some leaflets evident on lower leaves	6-10
4.	Lesions on lower and middle leaves but sever on lower leaves; defoliation of some leaflets evident on lower leaves	11-20
5.	Lesions present on all lower and middle leaves, over 50 per cent defoliation of lower leaves	21-30
6.	Severe lesions on lower and middle leave; lesions present but less severe on top leaves; extensive defoliation of lower leaves; defoliation of some leaflets evident on middle leaves.	31-40
7.	Lesions on all leaves but less sever on top leaves; defoliation of all lower and some middle leaves	41-60
8	Defoliation of all lower and middle leaves; sever lesions on top leaves; some defoliation of top leaves evident	61-80
9.	Almost all leaves defoliated, leaving bare stems; some leaflets may remain, but show sever leaf spots	81-100

the supernatants and make the volume to 50 ml with phosphate buffer. Take 1 ml of the above extract and add 1 ml of 20 % TCA. Keep it for half an hour and centrifuge at 8000 rpm for 20 min. wash the pellet with acetone twice and again centrifuge it. Discard the supernatant. Dissolve the pellet in 5 ml of 0.1 N NaOH and mix well till it gets dissolved. Take suitable aliquot (1 ml) of above solution and add to it 5 ml of freshly prepared alkaline copper sulphate reagent and mix properly then add 0.5 ml of Folin's reagent and mix the contents instantaneously and allow the colour to develop for 30 minutes. Then the colour development was read at 660 by using Spectrophotometer. Amount of protein per gram of the defatted seed was calculated using the standard curve.

3.5.2.8 DNA isolation

DNA was extracted based on a previously reported cetyltrimethylammonium bromide (CTAB) method (Saghai-Maroof *et al*., 1984) with some modification. Leaves were ground to fine powder in the presence of liquid nitrogen and transferred to a sterile tube containing 9 ml of preheated (65^0C) 2 x CTAB extraction buffer (100 mM Tris-HCL buffer pH 8, 700 mM NaCl, 20 mM ethylene di-aminetetraacetic acid (EDTA) pH 8, 2 per cent hexadecyltrimetyl – ammonium bromide, 1 per cent b-mercaptoethanol and 1 per cent sodium bisulphite). 200 mg polyvinylpyrrolidone 10 per g of leaf tissue was added and mixed gently. The contents were incubated for 90 min at 65^0C in a water bath with occasional shaking during incubation. The tubes were kept for 10 min to allow them to return to room temperature. An equal quantity (9 ml) of chloroform and amyl alcohol solution, prepared in a ratio of 24:1, was added to the tubes and they were centrifuged at 8000 r.p.m. At 4^0C for 20 min. The aqueous phase was transferred to a clean tube and the chloroform and amyl solution step was repeated. Nucleic acids were precipitated by adding 0.6 ml chilled isopropanol to the aqueous phase and incubating at -20^0C for 20 min. The DNA was hooked out and transferred to a new sterile tube containing 2 ml of $T_{50}E_{10}$ buffer (50 ml $T_{50}E_{10}$ + 1 ml Rnase 10mg/ml) and left overnight at room temperature. Phenol, chloroform, and amyl alcohol, prepared in a ratio of 25:24:1 was added to the tube and mixed gently and the tube was centrifuged at 8000 r.p.m at 10^0C. The clear phase was once again cleaned using another phenol; chloroform and amyl alcohol solution, then washed and centifuged at 2^0C. The aqueous phase was transferred to new tubes and DNA was precipitated using 2-4 ml of pure chilled ethanol. Tubes were kept at -20^0C fir 10 min. The DNA precipitate was removed and washed with 2 ml of 0.2 m sodium acetate in 76 per cent alcohol for 20 min followed by 1 ml 10 mM ammonium acetate in 76 per cent alcohol for 1 min. The DNA pellet was further washed with 70 per cent alcohol for 30 min and centrifuged again. The tubes were allowed to drain and dried at room temperature for 2 to 3 hr, they resuspended in 200 to 500 µl of Tris EDTA buffer. The quality and concentration of DNA was assessed by a spectrophotometer and also by gel electrophoresis using 0.8 per cent agarose with known concentration of uncut lambda DNA.

3.5.2.9 Setting polymerase chain reaction

3.5.2.9.1 Requirements for polymerase chain reaction

1. Random primers: commercial kits of random decamer DNA primers were obtained from Operon Technologies Inc. Alamedas, USA. A total of 20 random primers were used for the assay. The sequence details of the primers are presented in the Table 4

2. dNTPs : the four dNTPs *viz.,* dATP, dGTP, dCTP and dTTP were obtained from M/s Bangalore Genei Pvt. Ltd., Bangalore.

3. *Taq* DNA polymerase : Taq DNA polymerase and 10x Taq assay buffer were obtained from M/s Bangalore Genei Pvt. Ltd., Bangalore.

3.5.2.9.2 Preparation of the master mix for the PCR

Master mix was prepared for the 195 tubes by mixing different components in the proportion as shown in Table .5 the master mix was distributed to 195 tubes (19 µl/tube)

and 1 µl of template DNA from the respective genotypes was added making the final volume of 25 µl.

3.5.2.9.3 The thermo profile for PCR

Table 4. List of primers used for RAPD analysis

Sl. No.	Primer	Sequence (5'-3')
1	OPK 09	CCCTACCGAC
2	OPK 14	CCCGCTACAC
3	OPA19	CAAACGTCGG
4	OPC 15	GACGGATCAG
5	OPC 09	CTCACCGTCC
6	OPC 13	AAGCCTCGTC
7	OPB 11	GTAGACCCGT
8	OPF 09	CCAAGCTTCC
9	OPJ 06	TCGTTCCGCA
10	OPV 16	GGGCCAATGT
11	OPA 15	TTCCGAACCC
12	OPA 20	GTTGCGATCC
13	OPF 07	CCGATATCCC
14	OPA 12	TCGGCGATAG
15	OPJ 17	ACGCCAGTTC
16	OPC 03	GGGGGTCTTT
17	OPV 15	AGTCGCCCTT
18	OPC 06	GAACGGACTC
19	OPF 10	GCAAGCTTGG
20	OPA 17	GACCGCTTGT

Table 5. Components of master mix

Component	Quality (µl/tube)
10x assay buffer	2
dNTPs (10 mM)	2
Primer (5 pM)	2
Taq DNA polymerase	0.33
Nanopure water	12.67

Table 6: Thermo profile for the PCR

Sl No	Step	Temperature (^{0}C)	Duration (min)	No. of cycles
1.	Denaturation	94	5	1
2.	Denaturation	94	2	37
	Annealing	36	1	
	Primer extension	72	2	
3.	Final extension	72	10	1
4.	Dump	4		

The thermo profile for the PCR reaction was set as shown in Table 6. After the completion of the PCR, the products were stored at -4^{0}C until the get-electrophoresis was done.

3.5.2.9.4 Agarose Gel electrophoresis

The PCR product was mixed with 2 µl of loading dye (Bromophenol blue) and was loaded in 1.2 per cent agarose gel of 1x TAE buffer containing Ethidium bromide. Gel was run at 90 volts. The gel was photographed by using documentation system (Uvitech, Cambridge, England).

3.5.2.9.5 Scoring the amplified fragment

The amplified fragments were scored as 1 for the presence and '0' for the absence of a band generating the 0 and 1 matrix.

3.6 STATISTICAL ANALYSIS

3.6.1 Maximum Likelihood Method

The data on quantitative characters was analyzed by following residual maximum likelihood (REML) on GENSTAT 5.1 to estimate variable components and their approximate standard error. The method of maximum likelihood is a general method of estimating parameters of a population by values that maximize the likelihood (L) of a sample. The likelihood L of a sample of n observations x_1, x_2..., x_n, is the joint probability function $p(x_1, x_2, ..., x_n)$ when x_1, x_2, ..., x_n are discrete random variables. If x_1, x_2,..., x_n are continuous random variables, then the likelihood L of a sample of n observations, x_1, x2, ..., x_n, is the joint density function $f(x_1, x_2, ..., x_n)$.

Variance components and mixed model ANOVA is a specialized module for design with random effects and/or factors with many levels, options for handling random effects and for estimating variance components and also provided in the general linear models module. The variance components module will allow you to analyze designs with any combinations of fixed effects and random effects.

3.6.2 Principal component analysis (PCA) Sneath and Sokal (1973)

The morphological descriptor scale of ten characters *viz.,* pod beak, pod constriction, pod reticulation, growth habit, stem hairiness, branching pattern, stem pigmentation, leaf let shape, flower colour and seed colour and mean observations of agronomic traits (days to 50% flowering, shelling percent, sound mature kernel, yield per plant, test weight, *Aspergillus* seed colonization, late leaf spot, lectin activity and protein) for each accession were standardized by subtracting from each observation the mean value of the character and subsequently dividing by its respective standard deviation. This resulted in standardized values for each trait with average 0 and standard deviation of 1 or less. The standardized values were used to perform PCA on SPSS 8.0. The purpose of principal component analysis is to derive a small number of linear combinations (principal components) of a set of variables that retain as much information in the original variables as possible often a small number of principal components can be used in place of the original variables for plotting, regression, clustering and so on.

3.6.3 The simple matching coefficient (Sneath and Sokal, 1973)

It was used to calculate the similarity coefficient between each pair of genotypes for qualitative characters (pod beak, pod constriction, pod reticulation, growth habit, stem hairiness, branching pattern, stem pigmentation, leaf let shape, flower colour and seed colour) using the formula:

$$S_{SM} = = \frac{m}{m + u} \quad \frac{m}{n} \quad \frac{a + d}{a + b + c + d}$$

Where,

m = number of matches, i.e., agreements in character states

u = the number of mismatches

3.6.4 Euclidian Distance (Sneath and Sokal, 1973)

Euclidean or straight-line measure of distance is the most commonly used statistic for estimating genetic distance (GD) between individuals (genotypes or populations) by morphological data (days to 50% flowering, shelling percent, sound mature kernel, yield per plant, test weight, *Aspergillus* seed colonization, late leaf spot, lectin activity and protein). Euclidean distance between two individuals *i* and *j*, having observations on morphological characters (p) denoted by x_1, x_2, ..., x_p and y_1, y_2,..., y_p for *i* and *j*, respectively, can be calculated by the following formula:

$$d_{(ij)} = [(x_1 - y_1)^2 + (x_2 - y_2)^2 + \text{------} (x_p - y_p)^2]^{1/2}$$

3.6.5 The general Similarity Coefficient of Gower (Sneath and Sokal, 1973)

Gower (1971) proposed a similarity measure between individuals by combined data of both qualitative *i.e.,* pod beak, pod constriction, pod reticulation, growth habit, stem hairiness, branching pattern, stem pigmentation, leaf let shape, flower colour and seed colour and quantitative data *i.e.,* days to 50% flowering, shelling percent, sound mature kernel, yield per plant, test weight, *Aspergillus* seed colonization, late leaf spot, lectin activity and protein. The similarity coefficient between the *ith* and the *jth* individuals (*Sij*) for *K* variables is:

$$S_{ij} = \frac{\sum_{K=1}^{p} W_{ijk} S_{ijk}}{\sum_{K=1}^{P} W_{ijk}}$$

Where, *Wijk* is a weight given to the *ijkth* comparison, assigning values of 1 for valid comparisons and a value of 0 for l invalid comparisons (when the value of the variable is missing in one or both individuals); S_{ijk} is the contribution of the *K*th variable to the total similarity between individuals *I* and *j*, and it takes values between 0 and 1.

3.6.6 Shannon-Weaver phenotypic diversity index

The diversity index (H') of Shannon-Weaver (1949) was calculated and used as a measure of phenotypic diversity of each trait. The index was estimated for each character over all 195 entries and four botanical varieties. This phenotypic diversity index is based upon the frequency distributions for the ten qualitative characters. This index also included the nine quantitative characters transformed into four phenotypic classes defined by the four quartiles of the landrace collection. The Shannon-Weaver Diversity Index, H'_C, was estimated using,

$$H'_C = -\Sigma^{n}_{i=1} p_i \log_e p_i$$

Where,

C = characters

n = number of phenotypic classes (for the qualitative characters n = 2 to 13 descriptor states, and for quantitative characters n = 4 frequency classes)

p_i = the proportion of the total number of entries in the i^{th} class.

3.6.7 The coefficient of Jaccard (Sneath and Sokal, 1973)

RAPD fragments were scored as either present (1) or absent (0). The Jaccard's coefficient was used to calculate the similarity coefficients between each pair of genotypes for all polymorphic loci using the formula:

$$Sij = \frac{a}{(a+b+c)}$$

Where,

a = number of common bands (1,1)

b = number of bands present in the first accessions and absent in the second accessions (1,0)

c = is the number of bands absent in the first accessions and present in the second (0,1) the 0,0, matches were not considered useful because the lack of a RAPD bands in two genotypes may not be due to a common mutational event.

Genetic distance were reported as dissimilarity coefficient

$Dij = 1 - Sij$

IV. EXPERIMENTAL RESULTS

4.1 MORPHOLOGICAL CHARACTERIZATION

4.1.1 Distribution pattern

Data on ten morphological traits were collected, based on the descriptor (Anonymous, 1992). Each trait was scored and grouped into different classes. The frequency distribution for each character was estimated in the mini core and sub sets representing different botanical varieties (Table 7).

Growth habit

Out of seven possible classes of growth habit only four were observed in the mini core. The procumbent categories *viz.,* 1 and 2 were missing. As many as 76 accessions (38.9%) showed decumbent-3 followed by 70 (35.8%) with decumbent-2, 33 (17.0%) had erect growth habit and sixteen (8.2%) with decumbent-1. Decumbent-2 was predominant in. Valencia (51.3%), Virginia runner (51.5%) and Virginia bunch (52.1%) while Spanish bunch was distinct with high frequency of Decumbent-3 (56.0%) type. Decumbent-1 and erect growth habits were missing from Spanish bunch and Valencia, respectively.

Branching pattern

As many as 96 accessions (49.2%) showed sequential, followed by 78 (40.0%) alternate, 17 (8.7%) and 4 (2.1%) with irregular with out flowering on main stem and irregular with flowering on main stem, respectively. Alternate branching pattern was predominated in subsp. *hypogaea* group *i.e.,* Virginia runner (84.8%) and bunch (75.0%) where as subsp. *fastigiata* group *i.e.,* Valencia (74.4%) and Spanish bunch (74.7%) was characterized by sequential branching pattern. None of the genotypes belonging to Spanish bunch showed irregular with flowering on main stem. Irregular without a flower on main stem was slightly higher in Spanish bunch (12.0%) than others (3 to 10.3%).

Stem hairiness

Both abundant and scarce type of stem hairiness were observed in the mini core but hairiness was scarce in 115 (59.0%) and abundant 80(41.0%) accessions. Interestingly, twenty accessions (51.3%) in Valencia exhibited abundant stem hairiness and it appeared distinct from the other botanical types.

Stem pigmentation

As many as 150 accessions (76.9%) did not possess stem pigmentation. Absent pigmentation was predominant in Spanish bunch (92%), Virginia runner (84.8%) and Virginia bunch (91.7%) types while, Valencia was distinct by having majority of the accessions (77.0%) with pigmentation.

Leaf let shape

Though eleven classes were observed in the mini core, wide elliptic (36.4%), oblong elliptic (20.0%) and narrow elliptic (15.9%) shapes were predominant followed by orbicular (9.2%), obovate (6.2%) and sub orbicular (5.6%) while others were less frequent (1 to 2.6%). Wide elliptic was also most predominant (27.2 to 42.7%)in all the four botanical types followed by oblong elliptic in Valencia (35.9%) and Virginia bunch (22.9%), narrow elliptic in Spanish bunch (21.3%) and Virginia runner (18.1%) orbicular was frequent in Virginia bunch (18.8%) and Virginia runner (18.1%) rare in Spanish bunch (4%) and absent in Valencia. Elliptic shape was observed in *fastigiata* group *i.e.,* Valencia (2.6%) and Spanish bunch (4.0%) but not in hypogaea group.

Flower colour

Maximum number of accessions (35.3%) exhibited golden yellow followed by canary yellow (33.8%) and lemon yellow (30.8%). The canary yellow was predominant in Valencia (53.8%) and golden yellow in Virginia bunch (50.0%).

Table 7: Frequency distribution of accessions with different morphological characters in mini core

SL. No	Character	Descriptor	Mini Core		Valencia		Spanish Bunch		Virginia Runner		Virginia Bunch	
			Freq	%	Freq	%	Freq	%	Freq	%	Freq	%
1	**Growth habit**	Decumbent-1	16	8.2	05	12.8	00	0	10	30.3	01	02.1
		Decumbent-2	70	35.8	20	51.3	08	10.7	17	51.5	25	52.1
		Decumbent-3	76	38.9	14	35.9	44	56.0	03	09.0	17	35.4
		Erect	33	17.0	00	00	25	33.3	03	09.0	05	10.4
		Total	**195**	**100**	**39**	**100**	**75**	**100**	**33**	**100**	**48**	**100**
2	**Branching pattern**	Alternate	78	40.0	04	10.3	10	13.3	28	84.8	36	75.0
		Sequential	96	49.2	29	74.4	56	74.7	03	09.0	08	16.7
		Irregular with flowers on main stem	4	02.0	02	05.1	00	0	01	03.0	01	02.1
		Irregular without flowers on main stem	17	08.7	04	10.3	09	12.0	01	03.0	03	06.3
		Total	**195**	**100**	**39**	**100**	**75**	**100**	**33**	**100**	**48**	**100**
3	**Stem hairiness**	Scarce	115	59.0	19	48.7	43	57.3	22	66.6	31	64.6
		Abundant	80	41.0	20	51.3	32	42.7	11	33.3	17	35.4
		Total	**195**	**100**	**39**	**100**	**75**	**100**	**33**	**100**	**48**	**100**
4	**Stem pigmentation**	Absence	150	76.9	09	23.8	69	92.0	28	84.8	44	91.7
		Presence	45	23.1	30	77.0	6	08.0	05	15.1	04	08.3
		Total	**195**	**100**	**39**	**100**	**75**	**100**	**33**	**100**	**48**	**100**
5	**Leaf let shape**	Cuneate	02	01.0	00	0	01	01.3	01	3.03	00	0
		Elliptic	04	02.1	01	02.8	03	03.8	00	00	00	0
		Lanceolate	02	01.0	00	0	0	0	00	00	02	04.2
		Narrow-elliptic	31	15.9	04	08.3	16	21.3	06	18.1	05	10.4
		Oblong elliptic	39	20.0	14	35.9	10	13.3	04	12.1	11	22.9
		Obovate	12	06.2	01	02.8	05	06.7	05	15.1	01	02.1
		Orbicular	18	09.2	0	0	03	04.0	06	18.1	09	18.8
		Ovate	05	02.6	02	05.6	0	0	01	3.03	02	04.2
		Sub orbicular	11	05.6	03	08.3	05	06.7	01	3.03	02	04.2
		Wide elliptic	71	36.4	14	35.9	32	42.7	09	27.2	16	33.3
		Total	**195**	**100**	**39**	**100**	**75**	**100**	**33**	**100**	**48**	**100**

Contd…..

SL. No	Character	Descriptor	Mini Core		Valencia		Spanish Bunch		Virginia Runner		Virginia Bunch	
			Freq	**%**	**Freq**	**%**	**Freq**	**%**	**Freq**	**%**	**Freq**	**%**
6	**Flower colour**	Lemon yellow	61	30.8	13	33.3	25	33.3	10	30.3	13	27.1
		Golden yellow	69	35.3	05	12.8	28	37.3	12	36.3	24	50.0
		Canary yellow	65	33.3	21	53.8	22	29.3	11	33.3	11	22.9
		Total	**195**	**100**	**39**	**100**	**75**	**100**	**33**	**100**	**48**	**100**
7	**Pod beak**	Absent	60	30.7	10	25.6	33	44.0	10	30.3	07	14.6
		Slight	83	42.5	20	51.2	23	30.7	16	48.4	24	50.0
		Moderate	37	19.0	06	15.4	14	18.7	07	21.2	10	20.8
		Prominent	10	05.1	02	05.1	03	04.0	00	00	05	10.4
		Very prominent	05	02.5	01	02.6	02	02.7	00	00	02	04.2
		Total	**195**	**100**	**39**	**100**	**75**	**100**	**33**	**100**	**48**	**100**
8	**Pod constriction**	None	12	06.1	05	12.8	03	04.0	01	03.0	03	06.3
		Slight	92	47.1	21	53.8	36	48.0	19	57.5	16	33.3
		Moderate	59	30.2	12	30.8	25	33.3	06	18.1	16	33.3
		Deep	30	15.4	01	02.6	11	14.7	06	18.1	12	25.0
		Very deep	2	01.0	00	00	0	0	01	03.0	01	02.1
		Total	**195**	**100**	**39**	**100**	**75**	**100**	**33**	**100**	**48**	**100**
9	**Pod reticulation**	Slight	53	27.1	10	25.6	25	33.3	08	24.2	10	20.8
		Moderate	83	42.5	14	35.9	35	45.7	13	39.3	21	43.8
		Prominent	41	21.0	10	25.6	12	16.0	07	21.2	12	25.0
		Very prominent	18	09.2	05	12.8	03	04.0	05	15.1	05	10.4
		Total	**195**	**100**	**39**	**100**	**75**	**100**	**33**	**100**	**48**	100
10	**Seed colour**	Tan	80	41.0	10	25.6	52	69.3	07	21.2	11	22.9
		Off white	5	02.5	01	02.6	03	04.0	00	00	01	02.1
		Rose	50	25.6	00	0	07	9.33	20	62.5	23	47.9
		Dark red	15	07.6	06	15.4	04	5.33	02	06.2	03	06.3
		Red	11	05.6	07	17.9	01	01.3	01	03.1	02	04.2
		Light red	13	06.6	08	20.5	03	04.0	00	0	02	04.2
		Brown	5	02.5	0	0	0	0	02	06.2	03	06.3
		Dark purple	12	06.1	06	15.3	06	06.6	0	0	01	02.1
		Salmon with purple flecks	2	01.0	01	02.7	00	0	01	03.1	00	0
		Red with white flecks	2	01.0	0	0	0	0	0	0	02	04.2
		Total	**195**	**100**	**39**	**100**	**75**	**100**	**33**	**100**	**48**	**100**

Pod beak

Among the 195 genotypes maximum number of genotypes 83 (42.5%) showed slight pod beak, 60 (30.7%) were beakless, 37 (19.0%) with moderate and 5 (2.5%) exhibited very prominent pod beak. Spanish bunch was predominated by the category without any pod beak (44.0%) but other three types *i.e.,* Valencia (51.2%), Virginia runner (48.4%) and Virginia bunch (50.0%) were dominated by slight pod beak. None of the genotypes belonging to Virginia runner showed prominent and very prominent pod beak.

Pod constriction

In the mini core subset, 92 genotypes (47.1%) showed slight pod constriction, 59 (30.2%) moderate, 30 (15.4%) deep and 2 (1.0%) very deep pod constriction and twelve (6.2%) exhibited no constriction. Slight pod constriction was predominated in all the four botanical types. The moderate constriction was least in Virginia runner (18.1%) as compared to other types (30.2% to 33.3%). deep constriction was less frequent in Valencia (2.6%) than in others 14.7% to 25.0%). Very deep pod constriction pattern was observed exclusively in subsp. *hypogaea* group *i.e.,* Virginia runner (3.0%) and bunch (2.1%) but not in *fastigiata* group.

Pod reticulation

As many as 83 genotypes (42.5%) showed moderate, 53 (27.1%) slight, 41(21.0%) prominent and18 (9.2%) very prominent reticulation in the mini core. The pattern of distribution was similar in all the botanical types except in Spanish bunch where in the very prominent reticulation was less frequent (4.0%) than in others (12.8 to 15.1%).

Seed colour

Ten seed colours were observed in the mini core. Majority of the genotypes, 80 (41.0%) exhibited tan, followed by rose (25.6%), dark red (7.6%), light red (6.6%), dark purple (6.1%) and red (5.6%) while other categories were less frequent (1 to 2.5%). Tan was predominant in Spanish bunch (69.3%) and Valencia (25.6%) where as rose in Virginia runner (62.5%) and Virginia bunch (47.9%). Different categories namely light red (20.5%), red (17.9%), dark red (15.4%) and dark purple (15.3%) were frequently observed in Valencia as compared to other botanical types. None of the genotypes belonging to Virginia runner and Valencia showed off white and rose colour, respectively. Red with white flecks was observed in Virginia bunch but not in other types. Brown was found in sub sp. *hypogaea i.e.,* Virginia runner (6.2%) and bunch (6.3%) but absent in subsp. *fastigiata.*

4.1.2 Principal component analysis

Principal component analysis (PCA) of the data was performed to investigate the importance of different traits in explaining multivariate polymorphism. The percentage of variation explained by the principal components (PCs) and the eigen are given in (Table 8). Out of nine, first five PCs contributed significantly and explained 71.645% variation. The principal component analysis indicated contribution of nine out ten characters by the PCs. The accessions were separated based on the stem hairiness and branching pattern by PC I, pod beak, pod constriction and pod reticulation (PC II), stem pigmentation, flower colour (PC III), seed colour (PC IV), flower colour and leaf let shape (PC V) (Table 9). Growth habit did not contribute any variation in mini core subset.

4.1.3 Shannon-Weaver diversity index

The diversity index (H') was calculated to measure phenotypic diversity for ten morphological characters in mini core and four botanical types (Table 10). Among the ten morphological traits seed colour (0.914) had highest H' while it was low for stem hairiness (0.2955) in the mini core. Among four botanical types, seed colour in Valencia (0.911) and Virginia bunch (0.778) and leaf let shape in Spanish bunch (0.618) and Virginia runner (0.797) showed highest H'. The diversity index (H') was lowest for stem pigmentation in Spanish bunch (0.147), Virginia bunch (0.162) and Virginia runner (0.22) while it was least for stem hairiness in Valencia (0.2983). The average diversity was maximum in Valencia (0.505), followed by Virginia bunch (0.498), Virginia runner (0.469) and least in Spanish bunch

Table 8. Total variance explained by PCA analysis in qualitative characters

Component	Initial Eigen values			Extraction sum of squared loadings		
	Total	% of variance	Cumulative %	Total	% of variance	Cumulative %
1	2.294	22.936	22.936	2.294	22.936	22.936
2	1.447	14.470	37.405	1.447	14.470	37.405
3	1.313	13.130	50.535	1.313	13.130	50.535
4	1.097	10.967	61.562	1.097	10.967	61.562
5	1.014	10.143	71.645	1.014	10.143	71.645
6	0.840	8.403	80.048			
7	0.797	7.974	88.022			
8	0.690	6.903	94.925			
9	0.507	5.075	100.000			
10	1.143E-16	1.143E-15	100.000			

Table 9. Component matrix revealed by PCA analysis in qualitative characters

Characters	Components				
	1	2	3	4	5
Growth habit	0.243	9.162E-02	0.436	0.488	-0.382
Branching pattern	0.899	0.398	7.738E-02	-1.93E-02	6.868E-02
Stem hairiness	0.899	0.398	7.738E-02	-1.93E-02	6.868E-02
Stem pigmentation	-7.26E-02	-0.225	0.711	-0.213	0.101
Leaflet shape	-0.130	-4.19E-02	-0.446	0.284	0.685
Flower colour	1.986E-03	-7.402E-02	-0.544	0.265	-0.559
Pod beak	-0.460	0.607	0.117	0.142	0.101
Pod constriction	-0.353	0.584	-0.113	-0.124	-7.82E-02
Pod reticulation	-0.486	0.582	0.178	-2.86E-03	0.136
Seed colour	-0.148	-0.123	0.231	0.791	0.178

Table 10: Shannon-Weaver diversity indexes for qualitative characters

Characters	Mini core	Valencia	Spanish bunch	Virginia runner	Virginia bunch
Growth habit	0.5441	0.4246	0.4033	0.4754	0.4825
Branching pattern	0.4381	0.3304	0.3236	0.2595	0.3370
Stem hairiness	0.2955	0.2983	0.2978	0.2795	0.2823
Stem pigmentation	0.3302	0.5113	0.1469	0.2201	0.1622
Leaf let shape	0.7832	0.6546	0.6176	0.7972	0.7519
Flower colour	0.4879	0.3855	0.4736	0.4767	0.4884
Pod beak	0.5558	0.5065	0.5574	0.4447	0.5742
Pod constriction	0.5319	0.4661	0.4959	0.4908	0.5789
Pod reticulation	0.5496	0.5652	0.5119	0.5695	0.5518
Seed colour	0.9135	0.9111	0.4938	0.6894	0.7781
Average ± se	0.54298 ± 0.012	0.50536 ± 0.026	0.43218± 0.0153	0.4696 ± 0.024	0.4984 ± 0.03

Table 11. Cluster composition based on similarity for qualitative characters

Sl. No	Cluster	Total	Valencia		Spanish Bunch		Virginia Runner		Virginia Bunch		Total Frequency
			No.	Frequency (%)	No.	Frequency (%)	No.	Frequency (%)	No.	Frequency (%)	
1	I	17	01	05.8	02	11.7	05	29.4	09	52.9	100
2	II	14	01	07.1	03	21.4	06	42.8	04	28.5	100
3	III	48	02	04.1	05	10.4	16	33.3	25	52.1	100
4	IV	13	03	23.0	08	61.5	00	0	02	15.3	100
5	V	43	06	13.9	34	79.1	00	0	03	6.99	100
6	VI	16	14	87.5	02	12.5	00	0	00	0	100
7	VII	22	0	0	18	81.8	01	4.5	03	13.6	100
8	VIII	06	1	16.6	0	0	03	50.0	02	33.3	100
9	IX	16	11	68.7	03	18.7	02	12.5	00	0	100
	Total	195	39		75		33		48		

Table 12. Distribution of accession in different clusters based on similarity for qualitative characters

Clusters	Total	Valencia	Spanish bunch	Virginia runner	Virginia bunch
I	17	ICG 14127	ICG 6263,4955,	ICG 2511, 862, 928, 3992, 4156,	ICG 532, 9666, 513, 1668, 11322, 5663, 3053, 9961, 2772
II	14	ICG 397	ICG 12988, 1137, 4716	ICG 9037, 2925, 7243, 6813, 9905,10479,	ICG 6892, 6703, 7000, 13942,
III	48	MN 1-28, MN 1-35	ICG 9249, 5195, 9809, GPBD-4, 4543	ICG 76, 4389, 875, 7153,8490, 13723, 11457, 11109, 163, 2777,4343, 13941, 2773, 4412, 4998, 11219,	ICG 5662, 6402, 12672, 188,5286,13787, 5327, 12276, 10890, 6667, 14466, 6706, 10185, 15287, 4527, 5991, 14475, 5745, 4598, 8285, 14008, 14482, 721,1415,9249
IV	13	ICG 3673, 10036, 7181	ICG 6654, 118, 12697, 4911, 3746, 14118, 6263,12682		ICG 9777, 11426
V	43	ICG 1142, 8517, 14106,15042, 12000, M 28-2	ICG 3584, 1973, 4729, 4684, 1415, 8083, 2106, 3421, 1519, 3240, 4750, 434, 81, TAG 24, 11515, 2019, 334, 13603, 9157, 9418, 11249, 5494, 10554, 7969, 11687, JL 24, 8567, 12879, 10384, 5779,14985,15042,15419, R 9227		ICG 15309, 115, 111

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Clusters	Total	Valencia	Spanish bunch	Virginia runner	Virginia bunch
VI	16	ICG 9315, 10092, 4670, 6888, 332, 5475, 6201, 5609, 2381, 10474, 10565, 15190, 3681, 8106	ICG 6407, 3775		
VII	22		ICG 13491, 442, 36, 7906, 9507, 1711, 14630, 5236, 3343, 3102, 12621, 4543, 6236, 12189, 11651, 10554, 3027,13942	ICG 12370	ICG 11855, 4538, 9842,
VIII	6	ICG 11088		ICG 8760, 13099, 2857	ICG 6057, 6913
IX	16	ICG 13856, 13858, 12625, 1470, 5221, 1274, 11144, 156/M-13, 6027, 6646, 15042	ICG 7190, 6375, 2738	ICG 1862, 5827	

Table 13. Representation of various botanical types in different clusters based on similarity for qualitative characters

Clusters	Valencia		Spanish bunch		Virginia runner		Virginia bunch	
	No.	Frequency (%)	No.	Frequency (%)	No.	Frequency (%)	No.	Frequency (%)
I	1	02.5	2	02.6	5	15.1	9	18.7
II	1	02.5	3	04.0	6	18.1	4	08.3
III	2	05.1	5	06.6	16	48.4	25	52.1
IV	3	07.6	8	10.6	0	0	2	04.1
V	6	15.3	34	45.3	0	0	3	06.2
VI	14	35.8	2	02.6	0	0	0	0
VII	0	0	18	24.0	1	03.0	3	06.2
VIII	1	2.5	0	0	3	09.1	2	04.1
IX	11	28.2	3	04.0	2	06.0	0	0
Total	39	100	75	100	33	100	48	100

(0.432). Different subsets representing botanical types showed more diversity than mini core set for some morphological traits Virginia bunch recorded higher diversity for pod beak, pod constriction, pod reticulation and flower colour, Virginia runner for pod reticulation and leaf let shape, and Valencia for pod reticulation and stem pigmentation. Spanish bunch group did not show higher diversity for any of the traits.

4.1.4 Similarity coefficient

The observation recorded on ten qualitative characters *viz.,* growth habit, branching pattern, stem hairiness, flower colour, seed colour, pod beak, pod constriction and pod reticulation were subjected to simple matching coefficient. The genetic similarity between 195 genotypes for qualitative characters was assessed by similarity coefficient (Sij) and it ranged from 0.10 to 1.00.The SPSS hierarchical cluster analysis revealed nine clusters at Sij of 18.0. Maximum numbers of genotypes (48) were grouped in cluster III and minimum (6) in cluster VIII. The cluster VI (87.5%) and IX (68.7%) were dominated by Valencia types, cluster IV (61.5%), V (79.1%) and VII (81.8%) by Spanish bunch, cluster II (42.8%), VIII (50.0%) by Virginia runner, cluster I (52.9%) and III (52.1%)by Virginia bunch. (Table 11 & 12). Maximum number of Valencia genotypes (35.8%) was grouped in cluster VI, Spanish bunch (45.3%) in cluster V, Virginia runner (48.4%) and Virginia bunch (52.1%) in cluster III. (Table 13)

4.2 EVALUATION OF AGRONOMICAL CHARACTERS

4.2.1 Analysis of variance

Data on quantitative characters *viz.,* days to 50% flowering, shelling percent, sound mature kernel, yield per plant, *Aspergillus* seed colonization, late leaf spot, lectin activity and protein were analyzed for lattice square design following REML (residual maximum likelihood) analysis with replication as fixed and entries as random on GENSTAT 5.1. The REML analysis of data revealed significant variation due to genotypes for days to 50 per cent flowering, yield per plant, and test weight and lectin activity (Table 14).

4.2.2 Productivity traits

The mean and range for each quantitative character in mini core and four botanical varieties are listed in Table 15.

Days to 50% flowering

Virginia runner type took more days (35) for flowering followed by Spanish bunch (34 days), Valencia (32 days) and Virginia bunch (31 days) as compared to mini core (33 days). In mini core, days to flowering ranged from 28 days (ICG 188,532 and 3027) to 42 days (Fig.1) and the range for days to 50 % flowering in all four botanical types was comparable to mini core. Among the checks GPBD-4 (32 days) was late to flower followed by mutant 28-2 (30 days), TAG-24 (29days) and JL-24 (29 days).

Yield per plant

As an indicator of yield potential, Valencia (15.25) was much better than the mini core (13.77) and other three botanical types (11.96 to 12.21 g). Pod yield per plant ranged from 5.45 g (ICG 6892) to 33.50 g (ICG 1862) in mini core (Fig .1) and it was high in Spanish bunch (7.36 to 33.0 g) followed by Valencia (7.8 to 21.9 g), Virginia runner (5.6 to 20.31g) and Virginia bunch (5.44-19.46 g). Among the checks GPBD-4 (20.25 g) recorded highest followed by mutant 28-2 (18.31 g), JL-24 (18.06 g) and TAG 24 (11.99g). The Spanish bunch types *viz.,* ICG 12697 9(33.01 g), ICG 3240 (30.14 g), ICG 4716 (22.76 g), ICG 14985 (22.04 g) were superior to the best check, GPBD-4.

Shelling per cent

The mean shelling percent was high in Valencia (72.58%) followed by Spanish bunch (71.94%), Virginia bunch (68.46%) and Virginia runner (68.18%) as compared to the mini core set (70.89%). The shelling percent ranged from 61.23 (ICG 7243) to 81.75 (ICG 3673) in the mini-core (Fig.1) and it appeared to be similar in all four botanical types. Among the checks, GPBD-4 (78.75%) recorded highest shelling percent followed by JL-24 (77.88%), TAG 24

Table 14. Analysis of variance. Residual maximum likelihood analysis (REML)

Character	Genotype		Residual	
	Variance	Standard error (se)	Variance	Standard error (se)
Days to 50 % flowering	10.673**	1.202	12.320	1.020
Yield per plant	3.520*	1.500	16.690	1.760
Shelling per cent	1.050	2.140	27.590	2.910
Sound mature kernel	0.000	1.910	26.350	2.740
Test weight	11.760*	4.600	48.760	5.180
Aspergillus	0.035	0.028	1.012	0.113
Late leaf spot	0.156	0.106	1.238	0.132
Lectin activity	805812.0**	289592.0	2865940.0	312588.0
Protein	04.20	30.40	400.40	42.60

Table 15. Mean and range of quantitative characters in mini core and four botanical types

Sl. No.	Variable	Mini core			Valencia			Spanish bunch			Virginia Runner			Virginia bunch		
		Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
1	DFG	28	42	33.37	29	40.5	31.81	29	41	33.51	30	41	35.04	28	36	31.33
2	YPP (g)	5.44	33.50	13.77	7.8	21.90	15.25	7.36	33	11.96	5.62	20.31	12.21	5.44	19.46	11.94
3	SPP (%)	61.23	81.74	70.89	64.10	81.74	72.58	62.71	81.6	71.94	61.28	80.09	68.18	61.96	77.30	68.46
4	SMK (%)	75.79	98.13	93.15	81.79	97.74	94.16	75.79	98.13	93.32	83.52	96.51	92.12	86.36	96.92	92.96
5	TWT (g)	21.89	64.89	41.46	27.18	60.81	40.78	21.89	52.92	37.7	29.87	64.26	42.31	27.49	64.89	44.35
6	ASP	1	4	3.58	1.02	4.00	3.61	1.08	4	3.65	1.4	4.0	3.58	1.1	4	3.56
7	LLS	1.67	7.33	4.65	3.67	7.33	5.19	2.5	7.33	5.47	2.83	5.68	3.91	2.5	6.26	4.04
8	Lectin (HAU/mg of protein)	693.58	14360.52	4081.01	1244.66	11288.48	4606.37	1348.62	14360.52	3782.87	2409.96	11384.11	3839.89	2394.95	10188.18	4583.69
9	Protein (%)	11.9	28.8	16.3	18.1	28.8	16.5	11.9	20.4	14.8	12.4	19.5	16.6	13.7	20.4	17.2

DFG : Days to 50% flowering

YPP : Yield per plant

SPP : Shelling per cent

SMK : Sound mature kernel

TWT : Test weight

ASP : *Aspergillus* seed colonization

LLS : Late leaf spot

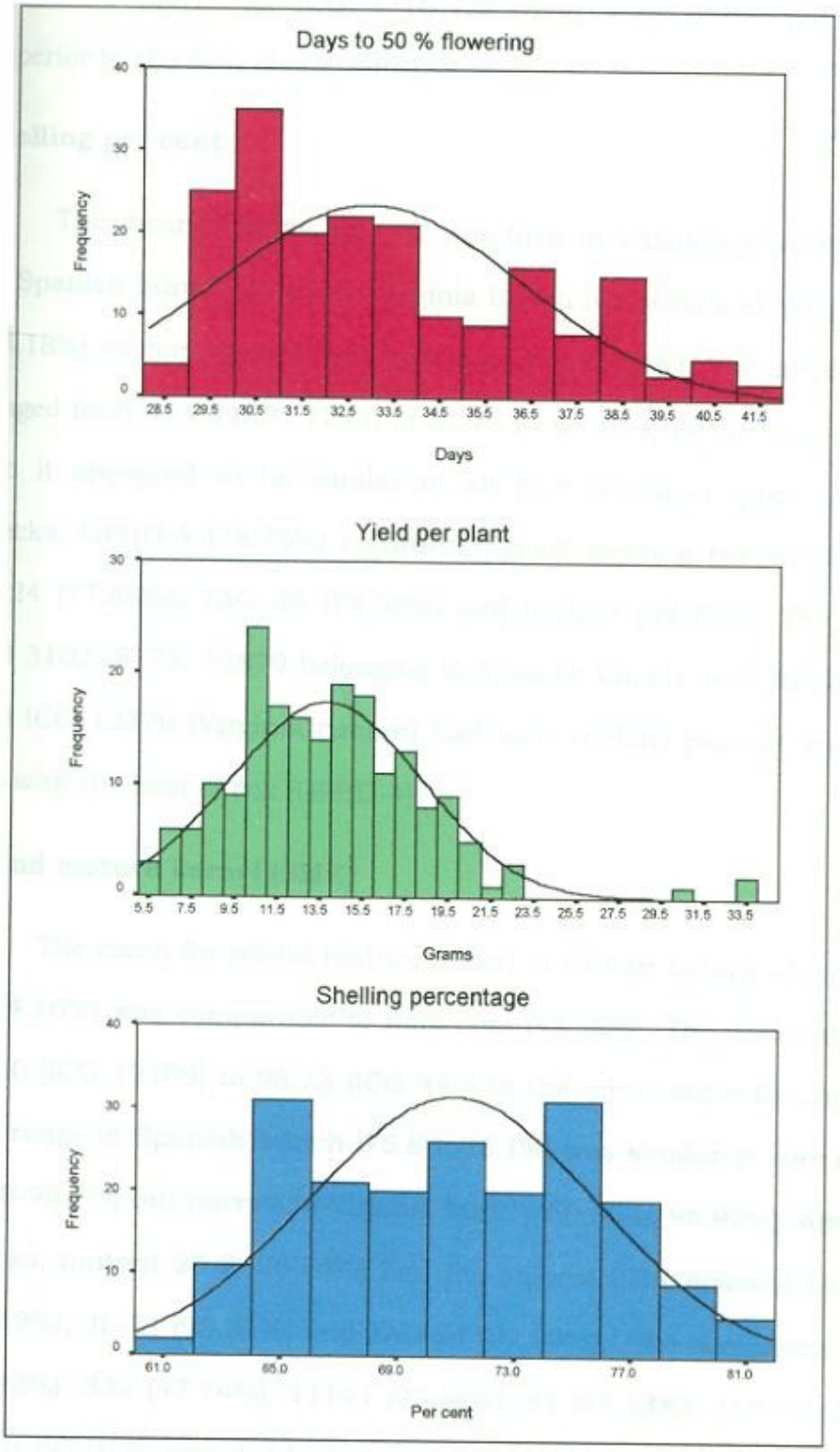


Fig. 1. Distribution of accessions for different quantitiatve traits in mini core

(74.20%) and mutant (69.73%). The genotypes ICG 3102, 5779, 12879 belonging to Spanish bunch, ICG 3673 (Valencia) and ICG 12370 (Virginia runner) had high shelling percent and were on par with the best check (GPBD-4).

Sound mature kernel (SMK)

The mean for sound mature kernel in all four botanical types (92.12 to 94.16%) was comparable to mini core (93.15%). The SMK ranged from 75.80 (ICG 12189) to 98.13 (ICG 442) in the mini core collection (Fig .1). The range in Spanish bunch (75.8 to98.1%) was similar to core collection (75.8-98.1%) but narrow in Virginia bunch (86.36 to 96.92%). Among the checks, mutant 28-2 (96.54%) had the highest SMK followed by GPBD-4 (95.19%), JL-24 (93.31%) and TAG 24 (92.29%). The accessions ICG 442 (98.13%), 332 (97.74%), 11141 (97.38%), 81 (97.23%), 115 (97.22%) and 11651 (97.01%) revealed high sound mature kernel percent.

Test weight (TWT)

The mean test weight was higher in Virginia bunch (44.35 g), followed by Virginia runner (42.31 g), Valencia (40.78 g) and Spanish bunch (37.7 g) in comparison to mini core (41.46 g). The overall test weight in mini core ranged from 21.90 g (ICG 1415) to 64.90 g (ICG 5662) (Fig .1). The range was different in four botanical types *viz.,* Valencia (27.18 to 60.81 g), Spanish bunch (21.89 to 52.92), Virginia runner (29.87 to 64.26 g) and Virginia bunch (27.49 to 64.89). Among the checks, mutant 28-2 (52.86 g) recorded high-test weight followed by JL-24 (50.25 g). The accessions ICG 6027 (60.82 g), MN 1-28 (64.11 g), MN1-35 (61.48 g) belonging to Valencia group, ICG 5662 (64.90 g), ICG 9777 (61.96 g) of Virginia bunch, and ICG 11219 (58.58 g), ICG 1862 (63.99 g), ICG 8760 (64.26g) of Virginia runner type were superior to the checks.

4.2.3 Disease resistance

Aspergillus seed colonization

The mean for Aspergillus seed colonization in four botanical types (3.56 to 3.65) was comparable to minicore (3.58). In the mini core, *Aspergillus* seed colonization ranged from 1 (ICG 8760) to 4 (Fig .1). The accessions, ICG 6027, ICG 3673, MN 1-35 of Valencia types (1.02 to 1.16), ICG 149854 of Spanish bunch (1.08), ICG 8760 of Virginia runner (1.0) and ICG 13787 of Virginia bunch (1.10) were found resistant by recording lower infection than the resistant check TG 49 (1.16) (Plate 4). While, moderate resistance to seed colonization was observed in ICG 12625 (2.04), ICG 1862 (2.16) of Spanish bunch, ICG 76 (2.40), ICG 2381 (2.32) of Virginia runner, ICG 6402 (2.24), ICG 12276 (2.29) of Virginia bunch and MN 1-28 of Valencia (2.08). Many genotypes were susceptible to *Aspergillus* seed colonization and were on par with the susceptible check TMV-2 (4.0).

Late leaf spot

The means in subsp. *fastigiata* viz. Valencia (5.19) and Spanish bunch (5.47) were slightly higher as compared to subsp *hypogaea viz.,* Virginia runner (3.91) and Virginia bunch (4.04). Late leaf spot ranged from 1.67 (MN 1-35) to 7.33 (ICG 397) in mini core (Fig .1) and it was also recorded in four botanical varieties *i.e.,* in Valencia (3.67 to 7.33), Spanish bunch (2.5 to 7.33), Virginia runner (2.83 to 5.68), and Virginia bunch (2.5 to 6.26). Among the checks GPBD-4 (1.62) recorded highest resistance followed by mutant (2.67) while TAG 24 was more susceptible (8.84). The accessions, ICG 12625 (2.33), 15419 (2.50), 12697 (2.50), 12682 (2.83) and ICG 4716 (3.00) of Spanish bunch, 76 (2.83), 8760 (2.84), ICG 2857 (3.00), ICG 4412 (3.00) and ICG 9905 (3.00) of Virginia runner, ICG 12672 (2.50), 13787 (2.50), 3027 (2.83) 532 (2.83), 6706 (3.00) and ICG 14475 (3.00) of Virginia bunch revealed resistance against late leaf spot. The genotypes MN 1-28 (1.84) and MN 1-35 (1.67) of Valencia were on par with GPBD-4 (1.62).

Contd.....

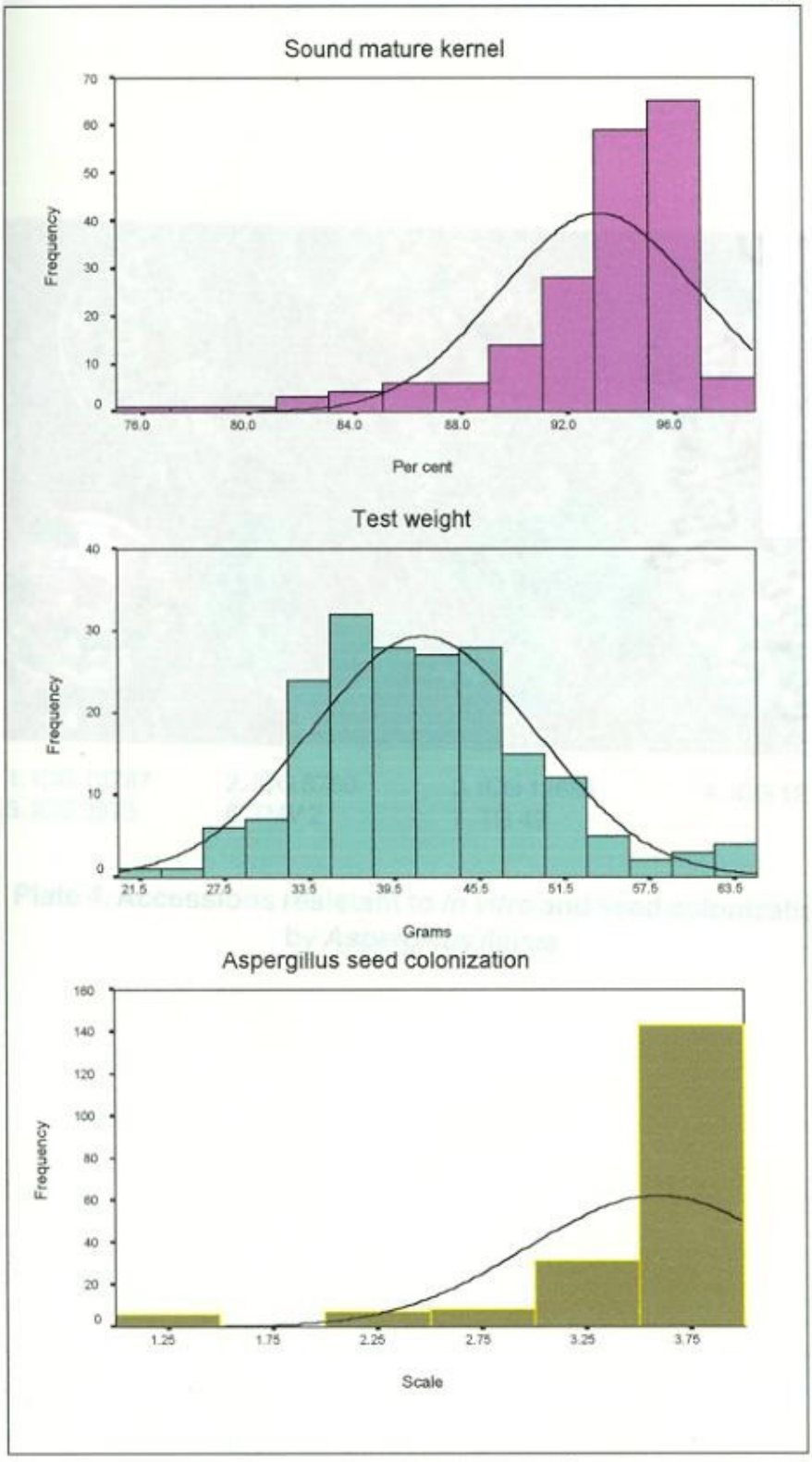
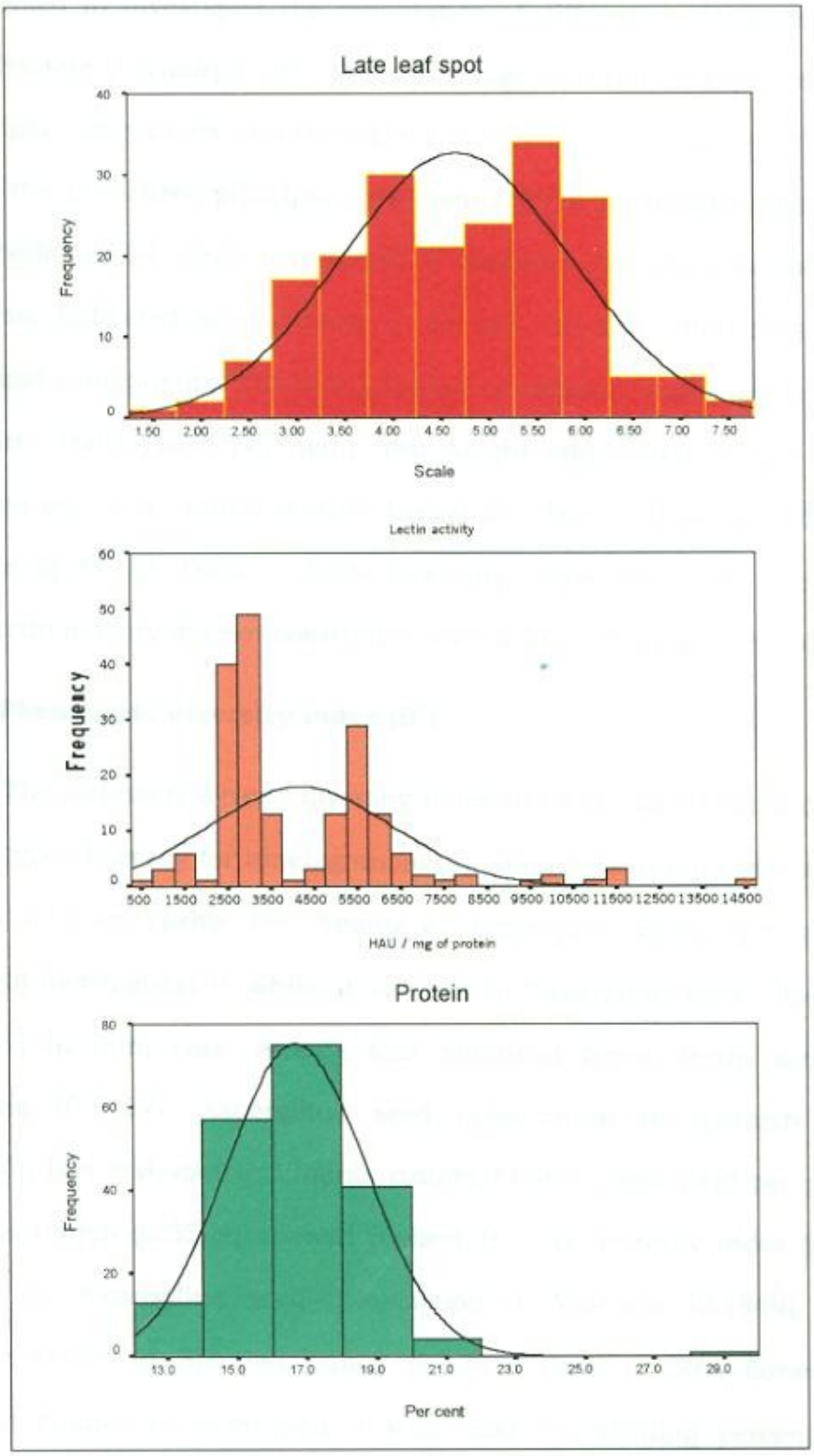

Sound mature kernel
Frequency
70
60
50
40
30
20
10
0
76.0
80.0
84.0
88.0
92.0
96.0
Per cent
Test weight
Frequency
40
30
20
10
0
21.5
27.5
33.5
39.5
45.5
51.5
57.5
63.5
Grams
Aspergillus seed colonization
Frequency
160
140
120
100
80
60
40
20
0
1.25
1.75
2.25
2.75
3.25
3.75
Scale

Plate 4. Accessions resistant to in vitro and seed colonization by Aspergillus flavus


Late leaf spot
Frequency
Scale
Lectin activity
Frequency
HAU / mg of protein
Protein
Frequency
Per cent

4.2.4 Chemical traits

Low lectin activity

The mean was highest in Valencia (4606.37 HAU/mg), followed by Virginia bunch (4583.69 HAU/mg), Virginia runner (3839.89 HAU/mg) and Spanish bunch (3782.87 HAU/mg). It ranged from 693.58 HAU/mg (ICG 11088) to 14360.52 HAU/mg (ICG 4955) (Fig.1) with a mean 4081.01 HAU/mg in mini core. The lectin activity range was different among the four botanical types *viz.,* in Valencia, (1244.66 to 11288.48 HAU/mg), Spanish bunch (1348.62 to14360.52 HAU/mg), Virginia runner (2409.96 to 11384.11 HAU/mg) and Virginia bunch type (2394.95 to 10188.18 HAU/mg). The accessions, ICG 11088 (693.58 HAU/mg), 1470 (1188.91 HAU/mg), 3681 (1244.66 HAU/mg), 6646 (1249.12 HAU/mg), 6027 (1259.62 HAU/mg), 6263 (1348.62 HAU/mg) and 12625 (1425.01 HAU/mg) revealed low lectin activity. ICG 81 (1448.33 HAU/mg) was on par with the best check JL 24 (1489.97 HAU/mg).

Protein content

The mean was highest in Virginia bunch (17.3%) followed by Virginia runner (16.6%), Valencia (16.5%) and least in Spanish bunch (14.8%). In mini core, the protein content ranged from 11.9% (ICG 7969) to 28.8% (ICG 8517) (Fig.1). The range was highest in Valencia (18.1% to 28.5%) as compared to other botanical types *i.e.,* Spanish bunch (11.9% to 20.4%), Virginia runner (12.4% to 19.5%) and Virginia bunch (13.7% to 20.4%). Among the checks, GPBD-4 revealed high protein content (18.4%) followed by mutant 28-2 (17.9%), TAG 24 (17.9%) and JL 24 (15.8%). The accessions, ICG 8517 (28.8%) of Valencia, 6703 (20.4%), 13941 (20.4%) of Spanish bunch, 513 (20.2%) and 14475 (20.1%) of Virginia bunch revealed higher protein content than the checks (15-18%).

4.2.5 Principal component analysis

Principal component analysis (PCA) of the agronomical data was performed to investigate the importance of different traits in explaining multivariate polymorphism. The percentage of variation explained by the principal components and the eigen values are given in (Table 16). Out of nine, the first three principal components (PCs) contributed significantly and explained 54.556% of variation in mini core. The principal component analysis indicated contribution of six out of nine characters by the Principal components. The first principal component separated accessions on three traits (yield per plant, test weight and sound mature kernel), shelling per cent, sound mature kernel and late leaf spot by PC II and protein by PC III. Days to 50 % flowering, *Aspergillus* seed colonization and lectin activity did not contribute any variation in mini core (Table 17).

4.2.6 Phenotypic diversity index (H')

The Shannon-Weaver diversity index (H') was calculated to compute phenotypic diversity for nine agronomical characters in mini core and four botanical types (Table 18). Among all agronomic traits, late leaf spot (0.6143) had highest H' while, it was low for Aspergillus seed colonization (0.3657) in mini core. Among four botanical types, lectin activity in Valencia (0.5157), Aspergillus seed colonization in Spanish bunch (0.5608), late leaf spot in Virginia runner (0.5890) and yield per plant in Virginia bunch (0.5504) showed highest H'. The diversity index (H') was lowest for Aspergillus seed colonization in Valencia (0.1849), sound mature kernel in Spanish bunch (0.1387), days to 50% flowering in Virginia runner (0.2338) and it was least for shelling percentage in Virginia bunch (0.2390). The average diversity was high in Virginia runner (0.4511) followed by Virginia bunch (0.4328), Spanish bunch (0.4261) and least in Valencia (0.2957). Spanish bunch (0.5608), Virginia runner (0.5224) and Virginia bunch (0.4065) recorded higher diversity for Aspergillus seed colonization than mini core (0.3657).

Table 16. Total variance explained by PCA in quantitative character

Component	Initial Eigenvalues			Extraction sum of squared loadings		
	Total	% of variance	Cumulative %	Total	% of variance	Cumulative %
1	1.983	22.028	22.028	1.983	22.028	22.028
2	1.828	20.307	42.335	1.828	20.307	42.335
3	1.100	12.221	54.556	1.100	12.221	54.556
4	0.937	10.409	64.965			
5	0.885	9.831	74.797			
6	0.699	7.767	82.564			
7	0.541	6.015	88.579			
8	0.529	5.880	94.459			
9	0.499	5.541	100.00			

Table 17. Component matrix revealed by PCA in quantitative character

Characters	Components		
	1	2	3
Days 50% flowering	0.321	-0.332	-0.377
Yield per plant	0.762	0.186	0.160
Shelling percentage	0.373	0.693	-2.80E-02
Sound mature kernel	0.534	0.538	0.191
Test weight	0.627	-0.378	0.352
Aspergillus	-0.492	0.401	-1.82E-02
Late leaf spot	-0.159	0.764	-7.81E-02
Lectin	-0.407	0.11	0.430
Protein	-0.205	-0.114	0.761

Table 18. Shannon-Weaver diversity indexes for quantitative characters in core collection

Characters	Mini core	Valencia	Spanish bunch	Virginia runner	Virginia bunch
Days to 50 % flowering	0.5543	0.2139	0.3389	0.2338	0.2863
Yield per plant	0.5749	0.2417	0.4959	0.5471	0.5504
Shelling per cent	0.6128	0.3649	0.2137	0.3133	0.2390
Sound mature kernel	0.5264	0.2812	0.1387	0.3540	0.3833
Test weight	0.6038	0.2664	0.5003	0.4524	0.5094
Aspergillus	0.3657	0.1849	0.5608	0.5224	0.4065
Late leaf spot	0.6143	0.2664	0.5549	0.5890	0.5421
Lectin activity	0.5729	0.5157	0.4788	0.5104	0.4683
Protein	0.5861	0.3740	0.4174	0.4270	0.4026
Average ± se	0.56298 ± 0.005	0.29577 ± 0.01	0.42617 ± 0.017	0.45114 ± 0.019	0.43287 ± 0.014

Table 19. Clusters composed based on the Euclidian distance matrix in quantitative characters

Sl. No	Cluster	Total	VL		SB		VR		VB		Total frequency
			No.	Frequency (%)	No.	Frequency (%)	No.	Frequency (%)	No.	Frequency (%)	
1	I	153	31	20.2	63	41.1	17	11.1	42	27.4	100
2	II	31	03	09.6	07	22.5	16	51.6	05	16.1	100
3	III	02	02	100	00	00	0	00	0	00	100
4	IV	08	03	37.5	05	62.5	0	00	0	00	100
5	V	01	00	00	00	00	0	00	01	100.0	100
	Total	195	39		75		33		48		

VL: Valencia SB: Spanish bunch VR: Virginia runner VB: Virginia bunch

Table 20. Representation of various botanical types in different clusters based on Euclidian distance for quantitative characters

Clusters	Valencia		Spanish bunch		Virginia runner		Virginia bunch	
	No.	Frequency	No.	Frequency	No.	Frequency	No.	Frequency
I	31	79.4	63	84.0	17	51.51	42	87.5
II	3	07.6	07	09.3	16	48.48	5	10.4
III	2	05.1	0	0	0	0	0	0
IV	3	07.6	05	06.6	0	0	0	0
V	0	0	0	0	0	0	1	02.0
Total	39	100	75	100	33	100	48	100

4.2.7 Euclidian distance

The data on nine quantitative characters *viz.,* days to 50% flowering, shelling percentage, sound mature kernel, test weight, yield per plant, *Aspergillus* seed colonization, late leaf spot, lectin activity and protein content were utilized to compute dissimilarity among the accessions. The genetic dissimilarity between 195 genotypes for quantitative characters was assessed by Euclidian distance. The SPSS hierarchical cluster analysis revealed five distinct clusters at the distance value of 5.2. Maximum number of genotypes (153) were grouped in cluster I and minimum (1) in cluster V (Table 19). Cluster III (100%) dominated by Valencia, cluster I (41.1%) and cluster IV (62.5%) by Spanish bunch, cluster II (51.6%) by Virginia runner and cluster V (100%) by Virginia bunch. Maximum number of Valencia genotypes (79.4%), Spanish bunch (84.0%), Virginia runner (51.5%) and Virginia bunch (87.5%) were grouped in cluster I itself (Table 20) A number of subgroups were observed in cluster I. The sub group of accessions, ICG 6057, 13942, 5327, 11858, 532, 4527, 188, 3053, 6913, 12672, 6766, 6667 and 5745 belonged to Virginia bunch type were resistance to late leaf spot. This group included ICG 532 that had highest test weight. The cluster I consisted the genotype, ICG 11088 with low lectin activity and ICG 442 with high sound mature kernel percent. The accession ICG 4955 with high lectin activity, ICG 5662 with high-test weight were found in cluster II. Cluster III included MN 1-28, MN 1-35 (Valencia type) and characterized for late leaf spot, *Aspergillus* resistance and high-test weight. The cluster IV included the variety GPBD-4 and ICG 8760, a source of late leaf spot and *Aspergillus* colonization resistance respectively. The genotype ICG 3673 with high shelling percentage also found in this cluster. The genotype ICG 13787 from Nigeria was distinctly grouped in cluster V apart from other genotypes and they well characterized by resistance to *Aspergillus* seed colonization resistance and high-test weight. There was no association of genotypes based on the geographical origin.

4.3 THE GOWER'S SIMILARITY COEFFICIENT

The genetic similarity between 195 genotypes for combined data of ten qualitative and nine quantitative characters was assessed by Gower's similarity coefficient (Sij). The hierarchical cluster analysis revealed nine clusters at the distance of 7.1. Maximum number of genotypes (82) were grouped in cluster II followed by cluster I (80), cluster III (14) and cluster IV (13) and other clusters had only one to two genotypes. The cluster I was dominated by Spanish bunch (72.5%), followed by Valencia (23.7%). The cluster II was predominant with Virginia bunch (48.7%) followed by Virginia runner (32.1%). The cluster III (50.0%) and cluster IV (53.8%) had more Valencia accessions (Table. 21). Nineteen (48.7%) of 39 accessions of Valencia and 58 (77.3%) of 75 Spanish bunch were grouped in cluster I, while 27 (81.81%) of 33 Virginia runner and 40 (83.33%) of 48 Virginia bunch grouped in cluster II (Table. 22) Most of the genotypes in cluster I had tan seed colour, wide elliptic leaf let shape, high shelling percentage, sound mature kernel and were more susceptible to *Aspergillus* seed colonization.

At a distance of 2.8, the cluster I was split into two sub groups that originated from the same geographical area (India). The first subgroup consisting of ICG 118, M 28-2 and 12697 differed distinctly from second subgroup *viz.,* ICG 2106, 3421 and 1519 in terms of late leaf spot resistance, days to 50 % flowering, growth habit and seed colour. ICG 6407, 9315 and 10092 showed the closest similarity among all the accessions (1.10) ICG 3584 and GPBD-4 were grouped together at distance of 3.1 based on same botanical type (Spanish bunch) and origin (India).

Cluster II included 40 accessions (47.6%) of Virginia bunch type followed by Virginia runner (27), Spanish Bunch (14) and Valencia (3). The majority of the genotypes in this group had low yield per plant and resistance to late leaf spot. ICG 3053 and 9961, which had sequential branching pattern, orbicular leaf let shape and rose seed color. The genotypes with highest sound mature kernel (ICG 442) and lectin activity (ICG 4955) were found in this cluster. In cluster III (a total 14 accessions), there are six accessions (ICG 1274, 11144, 13856, 13858, 2738 and 5221) from Valencia group including the two earliest flowering accession (ICG 13856 and 13858) from the same geographical origin (Uganda). ICG 11088 (accession from Peru) with low lectin activity was also included in this cluster.

Table 21. Clusters composed based on the Gower similarity coefficient in quantitative characters

Sl. No	Cluster	Total	Valencia		Spanish Bunch		Virginia Runner		Virginia Bunch	
			No.	Frequency (%)	No.	Frequency (%)	No.	Frequency (%)	No.	Frequency (%)
1	I	80	19	23.7	58	72.5	0	00	03	03.7
2	II	82	04	04.8	11	13.4	27	32.9	40	48.7
3	III	14	07	50.0	02	14.2	3	21.4	02	14.2
4	IV	13	07	53.8	02	15.4	2	15.4	02	15.4
5	V	01	0	0	00	0	0	00	01	100.0
6	VI	01	0	0	00	0	1	100	00	0
7	VII	01	1	100	00	0	0	00	00	0
8	VIII	02	1	50.0	01	50.0	0	00	00	0
9	IX	01	0	0	01	100	0	00	00	0
	Total	195	39		75		33		48	

Table 22. Representation of various botanical types in different clusters based on Gower similarity coefficient for quantitative characters

Clusters	Valencia		Spanish bunch		Virginia runner		Virginia bunch	
	No.	Frequency	No.	Frequency	No.	Frequency	No.	Frequency
I	19	48.7	58	77.3	0	0	3	06.2
II	4	10.2	11	14.6	27	81.8	40	83.3
III	7	17.9	2	02.6	3	09.0	2	04.1
IV	7	17.9	2	02.6	2	06.0	2	04.1
V	0	0	0	0	0	0	1	02.1
VI	0	0	0	0	1	03.0	0	0
VII	1	02.5	0	0	0	0	0	0
VIII	1	02.5	1	01.3	0	0	0	0
IX	0	0	1	01.3	0	0	0	0
Total	39	100	75	100	33	100	48	100

Cluster IV was dominated by Valencia type (7) and also represented by the major quantitative characters *viz.,* highest shelling percentage (ICG 3673), highest test weight (ICG 5662) and ICG 8760 with resistance *Aspergillus* seed colonization. MN 1-28 and MN 1-35 showed Decumbent-3 growth habit and grouped together. ICG 12625 and ICG 1862 with late leaf spot resistance grouped separately and formed a distinct cluster (VIII). Cluster V (ICG 188), VI (2925), VII (ICG 115) and IX (15419) formed distinct solitary clusters.

4.4 MOLECULAR DIVERSITY IN GROUNDNUT GERMPLASM

One-ninety five genotypes were subjected to RAPD assay to assess the molecular diversity. Twenty primers OPK 09, OPK 14, OPA 19, OPC 15, OPC 09, OPC 13, OPB 11, OPF 09, OPJ 06, OPV 16, OPA 15, OPA 20, OPF 07, OPA 12, OPJ 17, OPC 03, OPV 15, OPC 06, OPF 10 and OPA 17 were employed for the purpose.

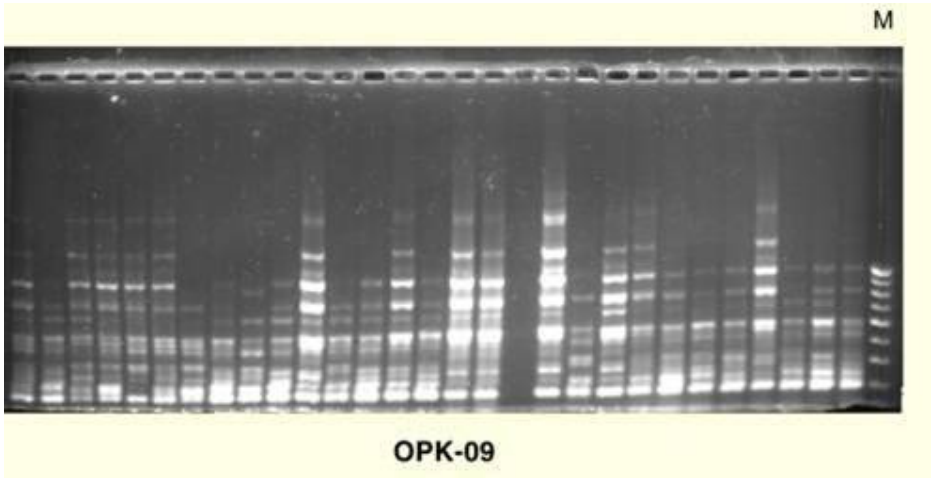
Number of amplified fragments ranged from 3 to 11 in a given primer. On an average, 6.7 bands per primer were amplified and all the bands were polymorphic. But when different sub groups representing botanical types were considered some bands in different primers failed to show polymorphism. The primers OPF 07 and OPV 15 have shown some monomorphic bands in all the four botanical varieties. The primers OPC 03 and OPA 17 in Valencia, OPF 07 and OPV 15 in Spanish bunch, OPA 17, OPC 03, and OPC 06 in Virginia runner and OPV 15, OPC 06, OPJ 17, OPA 17 (85.7%) and OPF 09 (87.5%) in Virginia bunch have shown monomorphic bands in different botanical types.

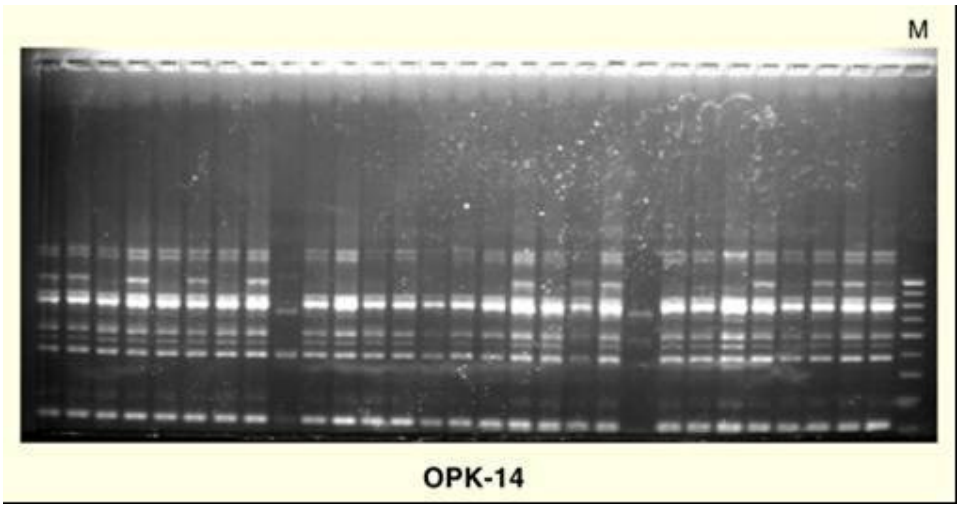
The band profiles obtained with 20 primers are summarized in Table 23 Twenty primers on the 195 genotypes generated a total of 135 amplified fragments, out of which 129 (6.45%) in Valencia, 132 (6.6%) in Spanish bunch, 130 (6.5%) in Virginia runner and 126 (6.3%) in Virginia bunch showed polymorphic bands. Two to four major fragments were amplified in any given sample, along with a number of bands of lesser intensity (Plate 5).

The matrix of genetic distances for molecular data was subjected to hierarchical cluster analysis in SPSS. A phonogram derived from Jaccard's similarity coefficient revealed considerable genetic dissimilarities were observed among the accession and at least six clusters were identified. The maximum number of genotypes were grouped in cluster I (88), followed by cluster III (40), cluster II (25), cluster IV (22), cluster V (19) and one in cluster VI (Table 24 and 25). Within each cluster many genotypes tended to group on the basis of botanical types. There were two accessions (ICG 8285 and 6667) belonging to Virginia bunch originating from the USA and were showing close similarity (2.0), the accessions (ICG 3102, 1519, 3421 and 1137) belonging to Spanish bunch from India were similar at 3, There were two accessions of Virginia bunch *viz.,* ICG 12276 and ICG 12672 from Bolivia with a similarity coefficient (1.0). The accessions ICG 14475 and ICG 14482 belonging to Virginia bunch and originating from Nigeria showed the closest similarity among all the accessions. Some accessions belonging to different botanical types but originating from the same geographical area were grouped together. For example ICG 11426 (Virginia bunch) and 2925 (Virginia runner) originating from India were grouped together. In contrast, the accessions ICG 2106 (India) and 10092 (Zimbabwe) belonging to Spanish bunch were grouped at 2.0. Similarly five Spanish bunch accessions (ICG 9157, 4729, 8567, 3343 and 11088) and one Virginia runner (ICG 7243) though originated from different places were similar at distance of 0.8. Two Virginia bunch accessions namely, 15190 (Peru) and 13982 (Brazil) and one Virginia runner (ICG 4412 from Brazil) were most similar at distance 0.9.

Table 23. Analysis of RAPD banding pattern

Primer	Valencia			Spanish bunch			Virginia runner			Virginia bunch		
	No. of amplified fragments	No. of polymorphic fragments	Per cent polymorphism	No. of amplified fragments	No. of polymorphic fragments	Per cent polymorphism	No. of amplified fragments	No. of polymorphic fragments	Per cent polymorphism	No. of amplified fragments	No. of polymorphic fragments	Per cent polymorphism
OPK 09	11	11	100	11	11	100	11	11	100	11	11	100
OPK 14	8	8	100	8	8	100	8	8	100	8	8	100
OPA19	3	3	100	3	3	100	3	3	100	3	3	100
OPC 15	8	8	100	8	8	100	8	8	100	8	8	100
OPC 09	6	6	100	6	6	100	6	6	100	6	6	100
OPC 13	8	8	100	8	8	100	8	8	100	8	8	100
OPB 11	7	7	100	7	7	100	7	7	100	7	7	100
OPF 09	8	8	100	8	8	100	8	8	100	8	7	87.5
OPJ 06	7	7	100	7	7	100	7	7	100	7	7	100
OPV 16	8	8	100	8	8	100	8	8	100	8	8	100
OPA 15	7	7	100	7	7	100	7	7	100	7	7	100
OPA 20	5	5	100	5	5	100	5	5	100	5	5	100
OPF 07	**6**	**5**	83.33	6	4	66.66	6	4	66.66	6	5	83.3
OPA 12	6	6	100	6	6	100	6	6	100	6	6	100
OPJ 17	7	7	100	7	7	100	7	7	100	7	5	71.42
OPC 03	5	4	80	5	5	100	5	4	80	5	5	100
OPV 15	5	4	80	5	4	80.0	5	4	80	5	3	60.00
OPC 06	5	5	100	5	5	100	5	4	80	5	3	60.00
OPF 10	8	8	100	8	8	100	8	8	100	8	8	100
OPA 17	7	4	57.14	7	7	100	7	4	57.14	7	6	85.71
Total	**135**	**129**		**135**	**132**		**135**	**130**		**135**	**126**	
Bands/primer	**6.75**	**6.45**		**6.75**	**6.6**		**6.75**	**6.5**		**6.75**	**6.3**	





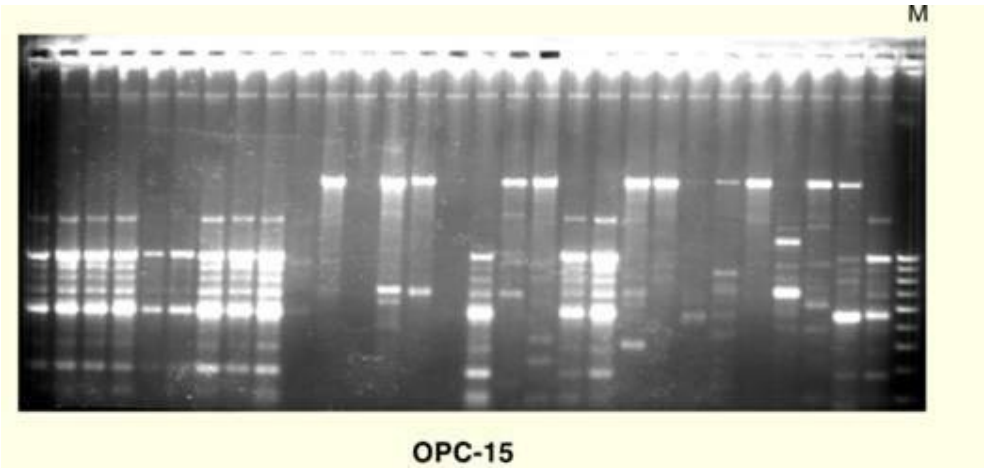


Plate 5. RAPD profile in mini core

Table 24. JACCARD MATRIX Frequency of each group genotype in each cluster

Clusters	Mini core		VL		SB		VR		VB	
	Total	Frequency	No.	Frequency	No.	Frequency	No.	Frequency	No.	Frequency
I	88	45.1	18	46.1	33	44.0	13	39.3	24	50.0
II	25	12.8	06	15.3	12	16.0	02	06.0	05	10.4
III	40	20.5	09	23.0	12	16.0	09	27.2	10	20.8
IV	22	11.2	01	02.5	13	17.3	05	15.1	03	06.2
V	19	9.7	04	10.2	05	06.6	04	12.1	06	12.5
VI	01	0.5	01	02.5	0	0	0	0	0	0
Total	195	100	39	100	75	100	33	100	48	100

Table 25: **Distribution of accessions in different clusters based on Jaccard similarity coefficient in molecular data**

Clusters	Total	Valencia	Spanish bunch	Virginia runner	Virginia bunch
I	88	ICG 3673, 4670, 8517, 7181, 3681, 5609, 6201, 6888, 6027, 6646, 5475, 11144, 9315, 14106, 13858, 10554, 10036, 10384	ICG 334, 1415, 5414, 3775, 3746, 4750, 434, 4543, 9249, 11322, 12682, 6375, 6654, 7190, 3584, 9809, 7969, 4955, 6407, 6236, 9157, 4729, 8567, 3343, 11088, 14118, 4684, 36, 81, 3240, 442, 15287, 1711	ICG 4412, 2777, 4343, 9037, 8490, 163, 5827, 7243, 11109, 2925, 3992, 2773, 862	ICG 15190, 13982, 111, 4527, 3053, 10185, 5662, 6057, 6703, 4598,5286, 6892, 4538, 5327, 5663, 9666, 9777, 8285, 6667, 11426, 6402, 2772, 1668, 513
II	25	ICG 332, 297, 397, 15309, 10566, 5221	ICG 4911, 118, 1862, 3102, 1519, 3421, 1137, 14985, 10565, 10890, 9418, 1399	ICG 13723, 875	ICG 532, 188, 3027, 721, 7963
III	40	ICG 10474, 13856, 1470, 14630, 14127, 1142, 115, MN 1-35, M 28-2	ICG 6263, 13941, 5195, 5779, 1415, 5236, 7906, 11249, 1973, 10092, 2106, TAG 24	ICG 9905, 4389, 928, 4998, 7153, 76, 11219, 6813, 4156	ICG 14008, 14475, 9842, 14466, 6706, 5745, 7000, 9961, 5991, 13942

Contd…..

IV	22	ICG 12625	ICG 11687, 11651, 12189, 12879, 13603, 13491, 11515, 12697, 12988, 9507, 8083, 12879, 12921	ICG 12370, 13099, 11457, 2857, 10479	ICG 12276, 12672, 11855
V	19	ICG 2738, 8106, 15042, MN 1-28	ICG 2019, 4716, R 9227, JL 24, GPBD-4	ICG 2511, 8760, 2857, 2381	ICG 12000, 14475, 14482, 13787, 14705, 6706
VI	01	ICG 1274			

V. DISCUSSION

Groundnut *(Arachis hypogaea* L.) is an annual legume and is grown primarily for high quality edible oil and easily digestible protein in its seeds. The main aim of plant breeding programs is to improve the plant traits for agronomic and economic value. Peanut breeding programs with a goal of rapid cultivar development have used mainly elite breeding lines and cultivars, which have resulted in the development of breeding material with a narrow genetic base. Utilization of entire germplasm resources in breeding programs is needed to enhance the diversity of cultivars. The knowledge about nature and extent of variability present in the germplasm accessions is important in planning a sound breeding strategy. The groundnut core collection is considered more effective in enhancing the utilization of peanut genetic resources (Holbrook, 1999). However, an even small subset of germplasm is needed for traits, which are difficult or expensive to measure. Upadhyaya and Ortiz (2001) sampled the core collection for developing a mini core subset that contain about 1 per cent of total accessions and to capture most of the useful variation of the crop. To enhance groundnut productivity, breeding of groundnut cultivars with high yield potential and resistance to various biotic and abiotic constraints is the main objective in most groundnut improvement programs in the world. The present investigation was envisaged to assess phenotypic and molecular diversity besides evaluation of agronomical traits and disease resistance in the mini core.

5.1 MORPHOLOGICAL CHARACTERIZATION

Morphological characterization continues to be the backbone of a taxonomic system at any taxonomic level. Morphological characters can contribute much to the study of relationships between botanical types and subspecies in groundnut. In the present investigation data on ten qualitative and nine quantitative characters was taken on mini core set of germplasm. The pattern of distribution of qualitative characters in different botanical types and contribution of both qualitative and quantitative characters to morphological diversity and differentiation of mini core is discussed.

5.1.1 Pattern of distribution of qualitative characters

The distribution pattern of the mini core and four botanical varieties for ten morphological characters is presented in the (Table 7) and (Fig. 2).

Four types of growth habits namely Decumnent-1, 2, 3 and erect were observed in the mini core. Decumbent-2 pattern was predominant in subsp. *hypogaea* (Virginia runner and Virginia bunch) and also Valencia while Spanish bunch was characterized by the predominance of Dcecumbent-3 followed by erect (Fig. 2). Branching pattern is the most important criteria employed in the classification of cultivated groundnut into subspecies. As expected sequential type of branching was predominant in subsp. *fastigiata* (Valencia and Spanish bunch) and alternate type in subsp. *hypogaea* (Virginia runner and Virginia bunch).

The stem colour and presence of hairs are important characteristics as descriptors for groundnut varieties. But both these traits are highly variable. Colour of the stem varies with the presence or absence and intensity of pigments in the stem tissue, which is highly influenced by the environment particularly exposure to the sunlight. Hence, recording of the typical colours becomes almost impossible; as a result stem colour was scored as present or absent. In the mini core, stem pigmentation was absent in Virginia runner, Virginia bunch and Spanish bunch but observed in Valencia (Fig. 2). Similarly hairiness was abundant in Valencia and scarce in other botanical types. Upadhyaya *et al* (2001b), Mallikarjuna Swamy (2001) and Upadhyaya *et al* (2003) obtained similar results on the core collection.

Leaf characters are important in the sub specific classification of groundnut. In the present study wide-elliptic pattern was most frequent in all the four botanical types besides oblong-elliptic in Valencia and Virginia bunch and orbicular in Virginia runner. High frequency of elliptic rather than wide elliptic as reported by Upadhyaya (2003) and Mallikarjuna swamy (2001) could be due to differences in scoring. The range of flower colour in *Arachis* is not great. Flowers are usually orange or yellow with or without marking. In the present study golden yellow colour was quite frequent in Spanish bunch, Virginia runner and Virginia bunch while canary yellow in Valencia types (Fig. 2). Other flower colours like garnet, lemon yellow,

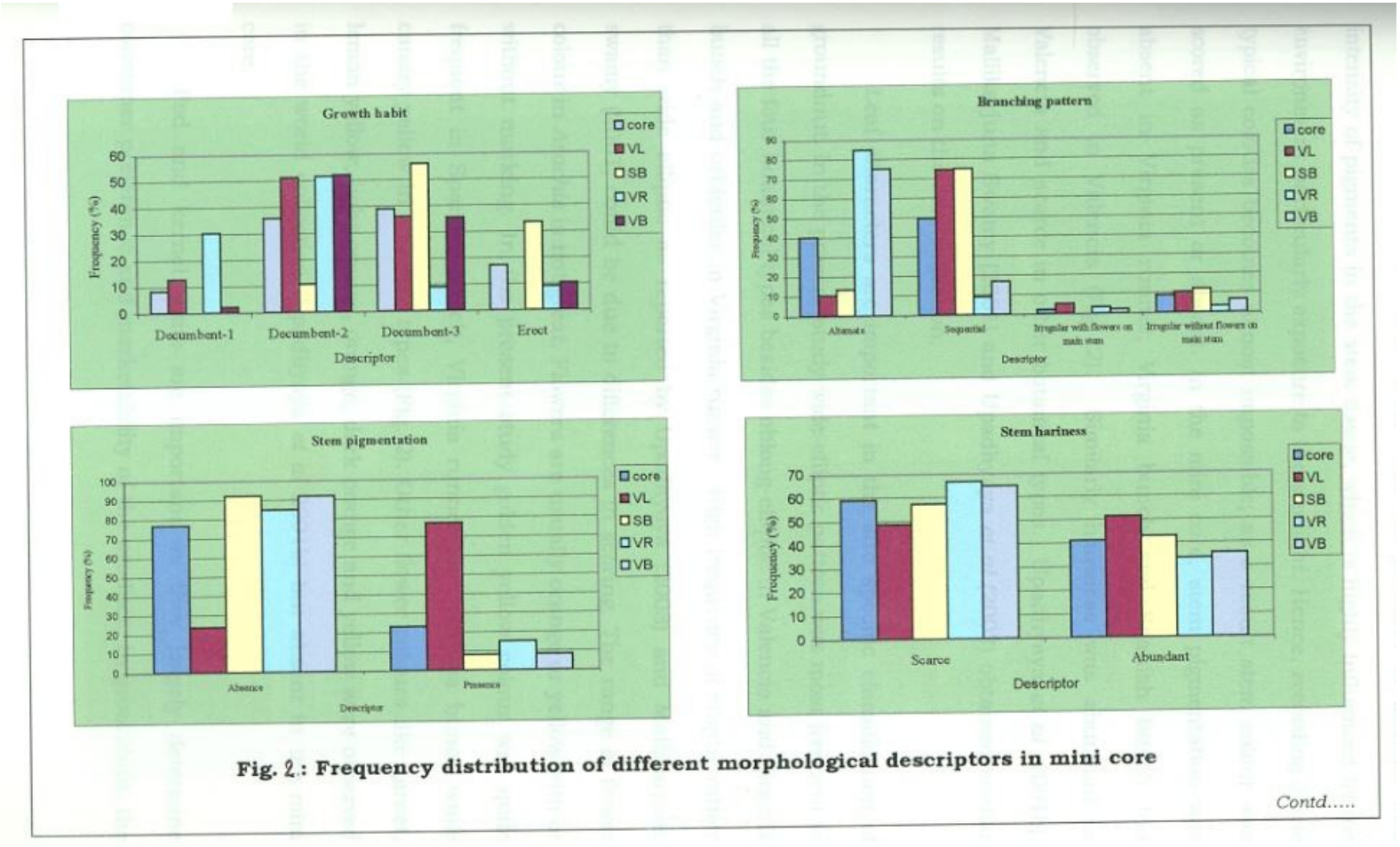
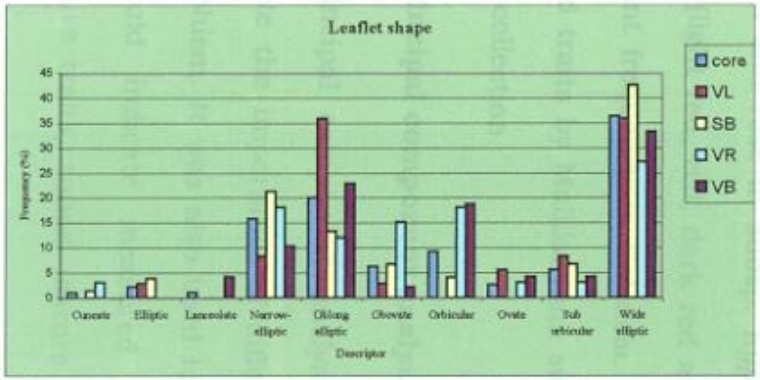
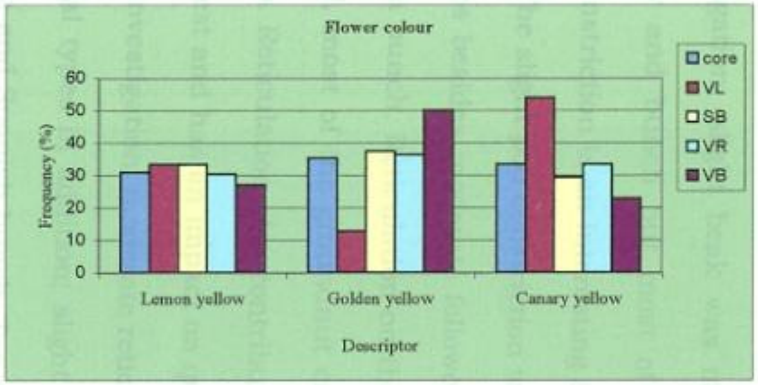
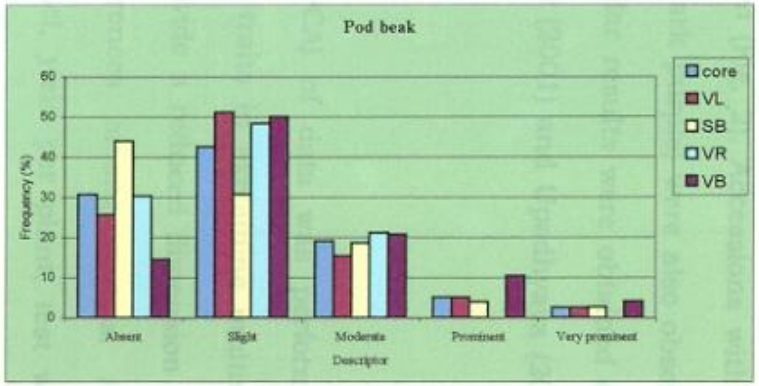
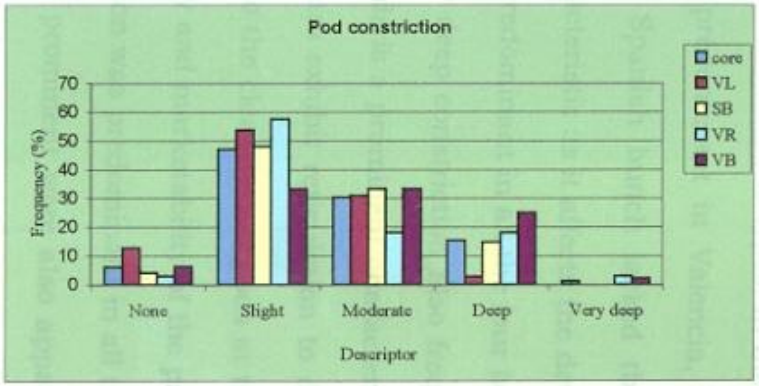


Fig. 2: Frequency distribution of different morphological descriptors in mini core

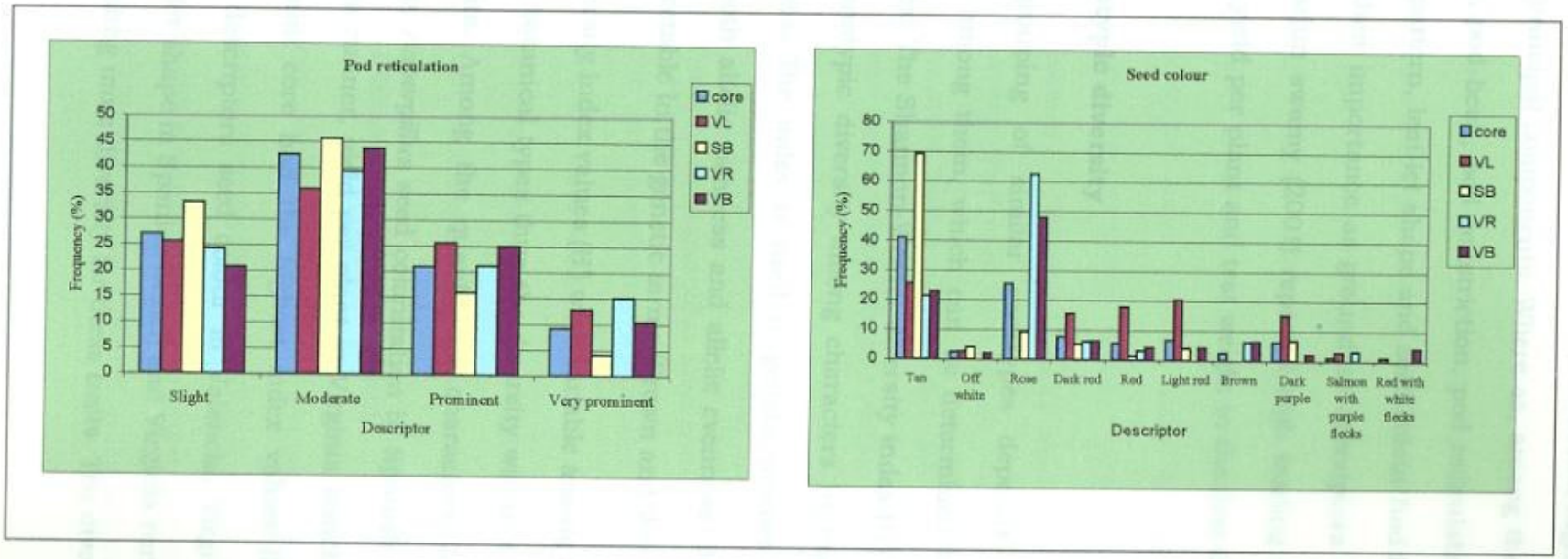

Leaflet shape
core
VL
SB
VR
VB
Frequency (%)
45
40
35
30
25
20
15
10
5
0
Cuneate
Elliptic
Lanceolate
Narrow-elliptic
Oblong elliptic
Obovate
Orbicular
Ovate
Sub orbicular
Wide elliptic
Descriptor


Flower colour
core
VL
SB
VR
VB
Frequency (%)
60
50
40
30
20
10
0
Lemon yellow
Golden yellow
Canary yellow
Descriptor


Pod beak
core
VL
SB
VR
VB
Frequency (%)
60
50
40
30
20
10
0
Absent
Slight
Moderate
Prominent
Very prominent
Descriptor


Pod constriction
core
VL
SB
VR
VB
Frequency (%)
70
60
50
40
30
20
10
0
None
Slight
Moderate
Deep
Very deep
Descriptor

Contd.....


Pod reticulation
Frequency (%)
50
45
40
35
30
25
20
15
10
5
0
Slight
Moderate
Prominent
Very prominent
Descriptor
core
VL
SB
VR
VB
Seed colour
Frequency (%)
80
70
60
50
40
30
20
10
0
Tan
Off white
Rose
Dark red
Red
Light red
Brown
Dark purple
Salmon with purple flecks
Red with white flecks
Descriptor
core
VL
SB
VR
VB

light orange, orange, dark orange and yellow were observed in the world collection (Upadhyaya *et al* 2001b) but absent in the mini core.

Pod and kernel traits are important as they largely determine consumer preference and marketability of the produce. In groundnut, the tip of the indehiscent fruit may end in an appearance called beak. The prominence of beak varies in different botanical types. In the present investigation, slight beak was more predominant in Valencia, Virginia runner and bunch but most of the Spanish bunch lacked the beak. Pod constriction is an interesting characteristic as it affects the developing seed. The slight pod constriction was predominant in all the four botanical varieties besides moderate followed by deep constriction also frequent in Virginia bunch. Reticulation on the pods is a prominent characteristic. In general, most of the groundnut cultivars exhibit reticulation to different degrees. Reticulation also contributes to the cleanness of pods at the time of harvest and has an impact on quality and marketability of the produce. In our investigation, moderate reticulation was predominant in all the four botanical types in addition; slight and prominent were also apparent in Valencia and Spanish bunch. Tan seed colour was predominant in subsp. *fastigiata* and rose in subsp. h*ypogaea* (Fig. 2). Accessions with bright colours (light red, red, dark red and dark purple) were also observed in significant frequency in Valencia. Similar results were obtained on pod and seed traits by Mallikarjuna swamy (2001) and Upadhyaya (2003) in the core collection.

5.1.2 Principal component analysis

Principal component analysis (PCA) of data was performed to investigate the importance of different traits in explaining multivariate polymorphism. It was also used to provide a reduced dimension model that would indicate measured differences among groups. Among quantitative traits, sound mature kernel, yield per plant, test weight, shelling percentage, late leaf spot and protein had higher loading values in first three principal components. Where as, among the ten morphological descriptors, pod beak, pod constriction, pod reticulation, stem hairiness, branching pattern, leaf-let shape and seed colour had high loading values indicating their importance as groundnut descriptors. Upadhyaya (2003) and Mallikarjun swamy (2001) reported high loading values for shelling percentage, yield per plant and test weight in the first five PCs in the core collection.

5.1.3 Phenotypic diversity

The grouping of similar genotypes depends on the level of dissimilarity among them, which can be determined by the phenotypic diversity index. The Shannon-Weaver diversity index (H') was calculated to compare phenotypic diversity among characters in mini core and four botanical types. The index is used in genetic studies as a convenient measure of both allelic richness and allelic evenness, but it may not be readily interpretable in the genetic terms (Brown and Weir, 1983).

The diversity index values (H') were variable among the traits in mini core and four botanical types thus the diversity within a group depended upon the traits. Among the quantitative characters, lectin activity in Valencia group, *Aspergillus* seed colonization in Spanish bunch, late leaf spot in Virginia runner, yield per plant in Virginia bunch types and late leaf spot in mini core had the highest index values (H'). Among the morphological descriptors seed colour in Valencia, Virginia bunch and mini core, leaf let shape in Spanish bunch and Virginia runner had shown highest H' revealing more diversity for these traits. The diversity index was lowest for *Aspergillus* seed colonization in mini core and Valencia, sound mature kernels in Spanish bunch, shelling percentage in Virginia bunch and days to 50% flowering in Virginia runner. Whereas across ten morphological descriptors phenotypic diversity index was lowest for stem pigmentation in Spanish bunch, Virginia runner and Virginia bunch and stem hairiness in Valencia had low H' indicating extremely unbalanced frequency classes for these traits and lack of diversity. In general mini core sub set exhibited more diversity than different botanical types indicating a need for sampling across botanical types to retain morphological diversity. Different subsets representing botanical types showed more diversity than mini core set for some morphological traits, Virginia bunch recorded higher diversity for pod beak, pod constriction, pod reticulation and flower colour, Virginia runner for pod reticulation and leaf let shape and Valencia for pod reticulation and stem pigmentation. While across quantitative characters, Spanish bunch and Virginia bunch

showed higher diversity for *Aspergillus* seed colonization. The average H' across qualitative and quantitative traits were similar among Spanish bunch, Virginia runner and Virginia bunch. Valencia showed higher diversity for qualitative traits but it was limited for quantitative traits indicating significant variation for qualitative traits.

5.2 MORPHOLOGICAL DIFFERENTIATION

The genetic similarity between 195 genotypes for qualitative characters was assessed by similarity coefficient and it ranged from 0.10 to 1.00. The hierarchical cluster analysis revealed nine distinct clusters at Sij 18.0. Different clusters did not conform to any set pattern relating to the geographical origin but each cluster represented predominantly by a particular botanical type. This indicated relevance of classification into botanical types and not the geographical origin as an important approach to capture the morphological diversity.

The cluster VI and IX were dominated by Valencia, cluster IV, V and VII by Spanish bunch, cluster II and VIII by Virginia runner, cluster III and I by Virginia bunch. Qualitative characters were also found more useful in differentiating each botanical type into more sub groups and their contribution to diversity. The accessions in clusters IV, VIII and IX were predominantly having slight beak, slight constriction and moderate pod reticulation. Majority of the genotypes with Decumbent-3 growth habit, sequential branching pattern and with stem pigmentation (light pink) were grouped in cluster VI and VIII. The majority of genotypes in cluster II showed Decumbent-1 growth habit, alternate branching pattern, abundant stem hairiness, moderate constriction and tan seed colour. Cluster VII was dominated by genotypes with moderate pod beak, pod constriction, pod reticulation and lemon yellow flower colour. To maintain the diversity due consideration could be given to botanical types as well as clusters (Table 12] in selecting the accessions for hybridization program.

The genetic dissimilarity assessed by Euclidian distance on quantitative data revealed five distinct clusters at 5.0 but majority of the accessions (153) were included in a single cluster (I). There was no correspondence with either geographical origin or botanical types. Thus quantitative characters were found less important in contributing to the morphological diversity.

Gower's similarity coefficient based on both qualitative and quantitative traits revealed abundant variability. The cluster analysis revealed nine distinct clusters with two major clusters comprising 162 accessions. Nineteen (48.7%) of 39 accessions of Valencia and 58 (77.3%) of 75 Spanish bunch were grouped in cluster I, while 27 (81.81%) of 33 Virginia runner and 40 (83.33%) of 48 Virginia bunch grouped in cluster II thus revealing differentiation specific to subspecies. Thus quantitative characters have not contributed to differentiation even when they are combined with qualitative characters.

5.3 MOLECULAR DIVERSITY

The molecular tools such as DNA markers are becoming increasingly important as effective tools in crop breeding programs but their application in groundnut is lagging behind because of limited knowledge of the genome. Extensive variation for morphological and physiological characteristics exists in both wild and cultivated groundnut (Halward *et al*., 1993), but low DNA polymorphism has been observed in cultivated species (Kochert *et al*., 1991 and Halward *et al*., 1993). Of late, studies using RAPDs, SSRs and AFLPs techniques have revealed the presence of DNA polymorphism in cultivated groundnuts (Dwivedi and Gurtu, 2002, Hopkins *et al*., 1999 and He and Prakash, 1997, He *et. al*., 2005). The observed polymorphism may be useful for developing molecular markers helpful in the genetic enhancement of the groundnut crop.

Variability using molecular markers has been examined in groundnut by different researchers Lanham *et al*., 1992 demonstrated that RAPD are a convenient and effective marker system for *Arachis* species. The RAPD assay was chosen in the present study since the procedure involved was simple, does not require probes or southern blot hybridization as in case of RFLP. Molecular diversity analysis by RAPD assay was carried out among the accessions of mini core, breeding lines (R 9227, MN 1-35, MN 1-28) and cultivars (GPBD-4, JL 24, TAG 24, M 28-2) as a means for better scientific exploitation of genetic resources.

DNA from the 195 genotypes was studied with twenty oligonucleotide primers for RAPD assay. A total of 135 DNA bands were amplified across all the accessions revealing an average of 6.75 bands per primer and 100% polymorphism was observed in the mini core. Even with in four botanical types, the extent of polymorphism was high and ranged from 57.4 to 100%. In contrast, several earlier studies have reported low to moderate level of polymorphism. It ranged from 8.73 to 33.08 per cent among eight primers in twenty-six accessions including interspecific derivates, land races and released cultivars (Dwivedi *et al*., 2001). Dwivedi and Varma., 2002 reported 11.7 to 55.0 per cent polymorphism in late leaf spot resistant accessions using eighteen primers. Ten primers produced 16.6 to 100 per cent polymorphism in late leaf spot and rust resistance material (Nagaraja Reddy *et al*., 2004). The polymorphism ranged from 12.5 to 76.9 per cent with eleven primers in late leaf spot and rust resistant material (Mondal *et al*., 2005). Low level of polymorphism was ascribed to limited number of accessions, narrow genetic base and limited number of primers employed in the earlier studies (Singh *et al*., 1998) High level of polymorphism in present study could be due to large number of accessions (195) representing wide genetic base. The material was a subset (mini core) of world germplasm representing the morphological and geographical diversity. Other reason could be use primers with potential for polymorphism. In the present study essentially the primers that were reported to be polymorphic and some with high G:C content were employed. The primers that were reported polymorphic by Subramanian *et al*., 2000 (OPA 20), Dwivedi *et al*., 2001 (OPA 19 and OPB 11), Dwivedi *et al*., 2002 (OPJ 17, OPV 15, OPV 16), Rajendraprasad, 2003 (OPF 09, OPF 10, OPF 07), Vijay, 2003 (OPF 09, OPF 10, OPK 14), Nagaraja Reddy *et al*., 2004 (OPF 09, OPF 10, OPA 15), and Pattanashetti, 2005 (OPA 15, OPK 14). The extent of polymorphism in these primers was much higher than reported by these authors indicating the importance of large as well as diverse material in exhibiting the polymorphism. Thus it is opined that RAPD assays can be efficient in identifying DNA polymorphism among germplasm.

The Jaccard similarity coefficient generated from pooled data of all the twenty primers. The genetic similarity coefficients of the genotypes ranged from 0.22 to 0.93 indicating substantial diversity present in the mini core. A few accessions showed high degree of genetic diversity in their DNA profile. These were ICG 8760 (Virginia runner from Zambia) with ICG 442 (Spanish bunch from USA), 1274 (Valencia from Indonesia) and 6402 (Virginia bunch from unknown origin). Similarly, GPBD-4 (Spanish bunch cultivar from India) with ICG 2381 (Virginia runner from Brazil) and ICG 1274 (Valencia from Indonesia) with ICG 12189 (Spanish bunch of unknown origin) had very high degree of genetic diversity. Thus there is a possibility of use of RAPD assay in understanding and manipulating the groundnut genome.

Although the cluster analysis grouped accessions into six different clusters, the accession in each cluster couldn't be associated with subspecies or botanical types. The accessions belonging to four botanical types were distributed in all the six clusters with slightly varying proportions. This indicated independence of molecular diversity with morphological diversity. In contrast, AFLP and SSRs (He and Prakash, 1997, He *et al*., 2005.) were found efficient in grouping different botanical types based on molecular diversity. This could be possibly due to association of AFLP and SSR and not RAPD markers with macro-evolutionary changes leading to morphological differentiation of subspecies and botanical types. In general major clusters did not show any association with geographical origin of the accessions. But with in each cluster some accessions belonging to the same or different botanical types but belonging to the same origin were included in sub clusters indicating their genetic similarity. This could indicate association of molecular diversity with micro evolutionary changes leading to regional adaptation.

The genetic diversity can be useful for organizing germplasm for conservation of genetic resources, for the identification of cultivars, for the selection of parents for hybridization, for predicting favorable heterotic combinations and for reducing the number of accessions to ensure sampling a broad range of genetic variability in breeding programs. Accessions with the most distinct DNA profiles are likely to contain the greatest number of novel alleles. As revealed by RAPD assay substantial genetic diversity exists in the mini core that could be exploited in crop improvement programs.

5.4 EVALUATION FOR QUANTITATIVE CHARACTERS

In the mini core, a good possibility exists of finding a range of desirable traits to meet demands for specific attributes requested by researchers.

The REML analysis of quantitative data *i.e.,* Days to 50 per cent flowering, shelling percent, sound mature kernel, yield per plant, test weight, *Aspergillus* seed colonization, late leaf spot, lectin activity and protein content has revealed significant variation for days to 50 per cent flowering, yield per plant, test weight and lectin activity indicating that the accessions included in the mini core displayed high variation among genotypes and hence are amenable to selection. Extensive variation was also evident by the wide range of values exhibited by the mini core for all the characters. Mallikarjuna swamy *et al*., 2003 reported significant difference for test weight, In contrast, Upadhyaya *et al* 2002, Holbrook *et al*., 1993 reported non-significant difference for yield per plant.

5.4.1 Productivity traits

Days to flowering

The mini core included genotypes with early as well as late flowering types. Earliness being preferred in view of per day productivity as well as to escape end season drought in groundnut. Virginia runner type took more days to flowering followed by Spanish bunch, Valencia and Virginia bunch. Twenty-two genotypes came to flowering with in 29 days, which included three (ICG 188, 532, 3027) that flowered at 28 days. These early flowering genotypes were distributed in different botanical types *viz.,* Valencia (10 genotypes), Virginia bunch (8) and Spanish bunch (4). None of the accessions belonging to Virginia runner flowered early (by 29 days). The early flowering genotypes originated from different places with as many as 9 accessions from India followed by USA (3) and Uganda (2) (Table 26).

Yield per plant

Eighteen genotypes recorded yield comparable to the best check (GPBD-4) and they belonged to different botanical types namely, Valencia (7) and Spanish bunch (8), Virginia runner (2) and Virginia bunch (1) traceable to different places of origin (Table. 26). Substantial genetic diversity (S_{ij} values ranging from 0.55 to 0.90) existed among them. Maximum genetic diversity was observed between the accession ICG 15042 (Valencia of unknown origin) and ICG 8285 (Virginia bunch from USA); the cultivar GPBD-4 (Spanish bunch from India) and M 28-2 (Valencia cultivar from India); M 28-2 (Valencia cultivar from India) and JL 24 (Spanish bunch cultivar from India); TAG 24 (Spanish bunch cultivar from India) and ICG 12697 (Spanish bunch from India); JL 24 (Spanish bunch from India) and ICG 11219 (Virginia runner from Mexico). The accessions ICG 8760 (Virginia runner from Zambia) and 15042 (Valencia) followed by ICG 12697 (Spanish bunch from India) and 4716 (Spanish bunch) clustered distinctly away from the other genotypes at S_{ij} 0.66 and 0.71, respectively. The accessions that have shown high genetic diversity could be utilized as parents to develop segregating populations with a potential for diversity.

While, some accessions have shown greater similarity for example ICG 4750 (Spanish bunch from Paraguay) and ICG 6201 (Valencia from Cuba) (Fig. 3). The accession ICG 1862 with highest yield per plant (33.50 g) was grouped together with genotypes ICG 15309, MN 1-35 and the best check GPBD-4 at S_{ij} 0.79. Similarly, the cultivars MN 1-28, JL 24 and R 9227 were grouped (at S_{ij}0.71) in one cluster indicating their genetic similarity and may share genes conferring higher productivity.

Shelling out turn

Pod yield is an important marketable product and is highly influenced by environment. Groundnut has to be shelled to facilitate further processing. Market price of groundnut pods is mainly fixed based on the shelling percentage of lots and farmers often face problems in maintaining high shelling percentage. Kernel recovery mainly depends on the shelling percentage and hence there is a need to identify genotypes with high shelling percent. [Higher mean performance for shelling percentage was observed in *fastigiata* group than subsp *hypogaea*.] Similar results were reported by Upadhyaya (2003). High potential genotypes comparable to the best check GPB-4 belonged to different botanical types *viz.,*

Table 26. Accessions selected based on numerical superiority for different traits

Characters	Valencia	Origin	Spanish bunch	Origin	Virginia runner	Origin	Virginia bunch	Origin
Days to 50 % flowering	ICG 332 1274 3681 5609 6201 7181 9315 13858 2738 115	Brazil Indonesia USA Srilanka Cuba India USA Uganda India India	ICG 3421 4729 10384 14985	India China Nigeria Unknown			**ICG 188** **532** **3027** 513 721 3053 4527 4538	India Unknown India India USA India Uganda India
Yield per plant	ICG 13856 15042 6201 332 5221 15309 6027	Uganda Unknown Cuba Brazil Argentina Brazil India	ICG 11687 4750 13491 14985 3240 12697 **4716** **1862**	India Paraguay Central African Republic Unknown Uaganda India Unknown Unknown	ICG 11219 8760	Mexico Zambia	ICG 8285	USA
Shelling percentage	ICG 5475 11144 6888 2738 3673	Kenya Argentina Brazil Tanzania Korea	ICG 8567 14118 7969 12921 11249 12987 9507 6375 12879 5779 3102	Uruguay United kingdom Zimbabwe Zimbabwe Tanzania Unknown Philippines Unknown Myanmar India India	ICG 12370	India	ICG 7963 13982	USA USA

Contd.....

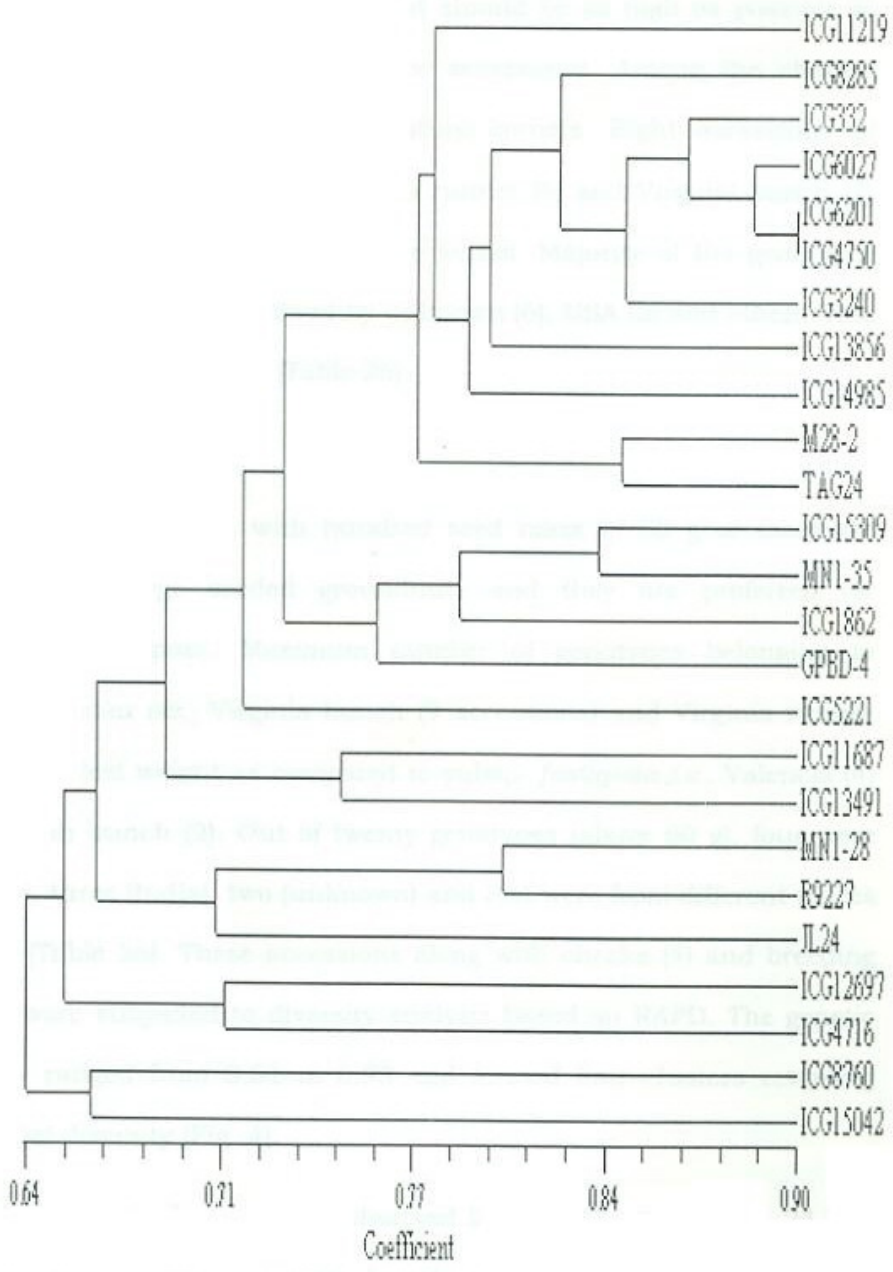
Sound mature kernel	ICG 10890 10565 8517 13856 1142 115 11144 332	Peru Unknown Bolivia Uganda Benin India Argentina Brazil	ICG 5779 1415 14118 15287 118 **14989** 5236 434 11651 81 442	India Senegal United kingdom Brazil India Unknown Chile USA China Unknown USA	ICG 2777 12370 11109 4389 13099 928	India India Taiwan India Unknown Unknown	ICG 5286 7963 14705 14475 3053 9961 11426	Zambia USA Cameroon Nigeria India Unknown India
Test weight	ICG 297 6646 6027 12625	USA Unknown Sudan Ecuador	ICG 118 **14985**	India Unknown	ICG 10479 2857 2381 11219 8760	Uruguay Argentina Brazil Mexico Zambia	ICG 3053 6667 15190 6057 3027 8285 13787 9777 5662	India USA Costa Rica USA India USA Nigeria Mozambique China
Aspergillus seed colonization	ICG 3673 6027 12625	Korea Sudan Ecuador	14985 **1862**	Unknown Unknown	ICG 8760 76 12276 2381	Zambia India Bolivia Brazil	ICG 13787 6402	Nigeria Unknown
Late leaf spot	ICG 12625	Ecuador	12682 12697 **4716** 15419	India India Unknown Ecuador	ICG 76 2857 4412 8760 9905	India Argentina USA Zambia Zambia	ICG 532 3027 6766 12672 14475	Unknown India USA Bolivia Nigeria

							13787	Nigeria

Contd….

Lectin	ICG 3681	USA	ICG 81	Unknown	4998	China	14482	Nigeria
	6027	Sudan	12682	India				
	6646	Unknown	12697	India				
	1470	Unknown	6263	Burkina Faso				
	12625	Ecuador	4955	India				
	11088	Peru						
	1399	Malawi						
	7181	India						
Protein	ICG 8517	Bolivia	ICG 13941	ICRISAT	ICG 163	Unknown	ICG 513	India
	1470	Unknown			862	India	5991	India
	115	India			2511	India	6057	USA
							6703	Paraguay
							12276	Bolivia
							14466	Nigeria
							14475	Nigeria

Fig. 3: Dendrogram depicting genetic diversity of selected accessions for yield per plant in mini core



Valencia (5), Spanish bunch (11), Virginia runner (1) and Virginia bunch (2). These accessions originated from different geographical places (Table 26).

Sound mature kernel

The extent of well matured kernels out of total number of kernel is indicated by sound mature kernel that should be as high as possible in view of both consumer and processor acceptance. Among the checks, M 28-2 was superior for sound mature kernels. Eight accessions of Valencia, Spanish bunch (11), Virginia runner (6) and Virginia bunch (7) were found superior for sound mature kernel. Majority of the genotypes originated from India (8) followed by unknown (6), USA (3) and others from different geographical areas (Table 26).

Test weight

Normally varieties with hundred seed mass of 60 g or more are considered as large seeded groundnuts and they are preferred for confectionary purpose. Maximum number of genotypes belonging to subsp. *hypogaea viz.,* Virginia bunch (9 accessions) and Virginia runner (5) had high test weight as compared to subsp. *fastigiata i.e.,* Valencia (4) and Spanish bunch (2). Out of twenty genotypes (above 60 g), four were from USA, three (India), two (unknown) and rest were from different places of origin (Table 26). These accessions along with checks (4) and breeding lines (3) were subjected to diversity analysis based on RAPD. The genetic similarity ranged from 0.51 to 0.93 and formed four clusters revealing substantial diversity (Fig. 4).

More genetic diversity was observed between the cultivar JL 24 and accession ICG 14985 (Spanish bunch of unknown origin); GPBD-4 (Spanish bunch from India) and M 28-2 (Valencia from India); M 28-2 (Valencia from India) and ICG 13787 (Virginia bunch from Nigeria); TAG 24 (Spanish bunch from India) and ICG 2381 (Virginia runner from Brazil); JL 24 (Spanish bunch from India) and R 9227 (Spanish bunch from India). The accession ICG 2381 of Virginia runner was distinct and away from the rest at Sij 0.62.

Highest genetic similarity was observed between accessions ICG 6057 (Virginia bunch from USA) and ICG 6027 (Valencia from Sudan). The accession ICG 5662 (Virginia bunch) with highest-test weight was grouped with TAG 24 and M 28-2 at 0.78 similarity coefficient. The cultivar JL 24 was grouped along with ICG 2857, 8760, 12625 and 3027 at S_{ij} 0.71 indicating genetic similarity. Similarly, ICG 13787 (Virginia bunch from Nigeria) was clustered together with the checks (MN 1-28, MN 1-35 and GPBD-4) at S_{ij} 0.75. These genetic relations could be of great importance in planning crossing programs.

5.3 RESISTANCE

The presence of chemical residues in food chain is increasingly attracting consumers concern. Most of the edible grade groundnut is generally grown under high input management, which includes the use of pesticides. Excessive use of pesticides can adversely affect the quality of the produce. In addition to causing substantial yield losses, they also affect seed quality adversely (Dwivedi *et al*., 1996). There is need to develop varieties with inbuilt resistance to pests and diseases which could minimize the problem of pesticide residues. *Aspergillus* seed colonization and late leaf spot are the major diseases of groundnut. Among the checks, GPBD-4 was resistant to late leaf spot while MN 1-35 had resistance to the late leaf spot and also for *Aspergillus* seed colonization.

Aspergillus seed colonization

Eleven genotypes were found superior (scoring lesser than 2.5 of 4) for *Aspergillus* seed colonization. Four accessions *viz.,* 6027, 14985, 8760 and 13787 have shown high resistance (scale ranged from 1 to 1.5) and seven were moderately resistant (from 2.1 to 2.5). These genotypes originated from different places and were distributed across all four botanical types namely, Valencia (3), Spanish bunch (2), Virginia runner (4), Virginia bunch (2) (Table 26). The similarity coefficient ranged (S_{ij}) from 0.54-0.90 indicating more diversity among them (Fig. 5). The cultivar TAG 24 and a resistant accession ICG 14985; the cultivar GPBD-4 (Spanish bunch cultivar from India) and accessions ICG 14985 (Spanish bunch of unknown origin); M 28-2 (Valencia cultivar from India) and ICG 13787 (Virginia bunch from Nigeria); TAG 24 (Spanish bunch cultivar from India) and ICG 2381 (Virginia runner from Brazil); JL 24 (Spanish bunch cultivar from India) and ICG 14985 (vulgaris of unknown origin)

Fig. 4: Dendrogram depicting genetic diversity of selected accessions for test weight in mini core

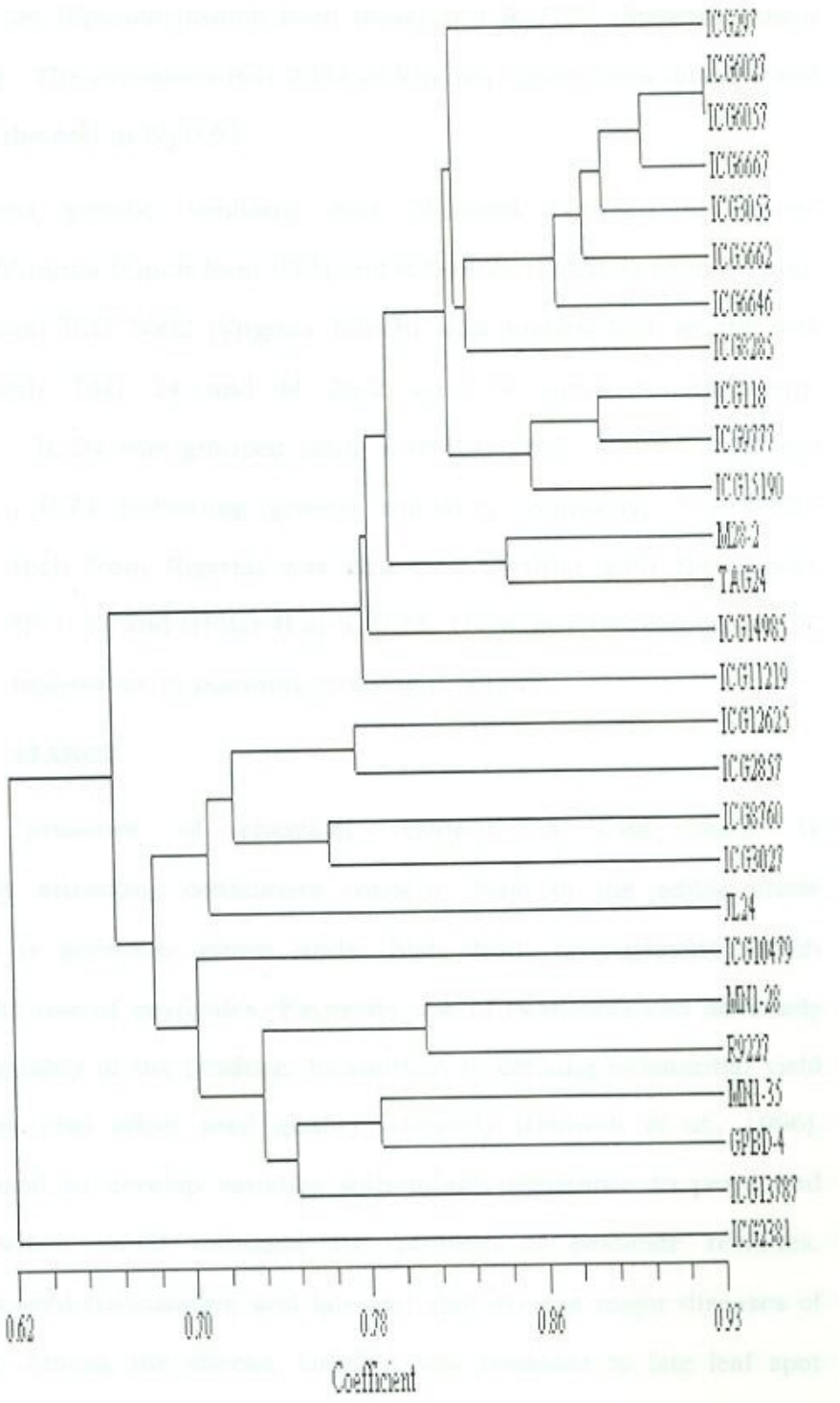
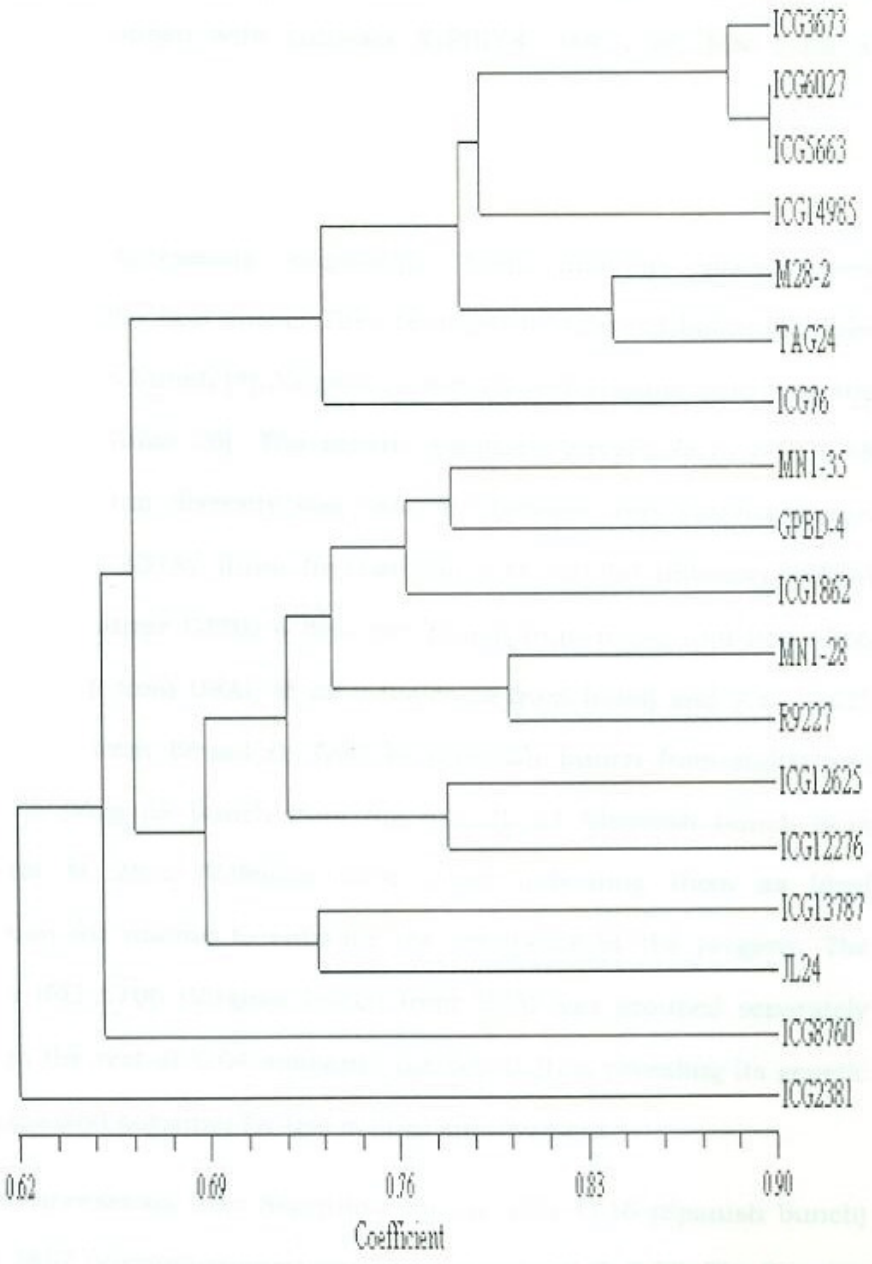


Fig. 5. Dendrogram depicting genetic diversity of selected accession for Aspergillus seed colonization in mini core



were found to be divergent and could form an ideal parents for developing mapping populations to identify DNA markers associated with resistance and in generating breeding populations. The two Virginia runner accessions ICG 8760 (Zambia) and ICG 2381 (Brazil) were highly distinct from all other accessions. Out of four highly resistant genotypes, ICG 6027 (Valencia from Sudan) and 14985 (Spanish bunch of unknown origin) were grouped closely away from the other two resistant genotypes namely, ICG 8760 (Virginia runner from Zambia) and 13787 (Virginia bunch from Nigeria).

The two resistant accessions ICG 6027 (Valencia from Sudan) and ICG 5663 (Virginia bunch from China) were found genetically most similar at 0.89 similarity coefficient. The accession from India ICG 1862 (Spanish bunch) was grouped with cultivars (GPBD-4, MN 1-28, MN 1-35) at S_{ij} 0.78.

Late leaf spot

Sixteen accessions originating from different places were comparable to the best check. They belonged to different botanical types namely, Spanish bunch (4), Virginia runner (5) and Virginia bunch (6) and *Peruviana* (1) (Table 26). The genetic similarity ranged from 0.57-0.88 (Fig. 6). Maximum diversity was observed between two Virginia bunch accessions ICG 13787 (from Nigeria) and ICG 532 (of unknown origin) besides, the cultivar GPBD-4 (Spanish bunch from India) and ICG 6766 (Virginia bunch from USA); M 28-2 (Valencia from India) and ICG 12625 (*aequatoriana* from Ecuador); TAG 24 (Spanish bunch from India) and ICG 14475 (Virginia bunch from Nigeria); JL 24 (Spanish bunch from India) and M 28-2 (Valencia from India) indicating them as ideal combination for mating to enhance the resistance in the progeny. The accession ICG 6706 (Virginia bunch from USA) was grouped separately away from the rest at 0.64-similarity coefficient thus revealing its genetic distinctness and potential for use in crop improvement.

Two accessions from Argentina namely, ICG 4716 (Spanish bunch) and ICG 2857 (Virginia runner) were most similar at S_{ij} 0.88. The Spanish bunch accession from Ecuador ICG 12625 with highest resistance to late leaf spot was grouped with Spanish bunch accessions from India (ICG 12697) at 0.75 similarity coefficient. The accessions belonging to different botanical types namely, ICG 12682 (Spanish bunch), ICG 4412 (Virginia runner) and ICG 532 (Virginia bunch) were grouped very closely at 0.85 similarity coefficient. The resistant genotypes MN 1-28, MN 1-35, GPB-4 and ICG 12672 were distinctly clustered from rest of the genotypes at S_{ij} 0.74.

5.4.4 Chemical traits

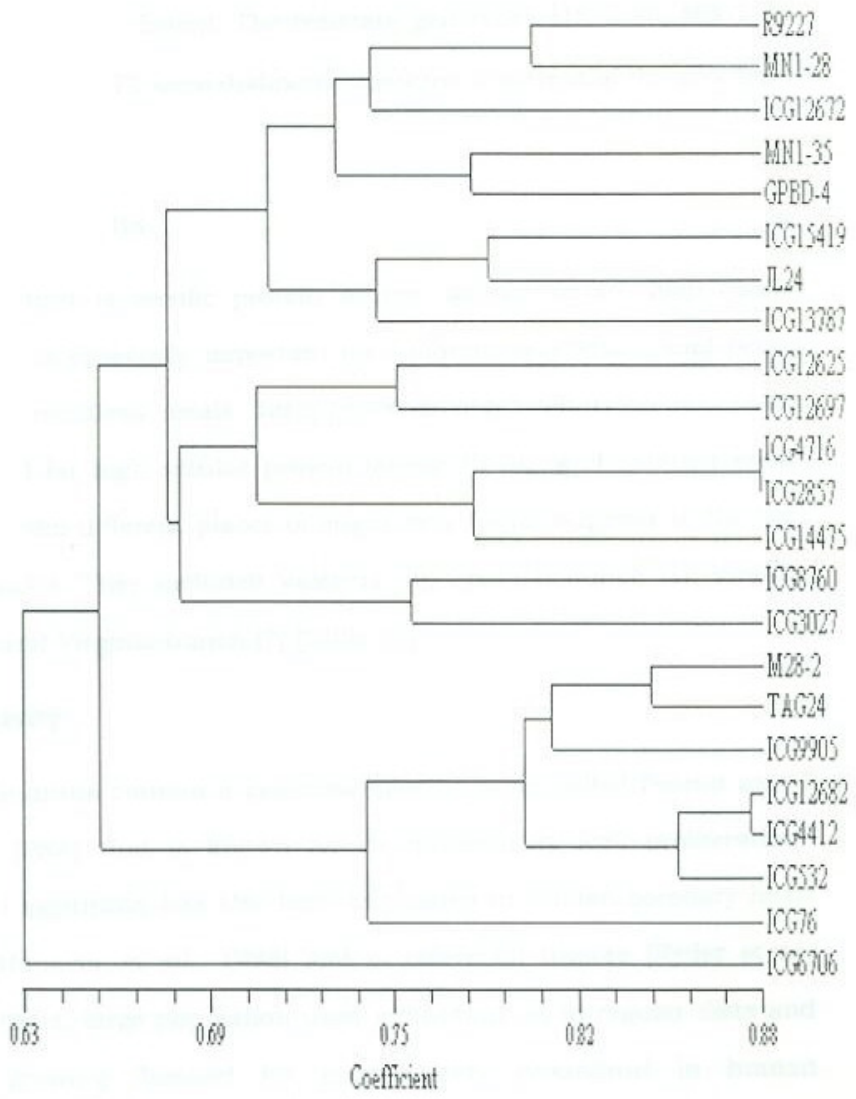
Groundnut is terrific protein source, which contain high quality protein. This is especially important for children, vegetarians and people eating more meatless meals (http//www.fao.org). Fourteen accessions were selected for high soluble protein (above 19 mg/g of defatted seeds) originating from different places of origin were found superior to the best check, GPBD-4. They included Valencia (3), Spanish bunch (1), Virginia runner (3) and Virginia bunch (7) (Table 26).

Lectin activity

Groundnuts contain a galactose specific lectin called Peanut anti-T agglutinin (PNA) that is known for its mitogenicity (cell proliferation). Groundnut agglutinin has also been implicated in human coronary heart diseases (Honavar *et. al*., 1998) and in colorectal tumors (Ryder *et al*., 1992). In India, large population used groundnut oil in regular diets and there is growing demand for good quality groundnut in human consumption.

Ten accessions with low lectin activity (693.58 to 2000 HAU / mg of protein) and six accessions with high lectin activity (10,803.11 to 14,360.52 HAU/ mg of protein) namely, ICG 1399, 4955, 4998, 7181, 8083, 14482 were identified as significant germplasm important for the trait. They originated from different geographical places and belonged to different botanical types namely Valencia (6), Spanish bunch (6), Peruviana (1), *acquetoriana* (1), Virginia bunch (1) and Virginia runner (1) (Table 26). The accessions with low lectin activity could be utilized for exploitation in future breeding program to enhance nutritional quality of groundnut. While, germplasm with extreme (low and high) activities could be utilized in the

Fig. 6: Dendrogram depicting genetic diversity of selected accessions for late leaf spot in mini core



investigation on the effects of lectin on nutritional quality of groundnut as well as in the identification of markers associated with the trait.

These genotypes along with cultivars (GPBD-4, M 28-2, TAG 24, JL 24 and MN 1-35) were subjected to diversity analysis on RAPD. The similarity coefficient (S_{ij}) ranged from 0.53 to 0.91 (Fig. 7). The highest genetic diversity was observed between ICG 14482 (high activity) and ICG 6027 (low activity) followed by ICG 4955 (high activity) and ICG 12697 (low activity). They can be conveniently used in the crossing program to generate mapping population to identify the markers associated with the lectin activity. The dendrogram constructed from the molecular data revealed distinct cluster of low lectin cultivar JL 24 (Spanish bunch cultivar from India) and ICG 14482 (Virginia bunch from Nigeria) away from the rest at 0.65-similarity coefficient. The accession with low lectin activity ICG 11088 (Peruviana) was grouped distinctly away from the cultivars (GPBD-4, MN 1-35 and JL 24) at S_{ij} 0.79. The *aequatoriana* accession ICG 12625 (Ecuador) and two Spanish bunch accessions namely, ICG 8083 (Russia and CISs) and ICG 12697 (India) were grouped together at 0.75 similarity coefficient.

Significantly superior accessions

Eleven accessions were significantly superior for different traits *viz.,* yield, test weight, *Aspergillus* seed colonization, late leaf spot and low lectin activity as compared to the best checks. They belonged to different botanical types namely, Valencia (2), Spanish bunch (5), Virginia runner (1), Virginia bunch (3) and different places of origin (Table 27). They constitute the most potential donors for crop improvement.

Three accessions (ICG 1862, 3240 and 12697) were superior for yield. ICG 1862 (Spanish bunch of unknown origin) had high-test weight with resistance to late leaf spot and *Aspergillus* seed infection. While, ICG 3240 (Spanish bunch from Uganda) had high shelling percentage and ICG 12697 (Spanish bunch from India) had resistance to late leaf spot and low in lectin activity.

Four accessions *viz.,* ICG 5662, 6027, 8760 and 9777 had high-test weight and among them ICG 6027 (Valencia from Sudan) and ICG 8760 (Virginia runner from Zambia) were comparable with the best check (GPBD-4) for yield and showed resistance to *Aspergillus* seed infection and late leaf spot. ICG 6027 was also found low in lectin activity. The accession ICG 9777 (Virginia bunch from Mozambique) showed moderate resistance to late leaf spot.

The accessions ICG 13787 and ICG 14985 showed high level of resistance to *Aspergillus* seed colonization. The accession ICG 13787 (Virginia bunch from Nigeria) was also resistant to late leaf spot and ICG 14985 (Spanish bunch of unknown origin) came to early flowering with comparable yield with the best check (GPBD-4).

An accession (ICG 11088) belonging to *Peruviana* botanical type and originating from Peru was found best with lowest lectin activity in the mini core.

Accessions with potential for multiple traits

Based on performance in comparison to checks twenty-nine germplasm accessions were found superior for more than one trait (Table 28). They belonged to different botanical types namely; Valencia (9), Spanish bunch (7), Virginia runner (6) and Virginia bunch (7) and originated from different geographical places. These accessions along with cultivars (GPBD-4, M 28-2, TAG 24, JL 24 and MN 1-35) were subjected to diversity analysis based on RAPD. The genetic similarity ranged from 0.63 to 0.93 indicating substantial diversity (Fig. 8).

The highest genetic diversity was observed between the accessions ICG 76 (Virginia runner from India) and ICG 14118 (Spanish bunch from United Kingdom); the cultivar GPBD-4 (Spanish bunch from India) and ICG 8760 (Virginia runner from Zambia); M 28-2 (Valencia cultivar from India) and ICG 14475 (Virginia bunch from Nigeria); TAG 24 (Spanish bunch cultivar from India) and ICG 11219 (Virginia runner from Mexico); JL 24 (Spanish bunch cultivar from India) and ICG 8760 (Virginia runner from Zambia); MN 1-35 (Valencia breeding line from India) and ICG 12682 (Spanish bunch from India). The dendrogram constructed from the molecular data revealed distinct cluster of accession ICG 2381 (Virginia runner from

Fig. 7: Dendrogram depicting genetic diversity of selected accessions for lectin activity in mini core

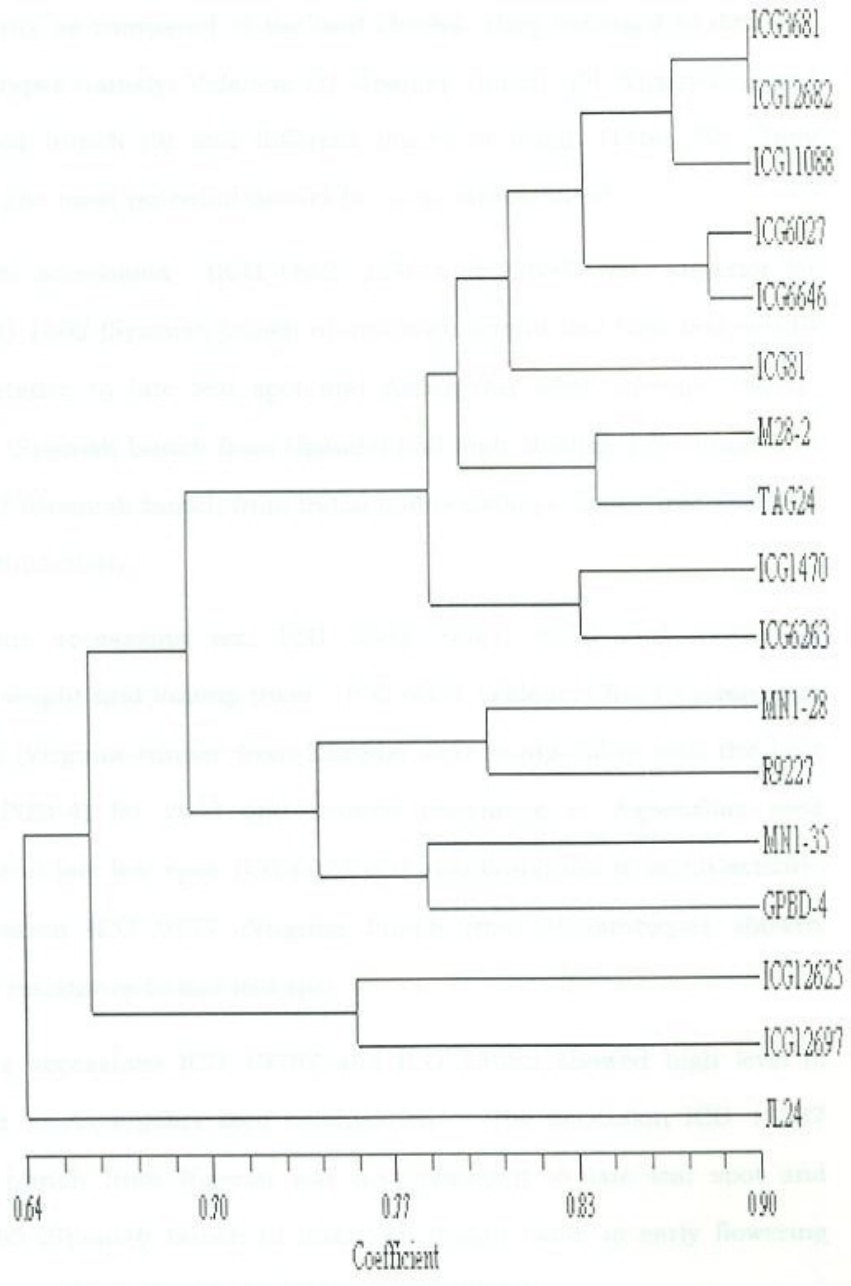


Table 27: Performance of accessions superior for different traits

Sl. No	Genotype	Type	DFG	YPP (g)	SPP (%)	SMK (%)	TWT (g)	ASP	LLS	Lectin (HAU/mg)	Protein (%)
1.	ICG 1862	SB	33.50	33.50	71.34	93.66	63.99	2.16	3.67	2495.06	18.8
2.	ICG 3240	SB	38.50	30.14	75.25	93.04	42.56	3.62	5.84	3191.87	14.7
3.	ICG 4716	SB	36.50	22.76	64.47	93.60	32.30	4.00	3.00	2607.99	17.9
4.	ICG 5662	VB	32.00	17.49	68.52	86.36	64.90	3.29	4.67	5609.72	16.7
5.	ICG 6027	VL	38.00	21.91	69.79	94.38	60.82	1.02	4.47	1259.62	18.7
6.	ICG 8760	VR	36.00	20.31	72.01	92.78	64.26	1.00	2.84	5127.45	18.3
7.	ICG 9777	VB	34.50	13.83	70.14	89.34	61.96	3.94	3.67	5724.05	16.4
8.	ICG 11088	PA	30.00	12.77	67.56	95.74	37.34	3.90	6.85	693.58	16.9
9.	ICG 12697	SB	40.00	33.01	72.09	94.91	28.84	2.96	2.50	1663.42	14.1
10.	ICG 13787	VB	36.00	10.51	65.61	87.35	57.57	1.10	2.50	2479.65	19.0
11.	ICG 14985	SB	29.00	22.04	71.44	96.83	52.93	1.08	6.17	2790.43	16.8
12.	GPBD-4	SB	32.00	20.25	76.75	95.19	42.38	3.84	1.62	2567.57	18.3
13.	M 28-2	VL	30.00	18.31	69.73	96.54	52.86	3.64	2.67	2636.60	17.8
14.	TAG 24	SB	29.00	11.99	74.20	92.29	44.67	3.70	6.84	2628.87	18.0
15.	JL 24	SB	29.00	18.06	77.88	93.31	50.25	3.88	5.33	1489.97	15.8
16.	MN 1-35	VL	31.00	19.73	69.77	96.28	61.48	1.16	1.67	2435.86	16.6

DFF : Days to 50% flowering

YPP : Yield per plant

SPP : Shelling percentage

SMK : Sound mature kernel

TWT : Test weight

ASP : *Aspergillus* seed colonization

LLS : Late leaf spot

VL : Valencia

SB : Spanish bunch

VR : Virginia runner

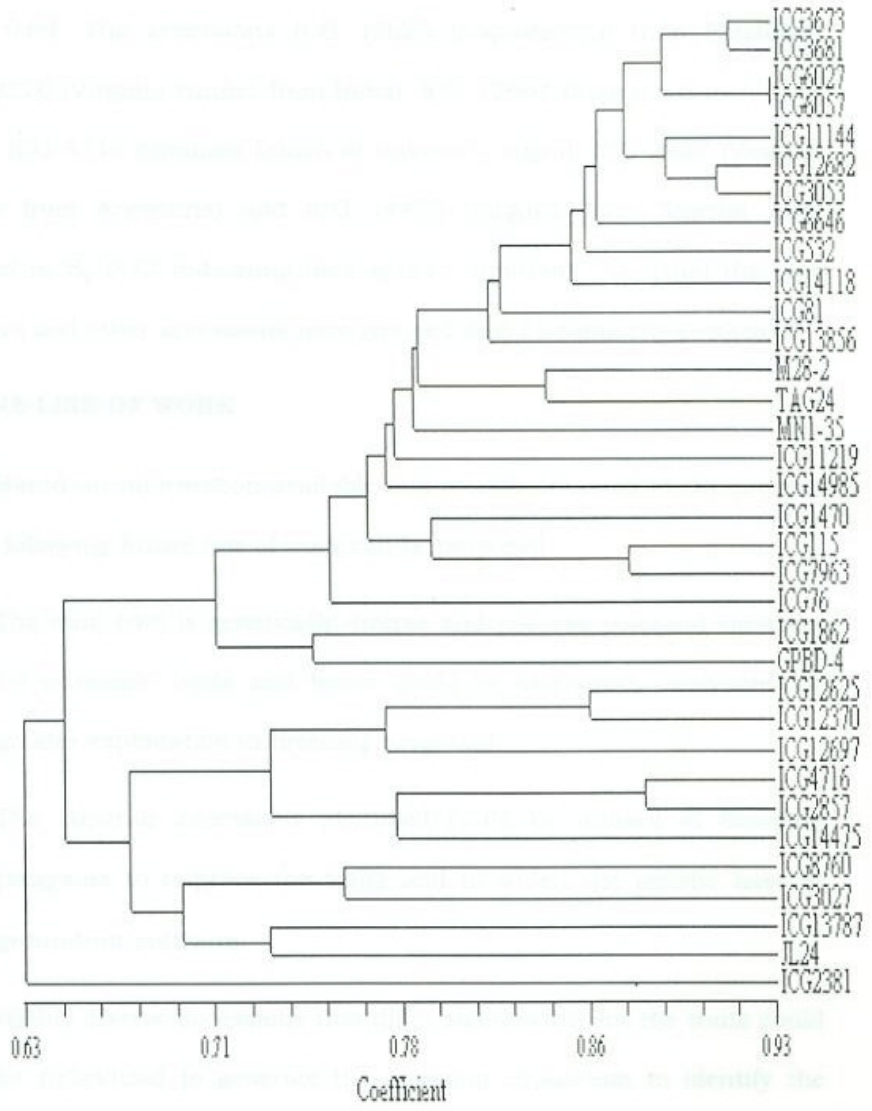
VB : Virginia bunch

PA : Peruviana

Table 28. Germplasm accessions with potential for multiple traits

	Accessions	Characters
Valencia	ICG 115	High protein and sound mature kernel
	ICG 1470	High protein and low lectin activity
	ICG 3673	High shelling percent and resistance to *Aspergillus* infection
		Early flowering and high protein
	ICG 3681	High test weight, yield per plant, protein content with resistance to *Aspergillus* infection
	ICG 6027	High test weight and low lectin activity
	ICG 6646	High shelling percent and sound mature kernel
	ICG 11145	High test weight, low lectin activity, resistance to late leaf spot and *Aspergillus* infection
	ICG 12625	High shelling percent and yield per plant
	ICG 13856	
Spanish bunch	ICG 81	High sound mature kernel percent and low lectin activity
	ICG 1862	High yield per plant and resistance to *Aspergillus* infection
	ICG 4716	High yield per plant and resistance to Late leaf spot
	ICG 12682	Low lectin activity and resistance to late leaf spot
	ICG 12697	High yield per plant, low lectin activity and resistance to late leaf spot
	ICG 14118	High shelling percent and sound mature kernel
	ICG 14985	Early flowering, high test weight and resistance to *Aspergillus* infection
Virginia runner	ICG 76	Resistance to late leaf spot and *Aspergillus* infection
	ICG 2381	High test weight and resistance to *Aspergillus* infection
	ICG 2857	High test weight and resistance to late leaf spot
	ICG 8760	High test weight, yield per plant, resistance to late leaf spot and *Aspergillus* infection
	ICG 11219	High yield per plant and resistance to *Aspergillus* infection
	ICG 12370	High shelling percent and sound mature kernel
Virginia bunch	ICG 532	Early flowering and resistance to Late leaf spot
	ICG 3027	Early flowering, high test weight and resistance to Late leaf spot
		Early flowering and high sound mature kernel
	ICG 3053	High protein and test weight
	ICG 6057	High shelling percent and sound mature kernel
	ICG 7963	High test weight, resistant to Late leaf spot and *Aspergillus* infection
	ICG 13787	High sound mature kernel, protein content and resistant to Late leaf spot
	ICG 14475	

Fig. 8: Dendrogram depicting genetic diversity of potendtial accessions for multi traits in mini core



Brazil) away from the rest at 0.63 similarity coefficient. Besides being potential donors of multiple traits these accessions are also useful in widening the genetic base of existing cultivars.

The accessions ICG 6027 (Valencia from Sudan) and ICG 6057 (Virginia bunch from USA) were found to be most similar at S_{ij} 0.92. The cultivar JL 24 was grouped along with the accessions ICG 13787, ICG 3027 and ICG 8760 and thus appeared to be distinct from the others at S_{ij} 0.69. The accessions ICG 12625 (*aequatoriana* from Ecuador), ICG 12370 (Virginia runner from India), ICG 12697 (Spanish bunch from India), ICG 4716 (Spanish bunch of unknown origin), ICG 2857 (Virginia runner from Argentina) and ICG 14475 (Virginia from Nigeria) were grouped at S_{ij} 0.72 indicating their genetic similarity. As usual the four cultivars and other accessions were grouped at 0.71 similarity coefficient.

FUTURE LINE OF WORK

Based on information available and results obtained in the present study, following future line of work can be proposed.

1) The mini core is genetically diverse and possess potential variation for economic traits and hence could be extensively evaluated for greater exploitation in breeding programs.

2) The superior accessions identified could be utilized in breeding programs to improve the traits and to widen the genetic base of groundnut cultivars.

3) Highly diverse accessions that differ significantly for the traits could be hybridized to generate the mapping population to identify the markers for use in crop improvement programs through marker-assisted selection.

4) Substantial molecular diversity existed in the mini core and hence more RAPD primers and advanced markers like SSRs and AFLPs should be screened to identify an adequate number of polymorphic primers to enhance its scientific exploitation.

VI. SUMMARY

The cultivated peanut or groundnut (*Arachis hypogaea* L.) is one of the most important vegetative oil seed crops in the world. Even though India is a leading producer, the productivity is very low as compared to other major producers like USA and China, which calls for sustained genetic improvement of the crop. Currently cultivated varieties have narrow genetic base there by necessitating the exploitation of variability in the germplasm. In any crop improvement program it is pre-requisite to maintain the genetic variability to identify the desirable genotypes and large germplasm collections are available for the purpose. But the resources available for evaluation of germplasm are limited and dwindling steadily. The mini core subset that represents 1 % of the entire collection but preserves the variation present in the core subset provides an easy approach to access the genetic resources that can be extensively examined for all agronomically important traits.

Keeping this in view, the present study was undertaken with an aim to characterize the mini core using morphological traits, evaluate for agronomical traits and assess the genetic diversity using Randomly Amplified Polymorphic DNA.

Morphological characterization

Study on morphological characterization revealed the uniqueness of Valencia type among four botanical types. Presence of stem pigmentation, abundant stem hairiness, canary yellow flowers and bright seed colours (light red, red, dark red and dark purple) were observed only in Valencia type while, Decumbent-3 growth habit and absence of beak were observed in Spanish bunch. Four morphological descriptors namely, sequential branching pattern, wide elliptic leaflet shape, slight constriction and moderate pod reticulation were found in subsp. *fastigiata* (Valencia and Spanish bunch). Wide elliptic followed by orbicular leaflet shape and slight pod constriction was characteristic to Virginia runner while wide elliptic followed by orbicular elliptic leaf let shape and slight followed by moderate and deep pod constriction were specific to Virginia bunch and remaining characters were common for both the Virginia runner and Virginia bunch. Distinct morphological features existing in different subspecies and botanical types could be exploited for developing cultivars with unique features that are required in the new PVP regime.

Principal component analysis

It is a reduced dimension model that would indicate measured differences among the groups. Among quantitative traits, sound mature kernel, yield per plant, test weight, shelling percentage, late leaf spot and protein content had higher loading values in first three principal components, where as, among the ten morphological descriptors pod beak, pod constriction, pod reticulation, stem hairiness, branching pattern, leaf let shape and seed colour hid high loading values indicating their importance as groundnut descriptors.

Phenotypic diversity

Shannon-Weaver diversity index was used to calculate the variation among the traits in mini core and four botanical types. It revealed diversity for some morphological traits, pod reticulation and stem pigmentation in Valencia; pod reticulation and leaf let shape in Virginia runner; pod beak, pod constriction, pod reticulation and flower colour in Virginia bunch. While, across nine quantitative charters, *Aspergillus* seed colonization in Spanish bunch and Virginia bunch showed more diversity in different botanical subsets representing mini core. Valencia showed higher diversity for qualitative traits but it was limited for quantitative traits indicating significant variation for qualitative traits.

Morphological differentiation

The genetic similarity between 195 genotypes for qualitative characters was assessed by similarity coefficient. The clusters did not conform to any defined pattern relating to the geographical origin but each cluster represented predominantly by a particular botanical type and each botanical type was differentiated into distinct clusters. Thus to maintain the morphological diversity due consideration should be given to botanical types as well as clusters based on qualitative traits in the selection of material for hybridization. The

genetic dissimilarity assessed by Euclidian distance on quantitative characters revealed lack of any correspondence with either geographical origin or botanical types. Gower's similarity coefficient based on combined data of qualitative and quantitative traits revealed differentiation specific to subspecies thus revealing lack of much contribution of quantitative characters to morphological diversity.

Molecular diversity

This study was carried out for better scientific exploitation of genetic resource by identifying diverse germplasm superior for multiple agronomical traits so that they can be intercrossed to develop potential breeding populations. Out of the twenty primers used, the primers OPK 14, OPA 19, OPC 15, OPC 09, OPC 13, OPB 11, OPF 09, OPJ 06, OPV 16, OPA 15, OPA 20, OPA 12, OPF 10 have shown high polymorphism across all four botanical types. The polymorphism per primer ranged from 57.14 to 100 per cent. The extent of polymorphism in these primers was much higher than earlier reports indicating the importance of large as well as diverse material in exhibiting the polymorphism. The accessions ICG 8760 with ICG 442, 1274, 6402 and GPBD-4 with ICG 2381 and ICG 1274 with ICG 12189 have shown high degree of genetic diversity in their DNA profile. Thus there is a possibility of use of RAPD assay in understanding and manipulating the groundnut genome.

Genetic similarity among the accessions ranged from 0.21 to 0.93. Clustering based on Jaccard similarity coefficients resulted in six distinct clusters. The accessions belonging to four botanical types were distributed in all the clusters with slightly varying proportions. This indicated independence of molecular diversity with morphological diversity.

In general major clusters did not show any association with geographical origin of the accessions. But with in each cluster some accessions belonging to the same or different botanical types but belonging to the same origin were included in sub clusters revealing their genetic similarity. This could indicate association of molecular diversity with micro evolutionary changes leading to regional adaptation.

Evaluation

The analysis of variance revealed significant genotypic differences for days to flowering, yield per plant, test weight and lectin activity indicating that the accessions included in the mini core displayed high variation among genotypes and hence are amenable to selection. Among four botanical types, Virginia bunch type came to flowering earlier than the other three types. Out of 195 accessions twenty-two accessions came to flowering with in 29 days, which included three (ICG 188, 532, 3027) that flowered at 28 days but none of the accessions belonging to Virginia runner flowered early (by 29 days).

Eighteen genotypes recorded yield comparable to the best check (GPBD-4). They belonged to different botanical types namely, Valencia (7) and Spanish bunch (8), Virginia runner (2) and Virginia bunch (1) and originated from different places. Among selected genotypes the maximum genetic diversity was observed between ICG 15042 and ICG 8285 that could be utilized as parents to develop segregating populations with a potential for diversity.

Kernel recovery mainly depends on the shelling percentage and thus there is a need to identify genotypes with high shelling percent. Nineteen accessions were found comparable to the best check (GPB-4) and they belonged to different botanical types *viz.,* Valencia (5), Spanish bunch (11), Virginia runner (1) and Virginia bunch (2). Sound mature kernel should be as high as possible in view of both consumer and processor acceptance. Thirty-two accessions belonging to Valencia (8), Spanish bunch (11), Virginia runner (6) and Virginia bunch (7) were found superior for that trait.

Normally varieties with hundred seed mass of 60 g or more are considered as large seeded groundnuts and they are preferred for confectionary purpose. Maximum number of selected accessions belonged to Subsp. *hypogaea viz.,* Virginia bunch (9 accessions) and Virginia runner (5) as compared to Subsp. *fastigiata i.e.,* Valencia (4) and Spanish bunch (2). These accessions revealed wide genetic diversity as indicated by RAPD assay. Maximum genetic diversity was observed between the cultivar JL 24 and ICG 14985. The accession

ICG 2381 of Virginia runner was distinct and away from the rest at 0.62 similarity coefficient. These genetic relations could be of use in planning crossing programs.

Excessive use of pesticides can adversely affect the quality of the produce. In addition to causing substantial yield loss, they also affect seed quality adversely. Hence. There is need to develop varieties with inbuilt resistance to pests and diseases which could minimize the problem of pesticide residues. *Aspergillus* seed colonization and late leaf spot are the major diseases of groundnut. Among the checks,GPBD-4 was resistant to late leaf spot while MN 1-35 had resistance to the late leaf spot and also for *Aspergillus* seed colonization.

Sixteen accessions selected for late leaf spot resistance belonged to different botanical types namely, Spanish bunch (4), Virginia runner (5) and Virginia bunch (6) and *Peruviana* (1). The maximum diversity was observed between two Virginia bunch accessions ICG 13787 and ICG 532. The accession ICG 6706 (Virginia bunch from USA) was grouped separately away from the rest at 0.64-similarity coefficient thus revealing its genetic distinctness and potential for use in crop improvement.

Four accessions *viz.,* 6027, 14985, 8760 and 13787 have shown high resistance (scale ranged from 1 to 1.5) and seven were moderately resistant (from 2.1 to 2.5) to *Aspergillus* seed colonization. These genotypes were distributed across all four botanical types namely, Valencia (3), Spanish bunch (2), Virginia runner (4), Virginia bunch (2). Among them the susceptible cultivar TAG 24 and a resistant accession ICG 14985 were found genetically most divergent. The two Virginia runner accessions ICG 8760 (Zambia) and ICG 2381 (Brazil) were highly distinct from all other accessions including cultivars. These accessions could be used in hybridization for developing mapping populations to identify DNA markers associated with resistance and generating breeding populations.

Groundnut is a good source of protein, which is of high quality in nature. Fourteen accessions were selected for high soluble protein (above 19 mg/g of defatted seeds) and were found superior to the best check, GPBD-4. They belonged Valencia (3), Spanish bunch (1), Virginia runner (3) and Virginia bunch (7). Similarly, Groundnuts contain a galactose specific lectin called Peanut anti-T agglutinin (PNA) that is known for its mitogenicity (cell proliferation). Ten accessions with low (693.58 to 2000 HAU / mg of protein) and six accessions with high (10,803.11 to 14,360.52 HAU/ mg of protein) lectin activity were identified as significant germplasm important for the trait. They belonged to different botanical types namely Valencia (6), Spanish bunch (6), Peruviana (1), *acquetoriana* (1), Virginia bunch (1) and Virginia runner (1). Diversity analysis on RAPD revealed wide genetic diversity between ICG 14482 (high activity) and ICG 6027 (low activity) followed by ICG 4955 (high activity) and ICG 12697 (low activity). They can be conveniently used in the crossing program to generate mapping populations to identify the markers associated with the lectin activity.

Significantly superior accessions

Eleven accessions were significantly superior for different traits *viz.,* yield, test weight, *Aspergillus* seed colonization, late leaf spot and low lectin activity as compared to the best checks. They belonged to different botanical types namely, Valencia (2), Spanish bunch (5), Virginia runner (1), Virginia bunch (3) indicated they constitute the most potential donors for crop improvement. Among them three accessions (ICG 1862, 3240 and 12697) were superior for yield, four accessions *viz.,* ICG 5662, 6027, 8760 and 9777 had high-test weight, two accessions ICG 13787 and ICG 14985 showed high level of resistance to *Aspergillus* seed colonization and two accessions ICG 6027 and ICG 11088 were found best with lowest lectin activity in the mini core.

Accessions with potential for multiple traits

Based on performance in comparison to checks, twenty-nine accessions were found superior for more than one trait and they belonged to different botanical types namely; Valencia (9), Spanish bunch (7), Virginia runner (6) and Virginia bunch (7). Diversity analysis based on RAPD revealed highest genetic diversity between ICG 76 and ICG 14118 and distinct clustering of ICG 2381 away from the rest at 0.63 similarity coefficient.

Besides being potential donors of multiple traits these accessions are also useful in widening the genetic base of the existing cultivars.

VII. REFERENCES

ANDERSON, W. F., L. C. WYNNE, C. C. GREEN, AND M. K. BEUTE, 1986. Combining ability and heritability f resistance to early and late leaf spot of peanut. *Peanut Science*, **31** : 588-593.

ANDERSON, W. F., HOLBROOK, C. C. AND BRENNEMAN, T. B., 1993, Resistance to *Cercosporidium personatum* within peanut germplasm. *Peanut Science*, **20** : 53-57.

ANDERSON, W. F., HOLBROOK, C. C. AND WYNNE, J. C., 1991, Heritability and early generation selection for resistance to early and late leafspot in peanut. *Crop Science*, **31** : 588-593.

ANDERSON, W. F., HOLBROOK, C. C., WILSON, D. M. AND MATHERON, M. E., 1995, Evaluation of pre harvest aflatoxin contamination in some potentially resistant peanut genotypes. *Peanut Science*, **22** : 29 – 32.

ANONYMOUS , 1992, Descriptors for groundnut. International Board for plant genetic resources, Rome, Italy, International crops research Institute for the semi-arid tropics. Patancheru, India.

ANONYMOUS, 2000, *Oil seeds Situation - A statistical compendium*, International crops Research Institute for the semi-arid tropics (ICRISAT). Patancheru, India

AQUINO, V. M., SHOKES, F. M., GORBET, D. W. AND NUTTER, F. W., 1995, Late leaf spot progression on peanut as affected by components of partial resistance. *Plant Disease,* **79** : 74-78.

BHAGAT, N. R., LALWANI, H. B., TASLIM AHMAD, DHAPWAL, G. S. AND NAGARAJ, G., 1984, Evaluation of groundnut collections from Zambia. *Indian Journal of Genetics and Plant Breeding*, **44** : 406-410.

BHAGAT, N. R., TASLIM AHMAD LALWANI, H. B. AND HARENDER SINGH, 1985, Status of National resources of cultivated groundnuts. *Indian Journal of Genetics and Plant Breeding*, **45** : 171-177.

BHAGWAT, A., KRISHNA, T. G. AND BHATIA, C. R., 1997, RAPD analysis of induced mutants of groundnut (*Arachis hypogaea*). *Journal of Genetics*, **76** : 201-208.

BLANKENSHIP, P. D., COLE, R. J. AND SANDERS, T. H., 1985, Comparative susceptibility of four experimental peanut lines and the cultivar, Floruner to pre – harvest aflatoxin contamination. *Peanut Science*, **12** : 70 – 72.

BOOKWALTER, N. R., WARNER, K., ANDERSON, R. A. AND BAGELY, E. B., 1979, Peanut – fortified food blends. *Journal of food sciences*, **44** : 820-825.

BOWEN, K. L. AND MACK, T. P., 1993, Relationship of damage from lesser corn stalk borer to *Aspergillus flavus* contamination in peanuts. *Journal of Entomological Science*, **28** : 29 – 41.

BROWN, A. H. D., 1988, The case for core collections. In A. H. D. Brown *et al.* (ed.). The use of plant genetic resources, Cambridge University. Press, Cambridge. 136-156 p.

BROWN, A. H. D., 1989a, Core collections: A practical approach to genetic resources management. *Genome,* **31** : 818-824.

BROWN, A. H. D., 1989b, The case for core collections. In : A. H. D. Brown (ed) *The Use of Plant Genetic Resources,* Cambridge University Press, Cambridge, England, pp. 136-155.

BROWN, A. H. D. AND WEIR, B. S., 1983, Measuring genetic variability in plant population. In: Tanksley A, Orton TJ (eds) *Isozymes in Plant Genetic and Breeding Part A*. Elsevier, Amesterdam, 219-238 pp.

BUROW, M. D., SIMPSON, C. E., PETERSON, A. M. AND STARR, J. I., 1996, Identification of peanut (*Arachis hypogaea* L) RAPD marker diagnostic of root knot nematode (*Meloidogyne arenaria* (Neal) Chitwood) resistance. *Molecular Breeding*, **2** : 369-379.

CAST, 1989, Mycotoxins: economics and health risks. *Task Force Report 116*, Council of Agricultural Sciences and Technology. Ames. IA.

CHANDRAN, K. AND PANDYA, S. M., 2000, Morphological characterization of Arachis species of section Arachis. *Plant Genetic Resource Newsletter*, **121** : 38-41.

CHITEKA, Z. A., GORBET, D. W., KNAUF, D. A., SHOKES, F. M. AND KUCHAREK, T. A., 1988a, Components of resistance to late leaf spot in peanut. II. Correlations among components and their significance in breeding for resistance. *Peanut Science,* **15** : 25-30.

COLE, R. J., DORNER, J. W. AND HOLBROOK, C. C., 1995, Advances in mycotoxin elimination and resistance. In: H. E. Pattee and H. T. Stalker (eds). *Advances in Peanut Science,* American Peanut Research Education Society, Stillwater, OK, pp. 456–474.

DWIVEDI, S. L. AND GURTU, S., 2002, Molecular diversity among accessions possessing varying levels of resistance to Early Leaf Spot in groundnut. *International Arachis News Letter*, **22** : 36-67.

DWIVEDI, S.L., NIGAM, S.N., NAGESWARA RAO, R.C., SINGH, U. AND RAO, K.V.S., 1996, Effect of drought on oil, fatty acids and protein contents of groundnut (*Arachis hypogaea L.*) seeds. *Field Crps Research,* **48**: 125-133.

DWIVEDI, S. L. AND VERMA, T. S., 2002, Molecular diversity among groundnut varieties differing in drought tolerance traits. *International Arachis Newsletter*, **22** : 34 – 36.

DWIVEDI, S. L., GURTU, S., CHARNDRA,S., YUEJIN,W. AND NIGAM,S. N., 2001, Assessment of genetic diversity among selected groundnut germplasm RAPD Analysis. *Plant Breeding*, **120** : 345-349.

DWIVEDI, S. L., NIGAM, S. N., JAMBUNATHAN, R., SAHRAWAT, K. L., NAGABHUSHANAM, G. V. S. AND RAGHUNATH, K., 1993, Effect of genotype and environment on oil content and oil quality parameters and their correlation in peanut (*Arachis hypogaea* L.), *Peanut Science*, **20** : 84-89.

EEDEN, C. F., VAN RENSBURG, J. B., VAN LINDE, T. K. AND VANDER., 1994, The role of insect damage in the colonization of groundnut (*Arachis hypogaea* L.) pods by fungi. *South African Journal of Plant and Soil*, **11** : 159 – 162.

ESQUIVEL, M., BARRIOS, M., WALON, L. AND HAMMER, K., 1993, Peanut (*Arachis hypogaea* L.) genetic resources in Cuba 1, collecting and characterization. *IBPGR Plant Genetic Resource Newsletter*, **92** : 9-15.

FAO 2004. Food and Agriculture Organization of the United Nations, FAOSTAT database. *http://www. FAO. ORG*.

FERGUNSON, M. E., BRAMEL, P. J. AND CHANDRA, S., 2002a, Genetic diversity assessment of cultivated peanut (*Arachis hypogaea* L.) botanical varieties from three continents using simple sequence repeats. In: *First International Conference on Legume Genomics and Genetics: Translation to Crop Improvement*, June 2-6, Minneapolis- St. Paul, MN.

FEGURSON, M. E., BUROW, M., SCHULZE, S., BRAMEL, P., PATERSON, A. AND KREOVICH, S., 2002b, Identification and characterization of micrisatellites from size fractionated genomic libraries of peanut (*Arachis hypogaea* L.). In: *First International Conference on Legume Genomics and Genetics: Translation to Crop Improvement,* June 2-6, Minneapols- St. Paul, MN.

FEGURSON, M. E., BUROW, M., SCHULZE, S., BRAMEL, P., PATERSON, A., KREOVICH, S. AND MITCHELL, S., 2004, Microsatellite identification and characterization in peanut (*Arachis hypogaea L.). Theoratical and Appklied Genetics,* **108** : 1064-1070.

FRANKEL, O. H., 1984, Genetic perspectives of germplasm conservation. In : W. K. Arer *et al.* (ed.) *Genetic Manipulation : Impact on Man and Society*. Cambridge University Press, Cambridge, UK, pp. 161-170.

FRANKEL, O. H. AND BROWN, A. H. D., 1984, Current plant genetic resources- a critical appraisal. In : V. L. Chopra, B. C. Joshi, R. P. Sharma and H. C. Bansal (eds). *Genetics: New Frontiers* Vol. VI. Oxfort and IBH Publ. Co., New Delhi, pp. 1-11.

GARCIA, G. M., STALKER, H. T. AND KOCHERT, G., 1995, Introgression analysis of an interspecific hybrid population in peanuts (*Arachis hypogaea* L.) using RFLP and RAPD markers. *Genome*, **38** : 166 – 176.

GEPTS, P., 1993, The use of molecular and biochemical markers in crop evolution studies. *In evolutionary biology*, volume 27 (Ed. Hecht), Plenum Press, New York.

GIBBONS, R. W., BUNTING, A. H., AND SMART, J., 1972, The classification of varieties of groundnut (*Arachis hypogaea* L.). *Euphytica*, **21** : 78-85.

GREEN, C. C. AND WYNNE, J. C., 1986, Field evaluation of the components of partial VALLS, J. F., RAMANATHA RAO. V., SIMPSON, C. E. AND resistance to early leaf spot in peanut. *Euphytica*. **35** : 561-573.

GREEN, C. C. AND WYNNE, J. C., 1987, Genetic variability and heritability for resistance to early leaf spot in four crosses of Virginia-type peanut. Crop Science, **27** : 18-21.

GREGORY, W. C. AND GREGORY, M. P., 1976, Groundnuts, in evolution of crop plants (ed. N. W. Simmonds), Longman Group Ltd., London, pp. 151-154.

GREGORY, W. C., GREGORY, M. P., KRAPOVICKAS, A., SMITH, B. W. AND YARBROUGH, 1973, Structures and genetic resources of peanuts In: Peanuts culture and uses. American Peanut Resource Education Society Stillwater, OK, pp. 47-133.

GREGORY, W. C., KRAPOVICKAS, A., AND GREGORY, M. P., 1980, Structures, variation, evolution and classification in *Arachis.* In : R. J. Summerfield and A. H. Bunting (eds), *Advances in Legume Science,* Royal Botanic Gardens, Kew, London.

GOWER, J. C., 1971, A general coefficient of similarity and some of its properties. *Biometrics*, **27** : 857-871.

HALWARD, T., STALKER, H. T., LARUE, E. AND KOCHERT, G., 1991, Genetic variation detectable with molecular markers among unadapted germplasm resources of cultivated peanut and related wild species. *Genome*, **34** : 1013-1020.

HALWARD, T., STALKER, H. T., LARUE, E. AND KOCHERT, G., 1992, Use of single–primer DNA amplification in genetic studies of peanut (*Arachis hypogaea* L.) *Plant Molecular Biology*, **18** : 315 – 325.

HALWARD, T., STALKAR, H. T. AND KOCHERT, G., 1993, Development of an RFLP linkage map in diploid peanut species. *Theory and Applied Genetics*, **87** : 379-384.

HARCH, B. D., LAWRENCE, P. K., CRUICKSHANK, A., TONKS, J., BASFORD, KE., DELACY, I. H. AND McLEOD, C., 1995, Diversity in the Australian groundnut germplasm collection. *International Arachis Newsletter*, **15** : 15.

HE, G. AND PRAKASH, S. H., 1997, Identification of polymorphic DNA markers in cultivated peanut (*Arachis hypogaea* L.). *Euphytica*, **97** : 143-149.

HE, G. AND PRAKASH, S. H., 2001, Evaluation of genetic relationships among botanical varieties of cultivated peanut (*Arachis hypogaea* L.) using AFLP markers. *Genetic Resources and Crop Evolution*, **48** : 347- 352.

HE, G. MENG, R., GAO, H., GUO, B., GAO, G., NEWMAN, M., PITTMAN, R. N. AND PRAKASH, S. H., 2005, Simple sequence repeat markers for botanical varieties of cultivated peanut (*Arachis hypogaea* L.) *Euphytica*, **142** : 131-136.

HE, G., MENG, R., NEWMAN, M., GAO, G., PITTMAN, N. R. AND PRAKASH, C. S., 2003, Microsatellites as DNA markers in cultivated peanut (*Arachis hypogaea* L.) *Biomedical central Plant Biology*, **3** : 3.

HOLBROOK, C. C., 1999, Testing and utilization of a core collection for the U. S. germplasm collection peanut. In : R. C. Johnson and T. Hodgkin (ed.), *Core Collection for Today and Tomorrow*. International Plant Genetic Resources Institute, Rome, Italy, pp. 68-73.

HOLBROOK, C. C., 2001, Status of the United States germplasm collection of peanut. *Peanut Science,* **28** : 84-89.

HOLBROOK, C. C. AND ANDERSON W. F., 1995, Evaluation of a core collection to identify resistance late leaf spot in peanut. *Crop Science*. **35** : 1700-1702.

HOLBROOK, C. C., ANDERSON, W. F. AND PITTMAN, R. N., 1993, Selection of a core collection from the U. S. germplasm collection of peanut. *Crop Science*. **33** : 859-861.

HOLBROOK, C. C. AND ISLEIB, T. G., 2001, Geographical distribution of genetic diversity in *Arachis hypogaea*. *Peanut Science*, **28** : 80-84.

HOLBROOK, C. C., WILSON, D. M., MATHERON, M. E. AND ANDERSON, W. F., 1997, *Aspergillus* colonization and aflatoxin contamination in peanut genotypes with resistance to other fungal pathogens. *Plant Diseases*, **81** : 1429 – 1431.

HONAVAR, P.M., KRITCHEVSKY, D. TEPS, S.A. AND KULRFIELD D.M., 1998, Lectin may contribute to the atherogencity of peanut oil. *Lipids*, **33**, 821-823.

HOPKINS, M. S., DASA, A. M., WANG, T., MITCHELL, S. E., KOCHERT, G. D., KRESOVICH, S., 1999, Discovery and characterization of polymorphic simple sequence repeats (SSRs) in peanut. *Crop Science*, **39** : 1243-1247.

http:// www. fao. org/compend/text/Reuirements for export and quality assurance. htm.

IROUME, R. N., AND KNAUFT. D. A., 1987a, Heritabilities and genetic, environmental, and phenotypic correlations for pod yield and leaf spot severity in peanuts (*Arachis hypogaea* L.): implications for early generation selection. *Peanut Science,* **14** : 46-50.

ISLEIB, T. G. AND WYNNE. J. C., 1983. Heterosis in test crosses of 27 exotic peanut cultivars. Crop Science, **23** : 832-841.

JENKINS, W. A., 1938, Two fungi causing leaf spot of peanut. *Journal of Agricultural Research*, **56** : 317 – 332.

JOGLOY, S., WYNNE, J. C. AND BEUTE, M. K., 1987. Inheritance of late leaf spot resistance and agronomic traits in peanut. *Peanut Science,* **26** : 85-94.

KATULE, B. K., THOMBARE, M. V., DUMBARE, A. D. AND PAWAR, B. B., 1992, genetic diversity in groundnut. *Journal of Maharashtra Agricultural Universities*, **17** : 302-303.

KISYOMBE, C. T., BEUTE, M. K. AND PAYNE, G. A., 1985, Field evaluation of peanut genotypes for resistance to infection by *Aspergillus parasiticus. Peanut Science*, **12** : 12 – 17.

KOCHERT, G. D. AND KRESOVICH, S., 1999, Discovery and characterization of polymorphic simple sequence repeats (SSRs) in peanut. *Crop Science*, **39** : 1243-1247.

KOCHERT, G., HALWARD, T., BRANCH, W. D. AND SIMPSON, C. E., 1991, RFLP, variability in peanut (*Arachis hypogaea* L.) cultivars and wild species. *Theoretical and Applied Genetics*, **81** : 565–570.

KORNEGAY, J. L., BEURE, M. K. AND WYNNE, J. C., 1980. Inheritance of resistance to *Cercospora arachidicola* and *Cercosporidium personatum* in six Virginia type peanut lines. *Peanut Science*, **7** : 4-9.

KRAPOVICKAS, A., 1969. The origin, variability and spread of the groundnut (*Arachis hypogaea*) (Englisn translation). In : Ucko, P. J. and Falk, I. S., *The Domestication and Exploitation of Plants and Animals,* Gerald Duckworth Co. Ltd., London, pp. 424-441.

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-Cor-ruentes. Academia Nacional de Agronomia y Veterinaria Tomo XLIX: 18-26 Buenos Aires, Argentina.

KRAPOVICKAS, A. AND GREGORY, W. C., 1994, Taxonomia del Arachis (*Leguminosae*). *Bonplandia,* **VIII** : 1-187.

KRICHEVSKY, D., TEPPS, S. A. AND KULRFIELD, D. M., 1998, Lectin may contribute to the autherogenicity of peanut oil. *Lipids,* **33** : 821-823.

LANHAM, P. G., FENNELL, S., MOSS, J. P. AND POWELL, W., 1992, Detection of polymorphic loci in *Arachis* germplasm using random amplified polymorphic DNAs. *Genome*, **35** : 885 – 889.

LIENER, I, E. AND HILL, E. G., 1953, The effect of heat treatment on the nutritive value and hemaglutinating activity of soybean oil meal. *Journal of Nutrition*, **49** : 609-620.

LOWRY, O. H., 1951, *Journal of Biological Chemistry*, **193** : 365.

LUSAS, E. N., 1979, Food uses of peanut protein. *Journal of American Oil Chemistry Society,* **56** : 425 - 430.

LYNCH, R. E. AND WILSON, D. M., 1991, Enhanced infection of peanut, *Arachis hypogaea* L. seeds with *Aspergillus flavus* group fungi due to external scarification of peanut pods by the lesser corn stalk borer, *Elasmopalpus lignosellus* (zeller). *Peanut Science*, **18** : 110 – 116.

MALLIKARJUNA SWAMY, B. P., 2001, Characterization of Asian core collection of groundnut (*Arachis hypogaea* L.). *M. Sc. (Agri.) Thesis*, University of Agricultural Sciences, Dharwad.

MALLIKARJUNA SWAMY, B. P., UPADHYAYA, H. D., KENCHANA GOUDAR, P. V., KULLISWAMY, B. Y. AND SINGH, S., 2003, phenotypic variation for agronomic characteristics in a groundnut core collection for Asia, *Field Crops Research*, **84** : 359-371.

MANIVEL, P., MATHUR, R. K., BANDYOPADHYAYA, A., SAMDUR, M. Y. AND MISRA, J. B., 2000, Evaluation of some confectionary types advanced breeding lines of groundnut. *International Arachis Newsletter*, **20** : 20 – 21

MEHAN, V. K., 1989, Screening groundnut for resistance to seed invasion by *Aspergillus flavus* and aflatoxin production. In : *Aflatoxin Contamination of Groundnut : Proceedings of the International Workshop*, 6-9 Oct 1987, ICRISAT Center, India (McDonald, D. and Mehan, V. K., eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics, pp. 323-334.

MEHAN, V. K. AND Mc DONOLD, D., 1980, Screening for resistance to *Aspergillus flavus* invasion and aflatoxin production in groundnut. *ICRISAT groundnut Improvement Programme Occasinal Paper,* No. 2. Patancheru, Andhra Pradesh, India, p. 15.

MEHAN, V. K., Mc. DONALD, D., HARAVU, L. J. AND JAYANTHI, S., 1991, The groundnut aflatoxin problem : Review and literature database. Patancheru, Andhra Pradesh, India: International Crops Research Institute for Semi Arid Tropics, p. 387.

MEHAN, V. K., Mc DONOLD. D. AND RAJAGOPALAN, K., 1987, Resistance of peanut genotypes to seed infection in *Aspergillus* field trials in India. *Peanut Science*, **14** : 17 – 21.

MEHAN, V. K., McDONOLD. D., RAMAKRISHNA, N. AND WILLIAMS, J. H., 1986, Effects of genotypes and date of harvest on infection of peanut seed by *Aspergillus flavus* and subsequent contamination with aflatoxin. *Peanut Science*, **13** : 46 – 50.

MEHAN, V. K., RAMAKRISHNA, N., RAO, R. C. N. AND Mc. DONALD, D., 1995, Pre harvest aflatoxin contamination of groundnuts subjected to terminal drought stress in post rainy season. *Mycotoxin Research*, **11** : 103 – 109.

MILLER, I. L., NORDEN, A. J., KNAUFT, D. A. AND GORBET, D. W., 1990. Influence of maturity and fruit yield on susceptibility of peanut to *Cercosporidium personaturm* (late leaf spot pathogen). *Peanut Science*, **17** : 52-58.

MIXON, A. C. AND ROGERS, K. M., 1973, Peanut accessions to seed infection by *Aspergillus flavus. Agronomy Journal*, **65** : 560 – 562.

MOHAN BENTUR, 2002, Stability analysis for yield and confectionary characteristics in large seeded groundnut (*Arachis hypogaea* L.) genotypes. *M. Sc. (Agri.) Thesis*, University of Agricultural Sciences, Dharwad.

MONDAL, S., GHOSH, S. AND BADIGANNAVAR, A. M., 2005, RAPD polymorphism among groundnut genotypes differing in disease reaction to late leaf spot and rust. *International Arachis Newsletter*, **25** : 27-30.

MORETZSOHN, M. C., HOPKINS, M. S., MITCHELL, S. E., KRESOVICH, S., VALLS, J. F. AND FERRERIA, M. E., 2004, Genetic diversity of peanut (*Arachis hypogaea* L.) and its wild relatives based on the analysis of hypervariable regions of the genome. *Biomed Central Plant Biology*, **4** (1) : 11.

MURARI SINGH, MEHAN, V. K. AND Mc DONALD, D., 1989, Screening groundnuts for seed resistance to Aspergillus flavus – statistical approaches to data evaluation. Aflatoxin contamination of groundnut: Proceedings of International Workshop, 6-9 October 1987, ICRISAT center, India, pp. 335- 344.

NADAF, H. L., HABIB, A. F. AND GOUD, J. V., 1986, Analysis of genetic diversity in bunch groundnut. *Journal of Oilseeds Research*, **3** : 37-45.

NAGABHUSHANAM, G. V. S. AND RAGHUNATH, K., 1993, Effect of genotype and environment on oil content and oil quality parameters and their correlation in peanut (*Arachis hypogaea* L.), *Peanut Science*, **20** : 84-89.

NAGARAJA REDDY, R., PARAMESHWARAPPA, K. G. AND NADAF, H. L., 2004, Molecular diversity for resistance to late leaf spot and rust in parents and segregating population of a cross in groundnut. *International Arachis Newsletter*, **24** : 31-33.

NAGUIB, K. H., NAGUIB, M. M., DAIB, M. M., SAHAB, A. F. AND AMRA, H., 1989, Occurrence of aflatoxins and aflatoxin producing strains of *Aspergillus flavus* in groundnut cultivars in Egypt. *Aflatoxin Contamination of Groundnut : Proceedings of International Workshop*, 6 – 9 Oct 1987, ICRISAT Center, India, pp. 311 – 317.

NATARAJAN, S, T., SATHIMOORTHY, M. R., VENKATESWARAN, A. N. AND RAMACHANDRAN, T. K., 1978, Estimation of genetic parameters correlation studies in bunch groundnut (*Arachis hypogaea* L.), *Madras Agricultural Journal*, **65** : 497-501.

NORDEN, A. J., SMITH, O. D. AND GORBET, E. W., 1982. Breeding of the cultivated peanut. In : pattee, H. E. and Young, C. T., *Peanut Science and Technology,* Amer. Peanut Res. And Educ. Soc. Inc., Yoakum, TX, pp. 95-122.

OMRAN, A. O., DEMIAN, K. R. AND ABDEL-AL-I. M., 1980, Flowering and fruiting pattern in postrate and semi- postrate type. *Agricultural Review*, **58** : 107-116.

PAIK- RO, O. G., SMITH, R. L. AND KNAUFT, D. A., 1992, Restriction fragment length polymorphism evaluation of six peanut species within the *Arachis* section. *Theoretical and Applied Genetics*, **84** : 201-208.

PALANISAMI, A., MANICKAM, A. AND NEELAKANTAN, S., 1990, Fungal flora and aflatoxin production in relation to post harvest practices in groundnut. *Madras Agricultural Journal*, **77** : 26 – 31.

PATTANASHETTI, S. K., 2005, Genetic analysis of mutational origin of diversity in groundnut (*Arachis hypogaea* L.). *Ph. D. Thesis*, University of Agricultural Sciences, Dharwad.

PHANDIS, B. A., EKBOTE, A. P. AND MARKER, A. R., 1973, Correlation studies in some metrical characters in groundnut (*Arachis hypogaea* L.). *Jawaharlal Nehru Krishi Viswa Vidyalaya Research Journal*, **7** : 68-72.

PUEPPKE, S. G., 1981, Multiple molecular forms of peanut lectin: classification isolectin distribution among genotypes of the genus Arachis. *Arch. Biochem. Biophys*, **212** : 254-261.

QIANG WANG, Lu- GANA YU, CAMPBELL, B. J., MILTON, J. D. AND RHODES, J. M., 1998, Identification of intact peanut lectin in peripheral venous blood. *The Lancet*, **352** : 1831-1832.

RADHAKRISHNAN, T., CHANDRAN, K. AND DOBARAIA, J. R., 2004, Genetic diversity of Indian groundnut cultivers. *Short Papers Presented at the National Symposium Enhancing Productivity of Groundnut for Sustaining Food and Nutritional Security*, NRCG, Junagadh, pp. 64-66.

RAINA, S. N., RANI, V., KOJIMA, T., OGIHARA, Y., SINGH, K. P. AND DEVARUMATH, R. M., 2001, RAPD and ISSR fingerprint as useful genetic markers for analysis of genetic diversity, varietal identification, and phylogenetic relationship in peanut (*Arachis hypogaea*) cultivars and wild species. *Genome*, **44** : 763-772.

RAJENDRAPRASAD, 2003, Genetic enhancement of host plant resistance to *Spodoptera litura* (Fab.) in groundnut. *Ph. D. Thesis*, University of Agricultural Sciences, Dharwad.

RAJGOPAL, K., CHANDRAN, K. AND LALWANI, H. B., 2004, Characterization of cultivated groundnut (*Arachis hypogaea* L.) germplasm for yield and related descriptor. *Short Papers Presented at the National Symposium Enhancing Productivity of Groundnut for Sustaining Food and Nutritional Security*, NRCG, Junagadhm, pp. 9-10.

RAJGOPAL, K., CHANDRAN, K., BHAGAT, N. R. AND BHALODIA, P. K., 1997, Morphological characterization of Valencia and Virginia bunch peanut (*Arachis hypogaea* L.) germplasm. *Plant Genetic Resources Newsletter*, **109** : 27-29.

RAJGOPAL, K., CHANDRAN MISRA, J. B., BHALODIA. AND MATHUR, R. S., 2000, Evaluation of bold seeded groundnut association for confectionary attributes. *International Arachis Newsletter*, **20:** 18-21.

RYDER, S. D., SMITH, J. A., AND RHODES, J. M., 1992, Peanut lectin: A mitogen for normal human colonic epithelium and human HT 29 colorectal cancer cells. *Journal of National Cancer Institute*, **84**(18) : 1410-1416.

SAGHAI-MAROOF, M. A., SOLIMAN, K. M., JORGENSEN, R. A. AND ALLARD, R. W., 1984, Ribosomal DNA spacer length polymorphism in barley: Mendelian inheritance, chromosomal location and populatiopn dynamics. *Proceedings of National Academy of Sciences,* USA, **81** : 8014-8018.

SANDHU, R. S. AND SANGHA, A. S., 1974, Analysis of diversity in groundnut: 8014-8018. (*Arachis hypogaea* L.) I. Bunch group. *Oilseeds Journal*, **14** : 1-8.

SANGHA, A. S., 1971, Analysis of groundnut (*Arachis hypogaea* L.) II. Spreading Group. *Madras Agricultural Journal*, **58** : 875-881.

SHANNON, C. E. AND WEAVER, W., 1949, The mathematically theory of communication. University of Illinis Press, Urbana.

SHOKES, F. K. AND CULBREATH, A. K., 1997. Early and late leaf spots. In: Kokalis Burelle, N., Porter, D. M., Rodriguez-Kababa, R., Smith. D. H. and Subrahmanyam. P. (eds.), Compendium of peanut diseases. 2nd edition APS Press, Am. Phytopath. Soc., St. Paul, MN, pp. 17-20.

SIMPSON, C. E., 1982, Collection of *Arachis* germplasm (1976-1982). *Plant Genetic Resources Newsletter*, **52** : 10-12.

SIMPSON, C. E., 1984, Plant exploration: Planning, Organization, and Implementation with special emphasis on *Arachis*, in conservation of crop germplasm – An International Perspective, (ed. W. L. Brown, T. T. Chang et al.), *Crop Science Society of America Special Publication*, No. **8**, pp. 1-20.

SINGH, A. K., SMART, J., SIMPSON, C. E. AND RAINA, S. N., 1998, Genetic variation vis-à-vis molecular polymorphism in groundnut, *Arachis hypogaea* L. *Genetic Resources and Crop Evolution,* **45** : 119-126.

SKINNER, D. Z., BAUCHAN, G. R., AURICHT, G. AND HUGHES, S., 1999, A method for the efficient management and utilization of large germplasm collections. *Crop Science,* **39** : 1237-1242.

SNEATH, P. H. A. AND SOKAL, R. R., 1973, *Numerical Taxonomy*, W. H. Freeman, San Francisco.

STALKER, H. T., 1990, A morphological appraisal of wild species in section *Arachis* of peanuts. *Peanut Science*, **17** (2) : 117.

STALKER, H. T., 1992, Utilizing *Arachis* germplasm resources. In: S. N. Nigam (Ed.), *Groundnut - A Global Perspective*. ICRISAT, Patancheru, India, pp. 281-296.

STALKER, H. T. AND BEUTE, M. K., 1993. Registration of four interspecific peanut germplasm lines resistant to *Cercospora arachidicola*. *Crop Science,* **33** : 1117.

STALKER, H. T., KOCHERT, G. D. ANDDHESI, J. S., 1995a. Variation within the species A. *duranensis*, a possible progenitor of the cultivated peanut. *Genome* **38** : 1201-1212.

STALKER, H. T. AND MOSS, J. P., 1987, Speciation, cytogenetics and utilization of *Arachis* species. *Advances in Agronomy*. **41** : 1-40.

STALKER, H. T. AND SIMPSON, C. E., 1995. Genetic resources in *Arachis*. In: Pattee, H. E. and Stalker, H. T (eds.). Advances in peanut science. Am. Peanut Research Education Society, Stillwater, OK, pp. 14-53.

SUBBARAO, P. V., SUBRAHAMANYAM, P. AND REDDY, P. M., 1990, A modified nine point disease scale for assessment of rust and late leaf spot of groundnut. In: Second International Congress of French Phytopathological Society, 28-30 November 1990, Montpellier, and France, p. 25.

SUBRAMANIAN, V., GURTU, S., NAGESWARA RAO, R. C. AND NIGAM, S. N., 2000, Identification of DNA polymorphism in cultivated groundnut using random amplified polymorphic DNA (RAPD) assay. *Genome*, **43** : 656-660.

THAKUR, R. P., RAO, V. P., REDDY, S. V. AND FERGUSON, M., 2000, evaluation of wild Arachis germplasm accession for in vitro seed colonization and aflatoxin production by *Aspergillus flavus*. *International Arachis Newsletters*, **20** : 44 – 46.

TOHME, J., JONES, S., BEEBE, S. AND IWANAGA, M., 1995, The combined use of agroecological and characterization data to establish the CIAT Phaseolus vulgaris core collection. In : T. Hodgkin *et. al*., (ed) Core Collections of plant genetic resources. International Plant Genetic Resources Institute (IPGRI). John Wiley and Sons, New York, pp. 95-108.

UPADHYAYA, H. D., 2003, Phenotypic diversity in groundnut (*Arachis hypogaea* L.) core collection assessed by morphological and agronomical evaluations. *Genetic Resources and Crop evolution,* **50** : 539-550.

UPADHYAYA, H. D., 2005, Variability for drought resistance related traits in the mini core collection of peanut. *Crop Science*, **45** : 1432 – 1440.

UPADHYAYA, H. D., BRAMEL, P. J., ORTIZ, R. AND SINGH, S., 2002, Developing a mini core of peanut for utilization of genetic resources. *Crop Science,* **42** (6) : 2150-2156.

UPADHYAYA, H. D., FERGUSON, M. E. AND BRAMEL, P. J., 2001a. Status of the *Arachis* germplasm collection at ICRISAT. Peanut Science, **28** : 89-96.

UPADHYAYA, H. D., NIGAM, S. N., MEHAN, V. K. AND LENNE, J. M. 1997. Aflatoxin contamination of grounnut: prospects for genetic solution through conventional breeding. Pages 81-85 *in* Aflatoxin contamination problems in groundnut in Asia. *Proceedings of the First Asia Working Group Meeting*, 27-29 May 1996, Ministry of Agriculture and Rural Development, Hanoi, Vietrnam (Mehan, V. K., and Gowda, C. L. L., eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

UPADHYAYA, H. D. AND ORTIZ, R., 2001, A mini core subset for capturing diversity and promoting utilization of chickpea genetic resources in crop improvement. *Theoretical and Applied Genetics,* **102** : 1292-1298.

UPADHYAYA, H. D., ORTIZ, R., BRAMEL, P. J. AND SUBE SINGH, 2001b, Development of groundnut core collection from Asia region. In 100 years of post-mendelian genetics and plant breeding – Retrospect and prospects. Diamond Jubilee Symposium, 6-9 November 2001. *Indian Society of Genetics and Plant Breeding*, New Delhi, India.

UPADHYAYA, H. D., ORTIZ, R., BRAMEL, P. J. AND SUBE SINGH, 2003, Development of a groundnut core collection using taxonomical, geographical and morphological descriptors. *Genetic Resources and Crop evolution,* **50** : 139-148.

VALLS, J. F., RAMANATHA RAO. V., SIMPSON, C. E. AND KRAPOVICKAS, A, 1985, Current status of collection and conservation of South American groundnut germplasm with emphasis on wild species of *Arachis.* In: J. P. Moss (ed). *Proceedings of International Workshop on Cytogenetics of Arachis*, October 31-November 2, 1983. International Crops research Institution of Semi Arid Tropics. Patancheru, A. P. India, pp. 15-35.

VARISAI MUHAMMAD, S., RAMANATHAN, T. AND RAMACHANDRAN, M., 1973a, Variation in morphological characters of *Arachis hypogaea* L. *Madras Agricultural Journal*, **60** : 1373-1379.

VARISAI MUHAMMAD, S., RAMANATHAN, T. AND RAMACHANDRAN, M., 1973b, Classification of *Arachis hypogaea* L. var. fastigiata. *Madras Agricultural Journal*, **60** : 1399-1402.

VIJAY, V., 2003, Evaluation of resistance to Sclerotium rolfsii Sacc. in groundnut (*Arachis hypogaea* L.). *M. Sc. (Agri.) Thesis*, University of Agricultural Sciences, Dharwad.

VISHWANATH, B. C., PATTANASHETTI, S. K., RAJENDRAPRASAD, M. N., SWAMY, B. M. AND GOWDA, M. V. C., 2004, Variation for lectin content and its Nutritional Relevance in groundnut, Short papers presented at the National Symposium Enhancing Productivity of groundnut for sustaining food and nutritional Security, NRCG, Junagadh, pp. 244 - 245.

WALIYAR, F., HASSAN, H., BONKOUNGOU, S. AND BOSC, J. P. 1994. Sources of resistance to Aspergillus flavus and aflatoxin contamination in groundnut in groundnut in West Africa. *Plant Disease,* **78** : 704-708.

WALIYAR, F., Mc DONALD, D., SUBBA RAO, P. V. AND REDDY, P. M., 1993. Components of resistance to an Indian Source of *Cercospora arachidicola* in selected peanut lines. *Peanut Science*. **20** : 93-96.

WALIYAR, F., SHEW, B. B., SHIDAHMED, R. AND BEUTE, M. K., 1995. Effect of host resistance on germination of *Cercospora arachidicola* on peanut leaf surfaces. *Peanut Science,* **22** : 154-157.

WEBER, J. L., 1990, Informativeness of human (dC- dA)n, (dG- dT)n polymorphism. *Genomics,* **7** : 524-530.

WILLAMS, D. E., 2001, New directions for collection and conserving peanut genetic diversity. *Peanut Science,* **28** : 135-140.

WILL, M. E., HOLBROOK, C. C. AND WILSON, D. M., 1994, Evaluation of field inoculation techniques for screening peanut genotypes for reaction to preharvest *Aspergillus flavus* group infection and aflatoxin contamination. *Peanut Science*, **28** : 135 – 140.

YOUNG, N. D., WEEDEN, N. F. AND KOCHERT, G., 1996, Genome mapping in legumes (Fam. Fabaceae). In: A. H. Paterson (ed), *Genome Mapping in Plants*, Landes Co., Austin, TX, pp. 211-227.

ZAMBETTAKIS, C., WALIAR, F., BOCKELEE – MORVAN, A. AND PINS, O. D. E., 1981, Results of four years of research on the resistance of groundnut varieties to *Aspergillus flavus*. *Oleagineux,* **36** : 377– 385.

ZHU, J. AND XU, H. M., 2000, Methods of constructing core collections by stepwise clustering with three sampling strategies based on the genotypic values of crops. *Theoretical and Applied Genetics,* **101** : 264-268.

Appendix I: List of mini core accessions and cultivars of Groundnut

S. No.	ICG	Origin	Species	Subsps	Varieties	Forma
1	36	India	hypogaea	fastigiata	vulgaris	
2	76	India	hypogaea	hypogaea	hypogaea	Type runner
3	81	Unknown	hypogaea	fastigiata	vulgaris	
4	111	Unknown	hypogaea	hypogaea	hypogaea	Type bunch
5	118	India	hypogaea	fastigiata	vulgaris	
6	163	Unknown	hypogaea	hypogaea	hypogaea	Type runner
7	188	India	hypogaea	hypogaea	hypogaea	Type bunch
8	297	USA	hypogaea	fastigiata	fastigiata	
9	332	Brazil	hypogaea	fastigiata	fastigiata	
10	334	China	hypogaea	fastigiata	vulgaris	
11	397	USA	hypogaea	fastigiata	fastigiata	
12	434	USA	hypogaea	fastigiata	vulgaris	
13	442	USA	hypogaea	fastigiata	vulgaris	
14	513	India	hypogaea	hypogaea	hypogaea	Type bunch
15	532	Unknown	hypogaea	hypogaea	hypogaea	Type bunch
16	721	USA	hypogaea	hypogaea	hypogaea	Type bunch
17	862	India	hypogaea	hypogaea	hypogaea	Type runner
18	875	India	hypogaea	hypogaea	hypogaea	Type runner
19	928	Unknown	hypogaea	hypogaea	hypogaea	Type runner
20	1137	India	hypogaea	fastigiata	vulgaris	
21	1142	Benin	hypogaea	fastigiata	fastigiata	
22	1274	Indonesia	hypogaea	fastigiata	fastigiata	
23	1399	Malawi	hypogaea	fastigiata	fastigiata	
24	1415	Senegal	hypogaea	fastigiata	vulgaris	
25	1519	India	hypogaea	fastigiata	vulgaris	
26	1668	USA	hypogaea	hypogaea	hypogaea	Type bunch
27	1711	Bolivia	hypogaea	fastigiata	vulgaris	
28	1973	India	hypogaea	fastigiata	vulgaris	
29	2019	India	hypogaea	fastigiata	vulgaris	
30	2106	India	hypogaea	fastigiata	vulgaris	
31	2511	India	hypogaea	hypogaea	hypogaea	Type runner
32	2772	Nigeria	hypogaea	hypogaea	hypogaea	Type bunch
33	2773	Tanzania	hypogaea	hypogaea	hypogaea	Type runner
34	2777	India	hypogaea	hypogaea	hypogaea	Type runner
35	2925	India	hypogaea	hypogaea	hypogaea	Type runner
36	3027	India	hypogaea	hypogaea	hypogaea	Type bunch
37	3053	India	hypogaea	hypogaea	hypogaea	Type bunch
38	3102	India	hypogaea	fastigiata	vulgaris	
39	3240	Uganda	hypogaea	fastigiata	vulgaris	
40	3343	India	hypogaea	fastigiata	vulgaris	
41	3421	India	hypogaea	fastigiata	vulgaris	
42	3584	India	hypogaea	fastigiata	vulgaris	
43	3673	Korea	hypogaea	fastigiata	fastigiata	
44	3681	USA	hypogaea	fastigiata	fastigiata	
45	3746	Argentina	hypogaea	fastigiata	vulgaris	
46	3775	Brazil	hypogaea	fastigiata	vulgaris	
47	3992	India	hypogaea	hypogaea	hypogaea	Type runner
48	4156	Unknown	hypogaea	hypogaea	hypogaea	Type runner
49	4343	India	hypogaea	hypogaea	hypogaea	Type runner
50	4527	Uganda	hypogaea	hypogaea	hypogaea	Type bunch
51	4538	India	hypogaea	hypogaea	hypogaea	Type bunch
52	4543	Unknown	hypogaea	fastigiata	vulgaris	

Contd……

S. No.	ICG	Origin	Species	Subsps	Varieties	Forma
53	4598	India	hypogaea	Hypogaea	hypogaea	Type bunch
54	4670	Sudan	hypogaea	fastigiata	fastigiata	
55	4684	USA	hypogaea	fastigiata	vulgaris	
56	4729	China	hypogaea	fastigiata	vulgaris	
57	4750	Paraguay	hypogaea	fastigiata	vulgaris	
58	4911	Malawi	hypogaea	fastigiata	vulgaris	
59	4955	India	hypogaea	fastigiata	vulgaris	
60	4998	China	hypogaea	hypogaea	hypogaea	Type runner
61	5195	Sudan	hypogaea	fastigiata	vulgaris	
62	5221	Argentina	hypogaea	fastigiata	fastigiata	
63	5236	Chile	hypogaea	fastigiata	vulgaris	
64	5286	Zambia	hypogaea	hypogaea	hypogaea	Type bunch
65	5327	USA	hypogaea	hypogaea	hypogaea	Type bunch
66	5475	Kenya	hypogaea	fastigiata	fastigiata	
67	5494	Malaysia	hypogaea	fastigiata	vulgaris	
68	5609	Sri Lanka	hypogaea	fastigiata	fastigiata	
69	5662	China	hypogaea	hypogaea	hypogaea	Type bunch
70	5663	China	hypogaea	hypogaea	hypogaea	Type bunch
71	5779	India	hypogaea	fastigiata	vulgaris	
72	5827	USA	hypogaea	hypogaea	hypogaea	Type runner
73	5991	India	hypogaea	hypogaea	hypogaea	Type bunch
74	6027	Sudan	hypogaea	fastigiata	fastigiata	
75	6057	USA	hypogaea	hypogaea	hypogaea	Type bunch
76	6201	Cuba	hypogaea	fastigiata	fastigiata	
77	6236	Burkina Faso	hypogaea	fastigiata	vulgaris	
78	6375	Unknown	hypogaea	fastigiata	vulgaris	
79	6402	Unknown	hypogaea	hypogaea	hypogaea	Type bunch
80	6407	Zimbabwe	hypogaea	fastigiata	vulgaris	
81	6646	Unknown	hypogaea	fastigiata	fastigiata	
82	6654	Unknown	hypogaea	fastigiata	vulgaris	
83	6667	USA	hypogaea	hypogaea	hypogaea	Type bunch
84	6703	Paraguay	hypogaea	hypogaea	Type bunch	
85	6706	USA	hypogaea	hypogaea	Type bunch	
86	6813	Senegal	hypogaea	hypogaea	Type runner	
87	6888	Brazil	hypogaea	fastigiata		
88	6892	USA	hypogaea	hypogaea	Type bunch	
89	6913	USA	hypogaea	hypogaea	Type bunch	
90	7000	USA	hypogaea	hypogaea	Type bunch	
91	7153	India	hypogaea	hypogaea	Type runner	
92	7181	India	hypogaea	fastigiata		
93	7190	Brazil	hypogaea	vulgaris		
94	7243	USA	hypogaea	hypogaea	Type runner	
95	7906	Zimbabwe	hypogaea	vulgaris		
96	7969	Zimbabwe	hypogaea	vulgaris		
97	8083	Russia and CISs	hypogaea	vulgaris		
98	8285	USA	hypogaea	hypogaea	Type bunch	
99	8490	Somalia	hypogaea	hypogaea	Type runner	
100	8517	Bolivia	hypogaea	fastigiata		

Contd….

S. No.	ICG	Origin	Species	Subsps	Varieties	Forma
101	8567	Uruguay	hypogaea	vulgaris		
102	9037	Cote d'Ivoire	hypogaea	hypogaea	Type runner	
103	9157	Puerto Rico	hypogaea	vulgaris		
104	9249	Mauritius	hypogaea	vulgaris		
105	9315	USA	hypogaea	fastigiata		
106	9418	Martinique	hypogaea	vulgaris		
107	9507	Philippines	hypogaea	vulgaris		
108	9666	India	hypogaea	hypogaea	Type bunch	
109	9777	Mozambique	hypogaea	hypogaea	Type bunch	
110	9809	Mozambique	hypogaea	vulgaris		
111	9961	Unknown	hypogaea	hypogaea	Type bunch	
112	10036	Peru	hypogaea	peruviana		
113	10092	Zimbabwe	hypogaea	fastigiata		
114	10185	USA	hypogaea	hypogaea	Type bunch	
115	10384	Nigeria	hypogaea	vulgaris		
116	10474	Cuba	hypogaea	fastigiata		
117	10565	Congo	hypogaea	fastigiata		
118	11088	Peru	hypogaea	peruviana		
119	11109	Taiwan	hypogaea	hypogaea	Type runner	
120	11144	Argentina	hypogaea	fastigiata		
121	11219	Mexico	hypogaea	hypogaea	Type runner	
122	11249	Tanzania	hypogaea	vulgaris		
123	11322	India	hypogaea	hypogaea	Type bunch	
124	11426	India	hypogaea	hypogaea	Type bunch	
125	11457	India	hypogaea	hypogaea	Type runner	
126	11515	China	hypogaea	vulgaris		
127	11651	China	hypogaea	vulgaris		
128	11687	India	hypogaea	vulgaris		
129	11855	Korea	hypogaea	hypogaea	Type bunch	
130	12189	Unknown	hypogaea	vulgaris		
131	12276	Bolivia	hypogaea	hypogaea	Type bunch	
132	12370	India	hypogaea	hypogaea	Type runner	
133	12625	Ecuador	hypogaea	aequatoriana		
134	12672	Bolivia	hypogaea	hypogaea	Type bunch	
135	12682	India	hypogaea	vulgaris		
136	12697	India	hypogaea	vulgaris		
137	12879	Myanmar	hypogaea	vulgaris		
138	12921	Zimbabwe	hypogaea	vulgaris		
139	12988	India	hypogaea	vulgaris		
140	13099	Unknown	hypogaea	hypogaea	Type runner	
141	13491	C.A.republic	hypogea	vulgaris		
142	13603	Indonesia	hypogaea	vulgaris		
143	13723	Niger	hypogaea	hypogaea	Type runner	
144	13856	Uganda	hypogaea	fastigiata		
145	13858	Uganda	hypogaea	fastigiata		

Contd……

S. No.	ICG	Origin	Species	Subsps	Varieties	Forma
146	14008	C.A.republic	hypogaea	hypogaea	Type bunch	
147	14106	United Kingdom	hypogaea	fastigiata		
148	14118	United Kingdom	hypogaea	vulgaris		
149	14127	United Kingdom	hypogaea	fastigiata		
150	14466	Nigeria	hypogaea	hypogaea	Type bunch	
151	14475	Nigeria	hypogaea	hypogaea	Type bunch	
152	14482	Nigeria	hypogaea	hypogaea	Type bunch	
153	14705	Cameroon	hypogaea	hypogaea	Type bunch	
154	GPBD-4	India	hypogaea	vulgaris		
155	5745	Puerto Rico	hypogaea	hypogaea	Type bunch	
156	2857	Argentina	hypogaea	hypogaea	Type runner	
157	1862	Unknown	hypogaea	vulgaris		
158	6993	Brazil	hypogaea	hypogaea	Type runner	
159	4412	USA	hypogaea	hypogaea	Type runner	
160	4716	unknown	hypogaea	vulgaris		
161	9842	Tanzania	hypogaea	hypogaea	Type bunch	
162	13942	ICRISAT	hypogaea	hypogaea	Type bunch	
163	14710	Cameroon	hypogaea	fastigiata		
164	10890	Peru	hypogaea	fastigiata		
165	15190	Costa Rica	hypogaea	hypogaea	Type bunch	
166	15309	Brazil	hypogaea	fastigiata		
167	13982	USA	hypogaea	hypogaea	Type bunch	
168	10554	Argentina	hypogaea	fastigiata		
169	14985	Unknown	hypogaea	vulgaris		
170	2381	Brazil	hypogaea	hypogaea	Type runner	
171	2738	India	hypogaea	fastigiata	fastigiata	
172	15287	Brazil	hypogaea	fastigiata	vulgaris	Type bunch
173	12000	Mali	hypogaea	hypogaea	hypogaea	
174	8106	Peru	hypogaea	fastigiata	fastigiata	
175	14630	Brazil	hypogaea	fastigiata	fastigiata	
176	1415	Senegal	hypogaea	fastigiata	vulgaris	
177	156/M-13	Unknown	hypogaea	fastigiata	vulgaris	
178	115	India	hypogaea	fastigiata	fastigiata	Type bunch
179	7963	USA	hypogaea	hypogaea	hypogaea	Type runner
180	4389	India	hypogaea	hypogaea	hypogaea	
181	6263	Burkina Faso	hypogaea	fastigiata	vulgaris	
182	13941	ICRISAT	hypogaea	fastigiata	vulgaris	Type runner
183	8760	Zambia	hypogaea	hypogaea	hypogaea	
184	15419	Ecuador	hypogaea	fastigiata	vulgaris	
185	15042	Unknown	hypogaea	fastigiata	fastigiata	
186	10566	Congo	hypogaea	fastigiata	fastigiata	Type runner
187	9905	Zambia	hypogaea	hypogaea	hypogaea	Type runner
188	10479	Uruguay	hypogaea	hypogaea	hypogaea	Type bunch
189	13787	Niger	hypogaea	hypogaea	hypogaea	
190	GPBD-4 *	India	hypogaea	fastigiata	vulgaris	
191	M 28-2 *	India	hypogaea	fastigiata	fastigiata	
192	TAG 24 *	India	hypogaea	fastigiata	vulgaris	
193	JL 24 *	India	hypogaea	fastigiata	vulgaris	
194	R 9227 **	India	hypogaea	fastigiata	vulgaris	
195	MN 1-28 **	India	hypogaea	fastigiata	fastigiata	
196	MN 1-35 **	India	hypogaea	fastigiata	fastigiata	

* Cultivar ** Breeding line

Appendix II: Monthly meteorological data for experimental year (2004) and average of past 54 years (1950-2004) of Main Agricultural Research Station, University of Agricultural Sciences, Dharwad

Months	Rainfall (mm)			Temperature (^{0}C)						Relative humidity (%)		
				Mean maximum			Mean minimum					
	2004	2005	1950-2004	2004	2005	1950-2004	2004	2005	1950-2004	2004	2005	1950-2004
January	Traces	Traces	0.086	29.6	29.9	29.15	14.7	15.0	19.23	54	55	63.37
February	Traces	Traces	1.161	32.5	34.0	34.52	16.4	16.8	16.02	53	50	51.18
March	Traces	Traces	0.147	36.5	35.3	35.73	19.6	18.5	18.81	49	45	56.47
April	24.4		48.45	37.4		37.00	19.8		21.32	51		76.98
May	61.1		81.40	33.6		36.52	21.4		21.48	66		66.71
June	43.8		109.14	28.8		29.50	21.5		21.21	80		81.69
July	24.8		150.77	29.2		22.06	21.0		20.95	79		87.46
August	160.7		95.30	27.0		22.01	20.3		20.62	83		86.51
September	222.1		100.54	28.6		28.75	19.9		20.16	77		82.40
October	64.6		130.99	30.1		30.12	18.4		19.30	65		76.44
November	0.6		32.04	30.2		29.46	15.9		15.50	52		68.13
December	0.0		54.50	29.4		29.18	12.5		13.44	45		63.81
Total	**602.10**		**750.52**									

EVALUATION OF A MINI CORE SET OF GERMPLASM IN GROUNDNUT (*Arachis hypogaea* L.)

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ABSTRACT

Groundnut breeding programmes have mainly used elite breeding lines and cultivars leading to narrow-genetic base. Lack of proper characterization and evaluation is the major reason for limited use of germplasm. Extensive evaluation of core collections facilitates better access to germplasm for use in crop improvement.

A minicore subset of world germplasm comprising 188 accessions was evaluated for morphological and agronomic characteristics to estimate phenotypic diversity and to identify potential germplasm for crop improvement. Seven morphological descriptors *viz*., stem hairiness, branching pattern, leaf let shape, seed colour, pod beak, pod constriction and pod reticulation and 6 agronomical traits viz., yield per plant, shelling percentage, sound mature kernel, test weight, late leaf spot and protein content contributed significantly to multivariate polymorphism. Shannon-Weaver diversity index revealed more diversity for pod reticulation and stem pigmentation in Valencia; pod reticulation and leaf let shape in Virginia runner; pod beak, pod constriction, pod reticulation and flower colour in Virginia bunch and *Aspergillus* seed colonization in spanish bunch and Virginia bunch. The accessions ICG 1862, 3240 and 12697 for yield, ICG 5662, 6027, 8760 and 9777 for test weight, ICG 13787 and ICG 14985 for resistance to *Aspergillus* and ICG 6027 and ICG 11088 for low lectin activity were found significantly superior for different traits.

The material was also subjected to RAPD assay using twenty primers to assess molecular diversity. The polymorphism per primer ranged from 57 to 100 percent. The genetic similarity (S_{ij}) ranged from 0.21 to 0.93. Some accessions with diverse DNA profiles (ICG 8760 with ICG 442, 1274, 6402 and GPBD-4 with ICG 2381 and ICG 1274 with ICG 12189) were identified for mapping and genetic enhancement in groundnut.