ABSTRACT

The 27 breeding lines with 2 released varieties evaluated in R.B.D for yield and its component traits grown in rabi seasons showed wide variation in all the 12 traits including yield. The genotypic source of variations were highly significant (at 1% level) for all the traits.

The P.C.V. and G.C.V. estimates were high for plant height, number of branches per plant, number of pods per plant, number of kernels per plant, hundred kernel weight, harvest index percentage, kernel yield per plant and pod yield per plant, thereby suggesting ample scope for improvement through selection among these advance breeding lines. Low values of G.C.V. was observed in days to 50% flowering, sound mature kernel percentage, shelling percentage and haulm yield per plant indicated the need to create variability either by hybridization or mutation followed by selection. High heritability estimates were observed for 100 kernel weight, shelling percentage, pod yield per plant, harvest index and number of pods per plant indicating less influence of environment on these characters. Along with high heritability, high genetic advance as percentage of mean has been noticed for pod yield per plant, hundred kernel weight, number of pods per plant and harvest index. Therefore it was clear that these four traits were less influenced by the environmental changes due to the presence of additive gene action in their
expressions. Hence improvement of these traits would be more effective through the selection. Low genetic advance along with low heritability estimate for sound mature kernel percent, and haulm yield per plant was observed in present study. This indicated the additive and non additive gene actions that have a role in their inheritance and phenotypic selection would be effective to some extent. Pod yield per plant and kernel yield per plant exhibited significant positive correlation and both these traits also exhibited significant positive correlation with plant height, number of pods per plant, 100 kernel weight, number of kernels per plant and harvest index at both phenotypic and genotypic level. This indicated the strong association of harvest index, number of kernels per plant and number of pods per plant with pod yield and kernel yield per plant in _rabi_ season. Both phenotypic and genotypic correlation coefficient for number of kernels per plant were highly significant and positive with plant height, number of pods per plant and harvest index. Number of pods per plant were significant and positively correlated with plant height and number of branches per plant both at phenotypic and genotypic levels. Hundred kernel weight was significant and positively correlated with harvest index percentage both at phenotypic and genotypic level. From this studies, it can be concluded that number of pods per plant and number of kernels per plant are two most important morphological traits for selecting better genotypes with high kernel yield and pod yield per plant. The highest phenotypic correlation as well as genotypic correlation was observed between kernel yield per plant and pod yield per plant. But the sound mature kernel percent is negatively correlated with number of kernels per plant, harvest index and both pod and kernel yield per plant. The path coefficient analysis showed low residual effect (0.089) during _rabi_ indicating that most of the major yield components were included in the study. Harvest index percentage had the highest direct positive effects on pod yield per plant followed by haulm yield per plant. Also kernel yield per plant, hundred kernel weight, number of pods per plant and plant height were observed to be the major indirect contributor towards pod yield through harvest index percentage. Present study thus indicated that prime emphasis should be given to harvest index percentage followed by number of kernels per plant and kernel yield per plant. The smallest $D^2$
estimate (9.044) was observed between ALG 234 and AG 2006-15, so these genotypes were much similar in many traits. The largest $D^2$ estimate (640.258) was obtained between ALG 234 and GPBD 5, which indicated the maximum diversity. On the basis of average $D^2$, shelling percentage contributed maximum divergence followed by harvest index, kernel yield per plant, hundred kernel weight and all other characters. Sound mature kernel percent, Plant height, pod yield per plant, days to 50% flowering, number of branches per plant, haulm yield per plant, number of pods per plant and number of kernels per plant contributed least to $D^2$ estimates. On the basis of critical $D^2$ values (83.614), 29 genotypes were classified into 8 clusters. Lowest intra-cluster distance was observed between cluster IV (24.933). Highest intra-cluster distance was observed within cluster VI followed by cluster V. Inter cluster distance is the main criterion for selection of genotypes. In this contest, the genotypes from cluster VI and VIII or cluster VI and VII could be selected as parents for hybridization. Thus the advance breeding line ALG 234 from cluster I and and GPBD 5 from cluster IV, exhibiting highest genetic diversity of 640.258 may be selected as parents in the future hybridization programme to give maximum high yielding segregates with desirable features. The canonical analysis revealed that values in both vectors (Vector I and II) for plant height, 100 kernel weight, kernel yield per plant and harvest index were positive. It was observed that the important characters responsible for genetic divergence were shelling percentage and kernel yield per plant in the first axis and harvest index and kernel yield per plant in the second axis. Such results indicated that these characters contributed maximum towards total divergence of the genotypes and it was also suggested that attention should be given for these five characters for yield improvement of groundnut.

In molecular analysis, a total of fourteen accessions of Groundnut (Arachis hypogaea L.) collected from different parts of Odisha and were used for genetic diversity analysis in the present study. A total of 27 bands were detected ranging in size from 200 bp to 2250 bp among the 14 genotypes. The number of bands produced with primer, OPW-1 is 10, OPE-19 is 9 and OPW-8 is 8 with a mean of
9 bands per primer. Of a total 27 RAPD bands, all 27 (100%) were found to be polymorphic. The maximum number of polymorphic bands (10) was obtained using OPW-1 primer whereas the minimum number of polymorphic bands (8) was obtained in Primer OPW-8. Genetic relationship between groundnut genotypes were determined on the basis of Jaccard’s pair wise similarity coefficients. The range of pair wise similarity varies from 0.00 to 0.833. Highest similarity coefficient was observed between ‘Dh-204’ and ‘Dh-209’ (0.833), indicating that they are belonging to similar genetic background, while minimum similarity was observed in between ‘ICGV-95401’ and ‘DhS-102’ (0.067). Following data analysis based on Jaccard’s similarity coefficient, a dendrogram was plotted for the data set. It depicted that, at genetic distance 0.00, two distinct clusters were formed having single root. Two major clusters (I-II) were identified from the dendrogram, which were found distinct from each other having only 0% similarity. The highest number of genotypes (13) was included in the Cluster I whereas cluster II was represented by only one genotypes. The genotypes namely ‘Dh-204’ and ‘Dh-209’ exhibited the maximum similarity (83.3%). From the clustering pattern, it showed that distinct genome specific group exists among the studied 14 accessions in groundnut. Principle component analysis was also done to visualize genetic relationships among the varieties. The results were very much similar to UPGMA results. Two Dimensional and Three Dimensional scaling showed four clusters (I to IV). Cluster-I had six accession i.e. “DhS-102, Dh-206, TCGS-159, Dh-108, CSMG-2014 and KGN-34”. Cluster-II had three accessions i.e. “Dh-216-1, ALG-234 and DRT-53”. Cluster-III had four accessions i.e. “Dh-8, Dh-209, Dh-204 and AG-2006-15”. Cluster-IV had only one accession i.e. “ICGV-95401”. Only difference is that, three Dimensional scaling shows vertical diversity between the accessions which is in cluster-I. Based on study the large range of similarity values for related accessions using RAPD provides greater confidence for the assessment of genetic diversity and relationships. This characterization of 14 genotypes will be very significantly helpful to the background selections during back cross of breeding programmes.
INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is one of the most important oilseed crops in India, occupying 5.19 m. ha area and contributing 6.94 million tones production of groundnut in shell with productivity of 1337 kg/ha during 2011-12 (ICAR, 2012). Minimum support price of groundnut in shell in 2012-13 increased to Rs.3700 per quintal from Rs.2700 per quintal in 2011-12. India stands first in terms of area among more than 100 countries where groundnut is grown, with a share of 19.21% in the world production, next only to China. The productivity level of groundnut in India is far below the world average of 1546 kg/ha mainly because it is predominantly grown as a rainfed crop (80% of total acreage) under various biotic and abiotic constraints that limit crop yield. It is native of South America which is cultivated in tropical, subtropical and warm temperate regions of the world confined to areas between 40° North and 40° South latitudes for commercial cultivation (Encyclopedia of Agricultural Science, 1994). It requires long warm growing season in a climate having well distributed rainfall in the range of 500-1000 mm and the mean daily temperatures are higher than 20°C. It grows best in temperature range of 25°C to 30°C. in sandy loam soil which permits easy entry and growth of pegs in soil and harvest of pods. The optimum pH for growth of groundnut is 6.0 - 6.5. The major groundnut growing states in India (Gujarat, Andhra Pradesh, Karnataka, Tamilnadu, Maharastra and Rajasthan) accounts for more than 90% of groundnut areas. Madhya Pradesh, Odisha, West Bengal are the other states having substantial areas under this crop.
Groundnut is the fourth most important source of edible oil and third most important source of vegetable protein in the world. The kernels contain 45-48% oil, 44-54% fat and 25-30% digestible protein. Groundnut oil is considered as stable and nutritive as it contains right proportions of saturated fatty acid (40-50%) and unsaturated fatty acid like linoleic acid (25-35%). Due to high oleic / linoleic ratio, groundnut oil has a longer shelf-life than those of most vegetables oils. It is the richest plant source of vitamins E, K and B. Moreover, groundnut oil contains tocopherol (approx. 0.9 mg/g oil) which being an antioxidant prevents development of rancidity. As groundnut helps to maintain blood cholesterol levels they have been recognized as heart friendly. Groundnut provides over 30 essential nutrients and is considered as a rich source of fiber, vitamins (niacin, folate, and vitamin E) and minerals (magnesium, manganese and phosphorus) and free from sodium. The foliage serves as an important fodder for animals and the meal that remains after oil extraction is made into animal feed (Paik Ro et al., 1991).

Groundnut belongs to the tribe Aeschynomeneae, subtribe Stylosantinae, family Leguminosae (Stalker, 1997). Groundnut is a member of genus *Arachis*, which posses a unique characteristic that distinguishes it from all other plants namely that it is flowering above ground and producing fruits below ground, hence the name groundnut (Holbrook and Stalker, 2003).

There are number of species, which are thought to be the progenators of *A. hypogaea* and amongst them *A. duranensis* and *A. ipaensis* are the most likely progenators (Kochert et al., 1996). Wynne and Coffelt, (1982) opined that qualitatively inherited traits were controlled by duplicated loci and added evidence
to the hypothesis that *A. hypogaea* is an allotetraploid species. Garcia *et al.*, (1995) concluded that most agronomically important traits in *A. hypogaea* were quantitatively inherited.

Groundnut was introduced into India in the first half of sixteenth century by the Spaniards. In 1884, the Mauritius variety was introduced to Pondicherry and Madras from Mauritius. During 1901-02, the Bombay Department of Agriculture introduced Pondicherry groundnut Mauritius from Madras, Spanish and Virginia from America, and Small Japan and Large Japan from Japan. The first improved variety, Spanish improved, a pure-line selection from Spanish groundnut, was released in 1905 from Dharwad of Karnataka state. All India Coordinated Research Project on Oilseeds (AICORPO) was established during 1967 to address the research needs of improving the production and productivity of nine oilseed crops, including groundnut. The National Research Centre for Groundnut (NRCG) was established in 1979 at Junagadh, Gujarat. The Technological Mission on Oilseed (TMOP) was launched in May, 1986. All India Coordinated Research Project on Groundnut was separated from AICORPO during 1992. So far more than 180 groundnut varieties belonging to different habit groups have been released. At present, the Spanish type groundnut occupies more than 50 % of the groundnut areas in India. Rathnakumar *et al.* (2010 ) highlighted the genetic improvement achieved over the years in yield and related traits in Spanish type groundnut varieties developed in India since the release of first improved variety Spanish Improved.
The genetic variability in genus *Arachis* is locked into three distinct gene pools: primary gene pool---landraces of *A. hypogaea* and it’s wild form *A. monticola*; secondary gene pool---diploid species from section *Arachis* that are cross compatible with *A. hypogaea*; and tertiary gene pool---species from section *Caulorrhizae, Erectoides, Extranervosae, Heteranthae, Procumbentes, Trierectoides, Triseminatae* and *Rhizomatosae*, which may be crossed with cultivated species using invitro techniques (Mallikarjuna and Sastri 2002; Mallikarjuna 2005; Mallikarjuna and Hoisington 2009). The wild *Arachis* species, unlike cultivated groundnut, are difficult to maintain under field condition due to their long generation time (from annual to perennial life cycle), pervasive nature (require more space to grow), deep to very deep peg/pod penetration into the soil which make it difficult to recover all the pods from the soil while harvesting; and few seeds are produced, thus, requiring unique facilities to regenerate many of these species. Further, accessions from *Rhizomatosae* have to be maintained vegetatively as these do not produce seeds.

Wild *Arachis* species have been extensively studied for resistance to many pests and diseases (Dwivedi *et al.* 2003, 2005, 2008) and for thermal stress tolerance (Nautyal *et al.* 2008). Peanuts are susceptible to *Aspergillus flavus* var. *columnaris* and this leads to the production of aflatoxins, which are carcinogenic (Holbrook and Stalker, 2003). It is because of this that testing of seeds has become a crucial exercise. Stalker (1990) was probably the first to characterize 73 wild species accessions from section *Arachis* for 56 morpho-reproductive traits, while Carvalho and Quesenberry (2009) reported morphological diversity among 34 *A. pintoi*
accessions. In contrast wild *Arachis* species have been extensively studied for molecular polymorphism, unraveling abundant molecular diversity among wild *Arachis* species (Koppolu et al., 2010). Both pre- and post-zygotic hybridization barriers have been shown to restrict crossing between *Arachis* species. These barriers are most severe when accessions from tertiary and quaternary gene pools are crossed with *A. hypogaea*, but such barriers may also be expressed in crosses with certain accessions of the secondary gene pool. Wild *Arachis* species harbor a range of genes conferring resistance to pests and diseases, oil and protein contents and oleic (O)/linoleic (L) fatty acid ratios. Some genotypes show very high levels of resistance to rust, ELS, LLS, nematodes, GRD, PBNV, thrips, jassids, leaf miner, *Spodoptera* and aphids. Accessions belonging to thirteen species in section *Arachis* show wide variation for most of the morphological traits (Chandran and Pandya, 2000). Directorate of Groundnut Research, Junagadh functions as the national repository of groundnut germplasm with a collection of over 8983 accessions and 105 wild relatives of *Arachis* species. Another huge collection (about 15000 accessions) of groundnut germplasm is available with International Crop Research Institute for Semi-Arid Tropics, Patancheru, Hyderabad. So far, only a fraction of this reservoir has been utilized for altering genetic constitution of groundnut plants to develop varieties possessing desirable attributes. The available variation can further be enhanced by pre-breeding, mutation, interspecific hybridization etc. Though there are examples where plant breeders have effectively exploited the exotic germplasm for incorporation of disease resistance or other single gene control traits (Stalker, 1980), use of exotic germplasm in improvement of
quantitative traits is rare. Halward and Wynne (1991) opined that groundnut improvement programmes aimed at rapid cultivar development rely mostly on established cultivars and elite breeding lines in developing breeding materials, rather than using exotic germplasm.

A limitation often groundnut breeders put forth to increase groundnut productivity is the availability of relatively low genetic variability in the germplasm commonly used in the breeding programmes. However, very little of the large genetic variability in the germplasm accession have been utilized in the crop improvement programme. The most groundnut cultivars have a very narrow genetic base. Progress has been made in genetic improvement of the Spanish type groundnut since organized breeding programmes began in India. As Spanish type variety contributes more than 50% of the total groundnut production of the country the genetic improvement gained in yield potential is really a commendable achievement for the groundnut breeders. The alternation in plant architecture with reduced height, erect growth habit, more number of pods, etc., are the main landmarks in the Spanish groundnut improvement. A rapid expansion phenophase, a short podding phenophase, a long filling phenophase and a high partitioning of assimilates to pods were considered to be the physiological criteria responsible for higher yields by Mc Cloud et al. (1980).

The molecular markers offer many advantages over morphological markers as they are phenotypically neutral, occur throughout the genome, neither influenced by environments nor by pleotropic and epistatic interactions, and expression is not dependent on plant age. Molecular markers also offer savings in time and cost for
introgression of genes into cultivars (Tanksley et al., 1989; Melchinger, 1990). The use of DNA markers could speed up this process by three plant generations as they allow the selection of offspring that contain the lowest amounts of the donor genome in every generation (Tanksley et al., 1989). Molecular markers are also useful in the assessment of genetic diversity for (i) the identification and removal of duplicates (ii) production of core collections (iii) evaluating genetic relationships between taxa (iv) selecting diverse parental genotypes required for (a) studying QTLs for complex traits (b) developing mapping populations.

Recent achievements in the development of marker protocols such as restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), inter simple sequence repeats (ISSR) and simple sequence repeats (SSRs) (also known as microsatellites) have revolutionized the genetic analysis and opened new possibilities in the study of complex traits in the crop plants. SSRs belong to the co-dominant marker class, are easy to manipulate, highly reproducible, and targets hypervariable regions of the genome. They are tandem repeats of DNA sequences of only a few basepairs (1-6 bp) in length (Gupta et al., 1996). Variation in the number of repeated core sequence of nucleotides at a SSR locus among different genotypes provides the basis for polymorphism that can be used in plant genetic studies. SSRs are therefore excellent choice of DNA markers for genetic mapping in plants. Unlike RFLPs, for instance SSR technology is PCR based, requires only nanograms of DNA, and is readily automatable. Unlike RAPDs, SSR markers have proven to be reliable and reproducible. Unlike AFLPs, they are co-dominant and
species specific. Moreover, they are both size and sequence specific. SSRs can be used in pedigree analysis to determine kinship among individuals, fingerprinting, forensics, genetic mapping, and phylogenetic analysis (Gupta et al., 1996).

In India, groundnut is cultivated largely in kharif season under rainfed conditions. In rabi, the crop is grown under residual moisture of rice fallows or river beds under minimal irrigation situations and also in summer as an irrigated crop. The area under rabi – summer groundnut system has expanded over the years.

Keeping in view all these aspects, the present study was conducted in groundnut with twenty seven advance breeding lines from all the major groundnut growing states and two high yielding standard varieties during rabi season.

The aims of the present investigation were to:

(i) Study the nature and magnitude of variability of morpho- metric traits among advance breeding lines of groundnut belonging to seven major groundnut growing states of India.

(ii) Study the nature and magnitude of character association among morphological traits.

(iii) Assess patterns of phenotypic diversity among advance breeding lines of groundnut belonging to several major groundnut growing states of India.

(iv) Identify agronomically beneficial breeding lines for broadening the genetic base of groundnut cultigens.
(v) Molecular characterization of intra-accession variation of selected groundnut genotypes and assessment of genetic diversity on the basis of polymorphic banding pattern.
REVIEW OF LITERATURE

2.1 Variability, Heritability and Genetic Advance

Upadhyaya et al. (2005) opined that in Asia, like elsewhere, the use of genetic resources has been limited in groundnut (Arachis hypogaea L.) breeding programs, resulting in a narrow genetic base of cultivars. Core collections, which generally contain 10% of total accessions and represent the diversity of the entire collection, have been suggested as a means to enhance the use of genetic resources. The groundnut core collection for Asia, consisting of 29 accessions of subspecies fastigiata var. fastigiata, 245 of subsp. fastigiata var. vulgaris, and 230 of subsp. hypogaea var. hypogaea, along with four control cultivars, was evaluated in multi environments for 22 agronomic traits to select diverse superior germplasm accessions for use as parents in improvement programs. Analysis of data indicated that variance components due to genotypes were significant for all 22 traits, and genotypes × environment interaction was significant for eight traits. Estimates of broad sense heritability ranged from 35.5% for pod yield per plant to 98.0% for days to cessation of flowering, indicating relative reliability of selection for different traits. The clustering by Ward's method indicated that the selected accessions were diverse from the control cultivars. These 60 diverse parents will provide the germplasm, which can be used in the improvement programs to broaden the genetic base of groundnut cultivars.
John (2005) studied variability and correlation among yield and yield component traits and stem necrosis disease in groundnut grown during kharif (rainfed) and post rainy (rabi-summer) seasons to select high yielding varieties. Twenty two genotypes were sown in randomized block design with three replications during rabi 2000–01. High genotypic and phenotypic variation was observed for final plant stand (730.40 and 1059.80), 100 pod weight (41.18 and 76.60). High genotypic and phenotypic coefficients of variation were recorded for pod yield per plant (23.06% and 33.20%) and plant stand maintained at harvest (22.75% and 27.41%). It indicates the presence of considerable amount of genetic variability for these characters. High heritability was observed for 100 pod weight (73.33%), plant stand maintained at harvest (68.92%) and PSND (63.61%). High genetic advance as percentage of mean was observed for plant stand maintained at harvest (38.91%), pod yield per plant (32.99%) and PSND incidence (20.90). The performance of additive genetic variance for plant stand maintained at harvest as showing high heritability, genetic advance and PSND incidence gives a lot of scope for improvement through selection. Moderate heritability and low genetic gain was observed for days to maturity and shelling out turn indicating the non-additive gene effects. Pod yield showed highly significant positive correlation with shelling out turn (0.272), kernel yield per plant (0.901) and weak negative correlation with peanut stem necrosis disease incidence (-0.063).

Sudhir Kumar et al. (2008) evaluated 64 genotypes of groundnut (39 accessions and 25 advance breeding lines) (Arachis hypogaea L.) for fourteen quantitative characters and revealed wide range of variability. The estimates of
PCV and GCV were moderate for plant height, matured pods per plants, pod yield per plant, kernel yield per plant, shelling percentage, 100 kernel weight, harvest index and specific leaf area. High heritability coupled with high genetic advance was noticed for all the characters under study except for days to 50% flowering, days to maturity, sound mature kernel percent and oil content. In the association analysis, oil yield per plant recorded positive and significant association with matured pods per plants and kernel yield per plant. Path analysis revealed high direct effects of kernel yield and oil content. He concluded it would be rewarding to pay due emphasis on the selection of these characters for rapid improvements in respect of oil yield.

Shoba (2009) made crosses to develop a foliar disease resistant groundnut line with acceptable pod and kernel traits using TMV 2 and three foliar disease resistant parents. Three $F_2$ cross derivatives and their four parents were used to study their mean performance, genetic variability, heritability and genetic advance as percentage of mean for yield and contributing characters. Among the crosses, TMV 2 x COG 0437 had higher mean performance for all the characters followed by TMV 2 x COG 438. Higher PCV and GCV values were also recorded by this cross. The cross TMV 2 x COG 0437 had high heritability and high to moderate GAM for most of characters followed by TMV 2 x COG 0438. Hence, based on mean and variability parameters, TMV 2 x COG 0437 is adjudged as best cross combination for further selection programme to evolve a promising progeny.
John et al. (2009) studied variability and character association in Spanish bunch groundnut and found high heritability along with high GAM for number of secondary branches per plant, number of immature pods per plant, shelling %, 100 kernel weight, SMK weight, total number of pods, total number of gynophores, maturity index, reproductive efficiency and pod yield. This showed additive type of gene action plays an important role. It indicates that phenotypic selection for these characters will be effective. Pod and kernel yields per plant showed significant and positive association with number of secondary branches per plant, number of mature pods per plant, SMK weight, SMK number, 100-kernel weight. So these characters have been considered as selection indices for the improvement of kernel and pod yields per plant.

Korat et al. (2009) studied on genetic variability in different genotypes of groundnut (Arachis hypogaea L.) by evaluating 80 diverse genotypes of bunch groundnut during summer 2006 for genetic parameters viz. variability and heritability and genetic advance. The estimates of PCV and GCV were high for number of secondary branches per plant and number of aerial pegs per plant. High heritability along with high genetic advance as percent of mean was observed for number of secondary branches per plant and number of aerial pegs per plant indicating that these traits are mainly governed by additive gene action and responsive to selection for further improvement of these traits.

Thaware (2009) studied on twenty four genotypes of groundnut and evaluated for stability of dry pod yield in groundnut (Arachis hypogaea L.). All the genotypes differ significantly revealed enough genetic variability for dry pod yield.
Linear component of genotype x environment interaction was higher than that of non-linear. All the twelve genotypes had above average mean, average responsive and genotype Number 123 was stable for dry pod yield, hence their use in future breeding programme is aimed at stable dry pod yield.

Rathnakumar et al. (2010) conducted a field study for two seasons under irrigated conditions using 49 varieties released during 1905-2002 in order to assess the genetic improvement in Spanish type groundnut varieties, which occupy more than 50% of groundnut area in India. Groundnut area in India has increased gradually from 2.6 m. ha in early 1930s to nearly 8.0 m. ha in the early 1990s and the production from 2.5 m. tones to around 7.5 m. tones during the same period. Though a number of niche specific varieties have been released coupled with development of suitable improved production technologies, the improvement in average productivity is not quite visible. The increments in pod and kernel yields and component traits over the years were studied. An annual increase of 9.4 kg/ha in pod yield and 6.2 kg/ha in kernel yield was observed. The trend in improvement was greater with 24.1 kg/ha pod yield increase when the pod yield in the best variety released during each decade of development was analyzed. The enhanced pod yield has resulted mainly from improvement in number of pods per plant, pod and seed weight. Improvement in shelling percent and sound mature kernel percent was not significant. Availability of sufficient variability in the germplasm for quality traits, pest resistance, drought tolerance, etc., should enable the breeders to incorporate these traits into breeding programmes and develop varieties endowed with higher yield potential in future.
Meta and Monpara (2010) studied on genetic variation and trait relationships in summer groundnut, \textit{(Arachis hypogaea} L.). Fifty elite genotypes of bunch groundnut, \textit{(Arachis hypogaea} L.) were evaluated in summer season to know the extent of genetic variability, nature and magnitude of association among the yield traits and their direct and indirect effects on pod yield. High magnitude of G.C.V. and P.C.V. for pods per plant, kernel yield per plant and pod yield per plant indicated large extent of genetic variability for these traits in the materials. High heritability was accompanied by high genetic advance for plant height and 100 pod weight; whereas moderate heritability was associated with high genetic advance and high G.C.V. for pods per plant and kernel yield per plant, indicating improvement of additive gene action for these traits. Pod yield/plant expressed high genetic advance with low heritability, however its high magnitude of G.C.V. suggested the scope of pod yield improvement. Pod yield per plant was associated strongly and positively with kernel yield per plant, pods/plant, shelling out turn and oil content but its correlation was significantly negative with 100 pod weight, days to 50% flowering and days to maturity. Pods/plant manifested maximum direct effects towards pod yield/plant followed by 100 pods weight and 100 kernel weights. Pods/plant and kernel yield per plant also contributed major share to pod yield/plant indirectly through other traits. Thus pods/plant and kernel yield/plant would be the important component traits of pod yield and should be considered as selection criteria for enhancing yield in summer groundnut.

Singh et al. (2010) evaluated thirty two groundnut genotypes of both spreading and bunch types for their yield, yield attributes, seed protein and oil
content to analyze the degree of genetic variability in quantitative and qualitative traits and to use as pedigree for further development of varieties with greater yield potential and seed quality. The genotypes showed the extent of variation from 550–1125 g m$^{-2}$ in biomass, 142–277 g m$^{-2}$ in pod weight, 91–216 g m$^{-2}$ in seed yield, 4–23 pods/plant, 1–3 seeds per pod, 245–594 g m$^{-2}$ in 1000 seed weight, 53–87 % in shelling percent, 11–27 % in harvest index, 20.8–28.9 % in protein and 39.6–49.1 % in oil contents of seeds. This degree of variation in seed yield and quality traits offer an opportunity to further evolve the promising groundnut varieties to boost both the seed and oil production in the country.

**Pradhan K. and Patra R.K. (2011)** studied four hundred sixty genotypes of groundnut germplasm in four different seasons for phenotypic variation, heritability, genetic advance and correlations among pod yield and yield components. Pod yield/ha was observed to be high during pre-rabi and rabi seasons, might be due to increase in yield contributing characters. The GCV estimates were low for shelling percentage and moderate for hundred pod weight and hundred kernel weight. High heritability coupled with high genetic advance as per cent of mean has been noticed for hundred pod weight and hundred kernel weight. Consistently positive association in all the seasons was observed for unfilled pods per plant with number of branches per plant and hundred pod weights with hundred kernel weight. Rabi and kharif performance of yield and yield contributing characters were positively correlated except unfilled pods per plant and plant height. Selection for high yield in groundnut could be made by inclusion of pod
weight, shelling percentage and hundred kernel weight as selection criteria along with plant height.

**John K. et al. (2012)** studied on estimates of genetic parameters for morphological, physiological, yield and yield attributes for moisture stress tolerance in groundnut (*Arachis hypogaea* L.). Among parents, TPT-4 showed the highest *per se* performance for number of well filled and mature pods per plant, shelling per cent, sound mature kernel per cent, 100 kernel weight, kernel yield per plant and pod yield per plant. The genotypes, ICGV-99029 for number of primary branches per plant, number of secondary branches per plant, transpiration rate, dry haulms yield per plant and harvest index, K-1375 for specific leaf area and water use efficiency and TCGS-647 for specific leaf weight exhibited the highest *per se* performance. Among F₁s, TPT-4 × ICGV-99029 produced more number of primary branches per plant, number of well-filled and mature pods per plant, shelling per cent, dry haulms yield per plant, kernel yield per plant, and pod yield per plant. High heritability and high genetic advance as per cent of mean was recorded for number of well filled mature pods per plant, high heritability and moderate genetic advance as per cent of mean observed for days to fifty per cent flowering. Moderate heritability and high genetic advance as per cent of mean was showed for water use efficiency and dry haulms yield per plant, whereas moderate heritability and moderate GAM was recorded for plant height, harvest index and kernel yield per plant and low heritability and moderate GAM for number of primary branches per plant and stomatal conductance indicating the importance of additive gene effects, selection for such characters may be rewarding.
Mohan Vishnuwardhan K. et al. (2013) studied on genetic variability for yield, yield attributes and resistance to foliar diseases in groundnut (Arachis hypogaea L.). Eight parents and their 28 cross combinations (crossed in an 8 X 8 diallel fashion without reciprocals) of groundnut were evaluated in randomized block design with three replications for variability, heritability, and genetic advance during kharif, 2009. Observations on sixteen characters were recorded. Analysis of variance revealed highly significant differences among the genotypes for all the characters studied. High GCV accompanied by high heritability and high GAM were obtained for number of secondary branches per plant, percentage of leaves affected by foliar diseases per plant and number of immature pods per plant indicating predominant role of additive gene action and amenability for phenotypic selection in early generations. Rust severity, number of mature pods per plant and pod yield per plant recorded high GCV and moderate heritability and GAM. Moderate GCV, moderate to low heritability and GAM were registered for number of primary branches per plant, kernel weight per plant, shelling out-turn, late leaf spot and harvest index indicating that additive and non-additive gene actions have a role in their inheritance and phenotypic selection would be effective to some extent. Days to 50 per cent flowering, days to maturity, plant height at harvest and sound mature kernel percentage recorded low GCV, high to moderate heritability and low GAM indicating larger role of non-additive gene action and selection would be effective in later segregating generations.

2.2 Character association and path coefficient analysis
Sumathi et al. (2007) studied on character association and path coefficient analysis in confectionery type 48 diverse groundnut genotypes for eleven characters and found pod yield per plant had significant positive association with kernel yield, sound mature kernel weight and 100 seed weight both at genotypic and phenotypic levels. The inter correlations of kernel yield with sound mature kernel weight, 100 seed weight were also positive and significant at both genotypic and phenotypic levels. The number of matured pods per plant exhibited positive, significant association with total number of kernels per plant and sound mature kernel number. Kernel yield per plant exerted maximum positive direct effect on pod yield per plant. Sound mature kernel weight, shelling percentage and 100 seed weight exerted high positive indirect effects through kernel yield per plant and contributed directly and positively to pod yield per plant.

Korat et al. (2010) observed higher genotypic correlation coefficients than the corresponding phenotypic correlations for seed yield and its component characters in groundnut indicating strong inherent association among the traits. Yield contributing characters like biological yield per plant, 100-kernel weight and harvest index had positive and significant association with pod yield per plant at phenotypic level. Phenotypic interrelationship between days to maturity and pod yield per plant was found negative and significant. Genotypic correlations of above said yield components with pod yield were also strong and with similar sign. The genotypic and phenotypic path analysis revealed the highest positive direct effects of biological yield per plant and harvest index towards pod yield. Hundred-kernel weight contributed indirectly via biological yield per plant and harvest index. Based
on correlation and path analysis, biological yield per plant, 100-kernel weight and harvest index were identified as the most important yield contributing characters.

**Bera et al. (2010)** evaluated forty four genotypes of groundnut in three years at two locations for path coefficient analysis. Path coefficient analysis indicated positive direct contribution of pods/plant and harvest index to the seed yield irrespective of locations and years effect. Their correlations with seed yield were also significantly positive. So pod yield/plant and harvest index can be used directly as selection criteria for improvement of seed yield in groundnut.

**Dhaliwal et al. (2010)** estimated inter trait associations along with direct and indirect effects by path analysis for dry pod yield and its components in groundnut. Dry pod yield had significant positive association with days to flowering, days to maturity, haulm yield per plant and kernel yield per plant. At genotypic level too these traits had high positive correlation with dry pod yield. Path analysis indicated high positive direct contribution of kernel yield per plant. Days to flowering, days to maturity and haulm yield per plant made indirect contribution to dry pod yield via kernel yield per plant. It was concluded that these traits must be given weight age during selection in the segregating generations for the improvement of dry pod yield in groundnut.

**Babariya C. A. and Dobariya K. L. (2012)** studied on correlation coefficient and path coefficient analysis for yield components in groundnut (*Arachis hypogaea* L.). The study was undertaken to estimate correlation coefficients and direct and indirect effects by path analysis for pod yield per plant and its components by using 100 genotypes of Spanish bunch groundnut. The pod
yield per plant was significantly and positively correlated with days to maturity, plant height, number of pods per plant, kernel yield per plant, number of mature pods per plant, 100-kernel weight, biological yield per plant and harvest index. Biological yield per plant and harvest index exhibited high and positive direct effects on pod yield per plant. Whereas, kernel yield per plant, number of pods per plant and days to maturity showed moderate and positive direct effects on pod yield per plant. Thus, these characters were identified as the most important yield components and due emphasis should be placed on these characters while selecting for high yielding genotypes in Spanish bunch groundnut.

2.3 Genetic diversity

Nearly all Arachis specis are diploid, but cultivated peanut is an allotetraploid (genome AABB). It is a member of the section Arachis, which also includes about 25 diploid and one tetraploid wild species (A. monticola). Arachis hypogaea is classified based on the presence or absence of flowers on the main axis into two sub-species, hypogaea and fastigiata. These two sub-species were further classified into 6 botanical varieties based on morphology and growth habits. Sub-species hypogaea was divided in two botanical varieties, hypogaea and hirsute, while sub-spp. fastigiata in the varieties fastigiata, vulgaris, aequatoriana and peruviana. The identity of the progenitor species of cultivated peanut has been of great interest. Several species have been suggested as putative A and B genome donors. RFLP analysis that included 17 diploid species of the section Arachis and 3 A. hypogaea accessions suggested a single origin for domesticated peanut and
ancestral species related to *A. duranensis* (A genome) and *A. ipaensis* (B genome) as the most likely progenitors of *A. hypogaea*. In situ hybridization analysis of 6 diploid species and one *Arachis hypogaea* accessions, and RAPD and ISSR (Inter-simple sequence repeat) analysis of 13 *A. hypogaea* accessions and 15 wild species, however suggested *A. villosa* (A genome) and *A. ipaensis* (B genome) as the progenitors of cultivated peanut.

Cultivated peanut exhibits a considerable amount of variability for various morphological, physiological and agronomic trials. However, little variation has been detected at the DNA level using techniques such as RAPDs, AFLPs and RFLPs. The low level of variation in cultivated peanut has been attributed to three causes or to combinations of them:

1. Barriers to gene flow from related diploid species to domesticated peanut as a consequence of the polyploidization event;
2. Recent polyploidization, from one or a few individual(s) of each diploid parental species, combined with self pollination;
3. Use of few elite breeding lines and little exotic germplasm in breeding programs, resulting in a narrow genetic base.

*Moretzsohn et al. (2004)* studied genetic diversity of peanut and its wild relatives based on the analysis of hyper variable regions of the genome. The genus *Arachis* is native to a region that includes Central Brazil and neighboring countries. Little is known about genetic variability of Brazilian cultivated peanut (*Arachis hypogaea*, genome AABB) germplasm collection at the DNA level. The
understanding of genetic diversity of cultivated and wild species of peanut (*Arachis* spp.) is essential to develop strategies of collection, conservation and use of germplasm in variety development. The identity of the ancestor progenitor species of cultivated peanut has also been of great interest. Several species has suggested as putative AA and BB genome donors to allotetraploid *A. hypogaea*. Microsatellite or SSR (Simple Sequence Repeat) markers are co-dominant, multi allelic and highly polymorphic genetic markers, appropriate for genetic diversity studies. Microsatellite markers may also to some extent, support phylogenetic inference.

A total of 67 new microsatellites markers (mainly TTG motif) were developed for *Arachis*. Only three of these markers, however, were polymorphic in cultivated peanut. These three new markers plus five other markers characterized previously were evaluated for number of alleles per locus and gene diversity using 60 accessions of *A. hypogaea*. Genetic relationships among these 60 accessions and a sample of 36 wild accessions representative of section *Arachis* were estimated using allelic variation observed in a selection set of 12 SSR markers. Result showed that the Brazilian peanut germplasm collection has considerable level of genetic diversity detected by SSR markers. Similarity groups for *A. hypogaea* accessions were established, which is a useful criteria for selecting parental plant for crop improvement. Microsatellite marker transferability was upto 76% for species of the section *Arachis*, but only 45% for species from the other eight *Arachis* sections tested. A new marker (Ah-041) presented a 100% transferability and could be used to classify the peanut accessions in AA and non-AA genome carriers.
The level of polymorphism observed among accessions of *A. hypogaea* analyzed with newly developed microsatellite markers was low, corroborating the accumulated data which show that cultivated peanut presents a relatively reduced variation at the DNA level. A selected panel of SSR markers allowed the classification of *A. hypogaea* accessions into two major groups. The identification of similarity groups will be useful for the selection of parental plants to be used in breeding programmes. Marker transferability is relatively high between accessions of selection *Arachis*. The possibilities of using microsatellite markers developed for one species in genetic evaluation of other species greatly reduces the cost of the analysis. Since the development of microsatellite markers is still expensive and time consuming. The SSR markers developed in this study could be very useful for genetic analysis of wild species of *Arachis*, including comparative genome mapping, population genetic structure and phylogenetic inferences among species.

**Mahalaxmi et al. (2005)** studied divergence analysis among fifty seven groundnut was carried out using Mahalanobis's $D^2$ statistic. The genotypes were grouped into seven clusters. The association between genetic and geographic diversity cannot be generalized. Maximum divergence was observed between clusters II and VII followed by clusters VI and VII. Days to first flowering followed by shelling percentage contributed maximum to the total divergence. The genotypes VRI 2 (cluster II) for shelling percentage and oil content ICG 3063 (cluster VI) for SMK number and pods and ICG 3254 (cluster VII) for maturity-index and reproductive efficiency were found as potential parents based on cluster mean and genetic diversity.
Upadhyaya et al. (2006) studied on identification of diverse groundnut germplasm as a source of early maturity in a core collection. A groundnut collection was evaluated in two seasons to identify 21 early maturing landraces. Phenotypic diversity of these 21 early maturing landraces was accessed in three rainy and five post-rainy seasons, along with 3 known sources of early maturity (Chico, Gangapuri, and JL 24). The new sources differed in 8 of 14 morphological traits studied. Of the 14 agronomic and 2 quality traits studied, 8 yield and yield component traits were evaluated at 2 harvest dates. The landraces matured in 80-90 days after sowing (DAS), similar to Chico and Gangapuri (80-90 DAS) and earlier than JL 24 (90-95 DAS). Four new early maturing landraces [ICG 4558 (India); ICG 4890 (Argentina); ICG 9930 (Zimbabwe); ICG 11605 (Bolivia)], with predominantly three to four seeds per pod, were identified as additional sources for breeding confectionery groundnut varieties. Correlation coefficient between the observations made at the two harvest dates for the seven yield traits were ≥ 0.71, indicating a observation is sufficient at 75 DAS or 90 DAS in initial characterization. Correlation between pod yield and 100 seed weight was significant in all the eight seasons individually and overall at 90 DAS. Presence of initial phenotypic diversity in the new early maturity landraces was detected. Principal Component Analysis (PCA) using the first 10 PC score delineated the 21 landraces into 3 clusters. The information of these clusters could neither be explained on the basis of geographic areas of landraces collection nor on the basis of botanical varieties. This might reflect the nascent variation acquired by the landraces in their secondary habitats, under ecologically similar conditions,
independent of their countries of origin. Landraces in cluster 2 and 3 showed a wide range for several agronomic traits, indicating their usefulness in breeding programs for developing early maturing high yielding broad based cultivars.

Laxmidevamma et al. (2006) studied eighty-one genotypes of groundnut (Arachis hypogaea L.) representing different groundnut centers for genetic divergence analysis utilizing Mahalanobis $D^2$ analysis. Based on the genetic distance ($D^2$ value) groundnut accessions were grouped into 16 clusters. Out of the 16 clusters formed, cluster I was the largest with 47 accessions followed by cluster II with 10 accessions. Test weight, days to maturity and oil content were the most potential traits contributing to the total divergence. Cluster XI and XVI had maximum inter-cluster distance suggesting wide diversity and by utilizing these accessions from these clusters desirable segregates may be evolved through hybridization. Cluster XII has genotype with most favorable characters and hence can be involved as potential parent for development of superior genotypes.

Aalami et al. (2007) studied on assessment of genetic diversity in Groundnut (Arachis hypogaea L.) germplasm using morphological traits. A field experiment carried out to evaluate genetic diversity in 76 peanut accessions (Arachis hypogaea L.), obtained from national plant gene bank of Iran, in the faculty of agriculture, the university of Gulian, Rasht, Iran. Eleventh major morphological traits were recorded using ICRISAT peanut Descriptor during 2002 growing season using a Randomized Complete Block Design (RCBD) with two replications. Application of Pearson correlation indicated that seed length, seed width, pod length and width had a direct positive relation to seed weight ($P \leq 0.01$). In addition, the number of pods
per plant had a positive correlation with numbers of seeds per plant and pod weight ($p \leq 0.01$). Three components derived from principal component analysis accounted for nearly 72% of whole variability in evaluated germplasm defined by number of seeds per plant, pod weight, seed length, seed width, seed weight, number of pods per plant, pod width and pod length. The accessions were divided into three groups by cluster analysis based on Ward’s method using Squared Euclidean distance coefficient. The studied accessions showed high similarity (0.19) therefore broadening genetic base of peanut germplasm and use of molecular markers could be suggested in order to complete the morphological traits-based classification and evaluation of genetic diversity in peanut germplasm.

Tang et al. (2007) used thirty-four SSR markers to assess the genetic variation of four sets of twenty-four accessions each from the four botanical varieties of the cultivated peanut. Among the tested accessions, ten to sixteen pairs of SSR primers showed polymorphisms. The maximum differentiation index, which was defined as the degree of genetic differentiation, was as high as 0.992 in the tested accessions. Each accession could be discriminated by a specific set of polymorphic SSR primers, and the intra-variety genetic distance was determined among accessions, with an average of 0.59 in var. *fastigiata*, 0.46 in var. *hypogaea*, 0.38 in var. *vulgaris*, and 0.17 in var. *hirsuta*. Dendrograms based on genetic distances were constructed for the four botanical varieties, which revealed the existence of different clusters. It was concluded that there was abundant intra-variety SSR polymorphism, and with more and more SSR markers being developed, the intrinsic genetic diversity would be detected and the
development of genetic map and marker-assisted selection for cultivated peanut would be feasible.

**Khote et al. (2010)** studied on genetic divergence among 30 groundnut (*Arachis hypogaea* L.) genotypes using Mahalanobis $D^2$ statistics. Based on genetic distance, these genotypes were grouped into six different clusters. Maximum intra cluster distance was in cluster V comprising two groundnut genotypes *viz.* RCM 556 and MS-48-1. Inter cluster distance was found to be maximum between cluster II and cluster V followed by cluster I and cluster V, cluster V and VI, cluster III and cluster VI, cluster II and cluster VI, cluster II and cluster V and cluster I and VI and were identified genetically diverse clusters could be used for hybridization programme in crop improvement in groundnut.

**Dolma et al. (2010).** Studied genetic divergence among 33 genotypes of groundnut (*Arachis hypogaea* L.) belonging to different eco-geographical regions by using Mahalanobis $D^2$ statistics. The analysis of variance revealed significant difference among the genotypes for all the traits. The 33 genotypes were grouped into 6 clusters, where as cluster I was the largest containing 18 genotypes followed by cluster II with 10 genotypes. The inter cluster distance was maximum between cluster IV and V followed by cluster III and V. Based on inter cluster distance and per se performance of genotypes, ISK-04-26, ISK-05-20 (cluster IV), ISK-04-11 (cluster V) and ISK-04-15 (cluster III) could be suggested for inclusion in the hybridization programme to evolve high yielding and late leaf spot resistant genotypes.
Venkataravana et al. (2010) analysed genetic divergence of sixty four genotypes (39 new germplasm accessions and 25 advance breeding lines) of groundnut and observed wide range of $D^2$ values ranging between 4.52 and 27.75 suggesting the presence of considerable amount of genetic diversity in the genotypes studied, which were grouped in to seven clusters where, cluster VII (28) was the largest followed by cluster I (24) and cluster VI (4). Maximum inter cluster distance was recorded between IV and VI representing wide divergence among these clusters. On the basis of inter cluster distance and cluster means the genotypes viz., ICGV-05033, ICGV-05052, PAFRGVT58, GG-20×ICGV-91114, ICGX-020063-F-B-SSD-P20-B, ICGX-020055-F-SSD-P37-B were widely diverse therefore may be considered for future breeding programmes.

Sudhir Kumar et al. (2010) collected sixty four groundnut genotypes from different sources and evaluated during late rainy season. The plant data recorded on fourteen characters were subjected to multivariate analysis to study the variability within the genotypes and to determine the efficiency of the methods in classifying genotypes. The first three axes both the factor analysis and principal component analysis (PCA) captured 59.52 % of the total variability and jointly identified final pod yield per plant, kernel yield per plant and oil yield per plant as characters contributing the most to the total variation. The first three axes of the canonical and discriminate analyses accounted for 99 and 95 % of the total variation respectively and identified in addition to the above characters oil content and branches per plant as important. Ward’s clustering method has grouped the genotypes into three
different distinct clusters. The effect of genetic divergence on the choice of parental stock and its improvement for breeding programme was discussed.

Sudhir Kumar et al. (2010) evaluated sixty four accessions of groundnut (Arachis hypogaea L.) under late kharif season and classified using principal component analysis (PCA) based on correlation matrix yielding eigen values and eigen vectors. Fourteen principal components (PC) have been extracted using the mean performance of the genotypes, first ten principal component contributed over 95 % of variation. Relative positive weights by each of the component to each single character, has shown pod yield per plant and kernel yield per plant being given high positive weight by first principal component. Biplot of first two principal components showed characters viz., plant height, harvest index, pod yield, kernel yield/plant and oil yield/plant distinguishing among the accession along the first principal component vector. Cluster analysis was performed based on first ten PC scores accounting more than 95 % of variation which classified the sixty four accessions into three clusters. Accessions in cluster 1 & 2 showed a wide range for several agronomic characters. This provides convenience in selecting superior accessions from each of these clusters for various yield contributing traits in the future breeding programs.

Zaman et al. (2010) studied the genetic divergence in groundnut. He conducted an experiment comprising 34 groundnut genotypes in a randomized block design with three replication during Rabi season for estimation of the multivariate analysis of divergence. The genotypes were grouped into five clusters.
Cluster III contained the highest number of genotypes (12) and the cluster II contained the lowest (2). The inter-cluster distances in all cases were larger than the intra-cluster distance which indicated that wider diversity is present among the genotypes of distant grouped. The highest intra-cluster distance was observed in cluster V and the lowest in II. The highest inter-cluster distance was observed between the cluster IV and III followed by V and III and the lowest between cluster V and I. Days to 50% flowering, days to maturity, number of branches per plant, number of matured nuts per plant and kernel size were the most important contributors based on the latent vector. But the highest cluster means for matured nuts per plant, 100 kernel weight, 100 nuts weight and yield per plant were obtained from the cluster II. With moderate yield but early maturity varieties were found in cluster IV. Therefore, more emphasis should be given on cluster VI for selecting genotypes as parents for crossing with the genotypes of cluster II and III for getting new recombinants with early maturity and higher yield.

Upadhyaya et al. (2011) evaluated a total of 269 accessions from 20 wild Arachis species belonging to 6 sections for 41 morpho-agronomic traits and 89 selected accessions for oil, protein and total sugar content. Six plants from each accession were grown in an open Arachis house in large cylindrical concrete structures during the 2002-05 seasons at Patancheru, India. REML analysis showed significance differences between species and accessions for most of the traits studied. Hierarchical cluster analysis, based on the first five principal component scores accounted for 82.5% variation, resulting in four clusters. Variation in genome relationships and ploidy levels had no bearing on the clustering pattern.
which was predominated by life forms: clusters 1 & 2 contained mostly annuals and cluster 3 & 4 perennials. A large range of variations were noticed among species for some of the agronomic traits: days to flowering, pod and seed characteristics, specific leaf area (SLA) and for SPAD chlorophyll meter reading (SCMR). *Arachis duranensis* showed the maximum intraspecific variation as revealed by a high diversity index for 23 of the 41 traits which included: days to flowering, primary branches, plant width, pod length, pod width, SCMR and SLA. The other species with desirable traits were *A. pusilla* (earliest flowering) and *A. villosa* (high SCMR at 60 days and 80 days after sowing). The latter species is cross compatible with cultivated groundnut, thus is a good source to enhance the trait value in the cultigens’ gene pool. The best 20 accessions with superior agronomic, nutritional quality and drought related traits combination have been identified for their use in introgression of diverse and unique alleles from wild *Arachis* species into *Arachis hypogaeae*.

Sonone *et al.* (2011) worked on multivariate studies in groundnut. They evaluated 40 accessions of groundnut (*Arachis hypogaea* L.) under multivariate analysis on the basis of pooled performance in 12 different environments created by monthly sowing from July 2006 to June 2007. The analysis of variance for morphological and yield traits in 40 genotypes on the basis of pooled performance over 12 environments showed the existence of distinct genetic differences among the genotypes for all the characters studied. Highest genotypic coefficient of variation and phenotypic coefficient of variation were recorded for kernel yield per plant. The heritability was high for pod length (99.3
The genetic advance as percent of mean was recorded highest for kernel yield per plant (25.6 g). All the 40 groundnut accessions were grouped into five clusters. The cluster I was the largest, involving 27 genotypes. In spite of variation in mean and $D^2$ values some genotypes *viz.* VRR-232 and 1792 constantly occupied cluster I in maximum of 12 environments including pooled over environment, indicating stable performance. The cluster I had minimum (0.43) intra-cluster distance and was found to be the most compact clusters. The cluster V ranked first in mean pod yield/plant (11.3g). The analysis for estimating the contribution of various characters towards the expression of genetic divergence on pooled basis indicated that pod length (25.6 %), plant height (15.1 %), shelling % (11.7 %), 100 seed weight (8 %) and number of kernels per plant (7.2 %) contributed maximum (67.5 %) towards total divergence in the material. Therefore these characters should be considered while selecting the parents for hybridization programme.

*Suneetha N. et al. (2013)* studied on genetic diversity analysis among released and pre-released cultures in groundnut. Diversity analysis among released/prerelease groundnut cultures was carried out to assess the genetic relationship which helps in the documentation of the differences in the context of intellectual property rights and to identify diverse parents for use in hybridization programme for improvement of yield and other desirable traits i.e. drought resistance and foliar disease resistance etc.. Twenty nine released and pre-release groundnut genotypes were grown during kharif season with protective irrigation in randomized block design with three replications. Data on morphological, yield and yield attributes were recorded. Diversity was analysed through Mahalanobis’s $D^2$
analysis. Twentynine genotypes were grouped into nine clusters. Clustering pattern showed relationship with pedigree and place of breeding. Maximum contribution to diversity was by harvest index, days to emergence and length of main axis and minimum contribution was by number of mature pods per plant.

2.4 EXPLOITING THE POTENTIAL OF GENETIC MARKERS IN GROUNDNUT

2.4.1 Biochemical markers

Identification of up to 17 polymorphic isozymes among wild species suggests that they may have the potential to follow gene introgression in interspecific hybrids and establish phylogenetic relationships in groundnut (Lacks et al., 1991. Lu and Pickersgill, 1993 and Stalker et al., 1994). However, only asparate amino transferase (AAT), glutamate oxaloacetate transaminase (GOT), isocitrate dehydrogenase (IDH) and phospho hexoseisomerase (PHI) were reported polymorphic in cultivated groundnut (Galgaro and Lopes, 1994; Grieshammer and Wynne, 1990 and Lacks and Stalker, 1993). Low polymorphism shown by isozyme markers in cultivated groundnut reveals their limited utility in genetic enhancement in groundnut.

Leticia Galgaro et al. (1997) reported that Arachis villosulicarpa is a perennial species cultivated for its soft and tasty seeds by indigenous inhabitants of
the Mato Grosso State, Brazil. Besides *A. hypogaea*, this species is considered as the only species of *Arachis* which represents a valuable food source for human consumption. Due to the lack of knowledge concerning the genetic diversity of *A. villosulicarpa*, this study was conducted to evaluate the genetic variability of the accessions from the Germplasm Collection of CENARGEN/EMBRAPA (Brazil) and IAC, Campinas, (Brazil). In addition, the genetic similarity between *A. villosulicarpa*, the related wild species *A. pietrarellii*, and the cultivated peanut *A. hypogaea* cv. Tatu was evaluated. From the entire sample analyzed of *A. villosulicarpa*, the accession from IAC, Campinas showed the highest indices of diversity for both enzymatic systems analyzed, pointing this accession as a promising source of genetic variability that must be preserved in the Germplasm Bank. A high level of genetic similarity was observed between *A. pietrarellii* and *A. villosulicarpa*, supporting previous suggestions that *A. pietrarellii* could be the ancestral progenitor species of *A. villosulicarpa* or that both species originated from a common ancestor.

**Motagi BN et al. (2007)** assessed Sixteen groundnut cultivars for protein (seed and hypocotyl) and glutamate oxaloacetate transaminase (GOT) polymorphism. Seed protein profiles produced six (SI-S6) electrophoretic phenotypes compared to three (GI-G3) of GOT. While, hypocotyl proteins produced twelve (H1-H12) electrophoretic patterns. Individually, seed and hypocotyl proteins can identify three and nine cultivars, respectively. However, combination of protein (seed and hypocotyl) and GOT isozyme profiles fingerprinted all the sixteen cultivars.
2.4.2 Molecular markers

Both RFLP and PCR-based markers have been used to assess polymorphic variation in cultivated and wild *Arachis* species in groundnut.

2.4.2.1 RFLPs (Restriction Fragment Length Polymorphism)

Marcos A. Gimenes *et al.* (2002) evaluated RFLP analysis of genetic variation in species of section *Arachis*, genus *Arachis* (Leguminosae). Four A-genome species of the genus *Arachis* (*A. cardenasii*, *A. correntina*, *A. duranensis*, *A. kempff-mercadoi*), three B genomes species (*A. batizocoi*, *A. ipaënsis* and *A. magna*), the AABB allotetraploid *A. hypogaea* (cultivated peanut) and introgression lines resulting from a cross between *A. hypogaea* and *A. cardenasii* were analyzed by RFLP. The A genome species (cytologically characterized by the presence of a small chromosome pair ‘A’) were closely similar to each other and shared a large number of restriction fragments. In contrast, the B genome species differed more from one another and shared few fragments. The results of this study indicate that the absence of the small chromosome pair is not a good criterion for grouping species of section *Arachis* as B genome species, since their genome might be quite distinct from the B genome of *A. hypogaea*. The lowest genetic variation was detected within accessions of *A. duranensis* (17 accessions), followed by *A. batizocoi* (4 accessions) and *A. cardenasii* (9 plants of accession GKP 10017). The high level of genetic variation found in *A. cardenasii* might indicate that not all accessions of wild species of *Arachis* are autogamous, as reported for *A. hypogaea*.

2.4.2.2 AFLPs (Amplified Fragment Length Polymorphisms)
Guohao He and Channapatna Prakash (2001) evaluated Forty-four accessions of cultivated peanut (*Arachis hypogaea* L.) representing six botanical varieties of two subspecies along with three accessions of the wild relative *A. monticola* Krapov et Rigoni for their genetic relationships using the AFLP marker technology. Fifteen AFLP primer pairs (*Eco*RI/*Mse*I) generated 28 distinct polymorphic markers that were employed to develop unique profiles of all accessions and to construct a phenogram. The results showed that the botanical varieties *aequatoriana* and *peruviana* were closer to subspecies *hypogaea* than subspecies *fastigiata* Waldr. to which they belong, and the wild *A. monticola* was not distinct from the cultivated *A. hypogaea*. Although the extent of genetic diversity in peanut is low compared to many other crops, our studies show that by employing the AFLP approach, sufficient DNA variation can be detected in the cultivated peanut germplasm to conduct evolutionary studies.

Liezel Herselman et al. (2003) studied in genetic variation among Southern African cultivated peanut (*Arachis hypogaea* L.) genotypes as revealed by AFLP analysis. The amplified fragment length polymorphism (AFLP) technique, employing two different rare cutters, *Eco*RI and *Mlu*I in combination with the frequent cutter *Mse*I, was used to assess genetic diversity and relationships among 21 closely related cultivated Southern African peanut genotypes. A dendrogram was constructed using Jaccard’s coefficient and the UPGMA clustering method. Low levels of polymorphism (on average 2.78%) were detected. Results indicated that both *Eco*RI/*Mse*I and *Mlu*/Mse*I AFLP enzyme combinations efficiently detected polymorphism within closely related cultivated peanut, although the
EcoRI/MseI enzyme combination detected more fragments per primer combination (on average 67.8) as opposed to 29.7 by the MluI/MseI enzyme combination. All 21 genotypes could be uniquely distinguished from each other with a minimum of three MluI/MseI primer combinations. Genetic data correlated well with known species and pedigree data, dividing the 21 genotypes into two main groups corresponding to the two subspecies of *Arachis hypogaea* namely *fastigiata* and *hypogaea*. Divisions within the two main groups correlated with botanical types and pedigree data. This is the first report where MluI/MseI primer combinations were used on cultivated peanut and also the first successful detection of polymorphisms between closely related cultivated peanut genotypes worldwide.

### 2.4.2.3 SSRs (Simple Sequence Repeats)

**Ronghua Tang et al. (2007)** used thirty four SSR markers to assess the genetic variation of four sets of twenty-four accessions each from the four botanical varieties of the cultivated groundnut. Among the tested accessions, ten to sixteen pairs of SSR primers showed polymorphisms. The maximum differentiation index, which was defined as the degree of genetic differentiation, was as high as 0.992 in the tested accessions. Each accession could be discriminated by a specific set of polymorphic SSR primers, and the intra-variety genetic distance was determined among accessions, with an average of 0.59 in var. *fastigiata*, 0.46 in var. *hypogaea*, 0.38 in var. *vulgaris*, and 0.17 in var. *hirsuta*. Dendrogrames based on genetic distances were constructed for the four botanical varieties, which revealed the existence of different clusters. It was concluded that there was abundant intra-
variety SSR polymorphism, and with more and more SSR markers being developed, the intrinsic genetic diversity would be detected and the development of genetic map and marker-assisted selection for cultivated groundnut would be feasible.

Carla M.L.C.D. Angelici et al. (2008) genetic diversity in section Rhizomatosae of the genus Arachis based on microsatellite markers. The genus Arachis (Fabaceae) native to South America, contains 80 species divided into nine sections, three of which contain species of special economic importance such as the cultivated peanut (Arachis hypogaea), belonging to the section Arachis, and some perennial forage species from sections Caulorrhizae and Rhizomatosae. We used microsatellite markers to assay genetic variability among 77 accessions of four species from section Rhizomatosae, the diploid Arachis burkartii (2n = 2x = 20) and the tetraploid Arachis glabrata, Arachis pseudovillosa and Arachis nitida (2n = 4x = 40). A total of 249 alleles were found in the fifteen loci analyzed and a high degree of intra and interspecific polymorphism was detected. The lowest intraspecific variation occurred in Arachis burkartii, while the smallest estimated interspecific value was between A. nitida and A. pseudovillosa and the largest was between A. burkartii and A. nitida. High observed heterozygosity was detected in A. glabrata. The diploid accessions grouped in one cluster and the tetraploid accessions in another. It was possible to distinguish all 77 accessions and the genetic distance between accessions could not be correlated with geographic origin.

Varshney R. K. et al. (2009) high level of natural variation in a groundnut (Arachis hypogaea L.) germplasm collection assayed by selected
informative SSR markers. The ability to identify genetic variation is indispensable for effective management and use of genetic resources in crop breeding. Genetic variation among 189 groundnut (*Arachis hypogaea* L.) accessions comprising landraces, cultivars, a mutant, advanced breeding lines and others (unknown genetic background) representing 29 countries and 10 geographical regions was assessed at 25 microsatellite or simple sequence repeat loci. A high number of alleles (265) were detected in the range of 3 (Ah1TC6G09) to 20 (Ah1TC11H06) with an average of 10.6 alleles per locus. The polymorphism information content value at these loci varied from 0.38 (Ah1TC6G09) to 0.88 (Ah1TC11H06) with an average of 0.70. A total of 59 unique alleles and 127 rare alleles were detected at almost all the loci assayed. Cluster analysis grouped 189 accessions into four clusters. In general, genotypes of South America and South Asia showed high level of diversity. Extraordinary level of natural genetic variation reported here provides opportunities to the groundnut community to make better decisions and define suitable strategies for harnessing the genetic variation in groundnut breeding.

**Jiang Hui-Fang et al. (2010)** reported that mini core collection plays an important role in evaluating genetic resources of groundnut (*Arachis hypogaea* L.). This study aimed at comparing the genetic diversities of a domestic and exotic groundnut mini core collections and providing basic data for germplasm introduction and groundnut breeding. The exotic mini core collection, which was introduced from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT, Patancheru, India), consisted of 168 accessions, whereas the
Chinese mini core collection consisted of 298 accessions. A total of 26 polymorphic simple sequence repeat (SSR) markers were used to evaluate the genetic diversity of these genotypes and their similarities. The similarity coefficient ranged from 0.49 to 0.99 among the total 466 accessions and the largest genetic distance was between “L2 Gangguo” from the Chinese core collection and “ICG12625” from the ICRISAT core collection. Among the 6 botanical types of peanut, the fastigiata type had the largest genetic diversity indices in both Chinese and ICRISAT mini core collections, which were 1.11 and 0.97, respectively. The hypogaea type had the second largest diversity with genetic diversity indices of 0.89 and 0.88 for Chinese and ICRISAT mini core collections, respectively. The genetic distance between the 2 mini core collections was larger than that within a mini core collection. Particularly, the aequatoriana genotype ICG12625 from ICRISAT had the largest differences to the Chinese genotypes. According to similarity coefficient and genetic diversity index, the genetic diversity was greater among genotypes from the Chinese mini core collection than that from the ICRISAT mini core collection.

**Shoba D. et al. (2010)** studied on genetic diversity analysis of groundnut genotypes using SSR markers. Groundnut (*Arachis hypogaea* L.), an important oilseed crop is a rich source of oil and protein. Molecular marker technologies are the effective tools and they are used for the assessment of genetic variability because they are not influenced by the environment. Among the molecular markers, Simple Sequence Repeat (SSR) has proved to be the most powerful tool for variety identification in groundnut and has much potential in genetic and breeding studies. Among the 17 SSR primer pairs used for assessing the genetic diversity, 6 primer
pairs (24.0 per cent) were polymorphic. The genotype TMV 2 was susceptible to rust and late leaf spot diseases and it was separately clustered in the dendrogram and among the eleven foliar disease resistant genotypes, the genotypes viz., COG 0423, COG 0436 and COG 0432 were distantly clustered from TMV 2. Hence, by using the genotypes viz., TMV 2, COG 0423, COG 0436 and COG 0432, three combinations viz., TMV 2 x COG 0423, TMV 2 x COG 0436 and TMV 2 x COG 0432 could be made for further studies.

Padmalatha Koilkonda et al. (2011) reported that large-scale development of expressed sequence tag simple sequence repeat (EST-SSR) markers was performed in peanut (Arachis hypogaea L.) to obtain more informative genetic markers. A total of 10,102 potential non-redundant EST sequences, including 3,445 contigs and 6,657 singletons, were generated from cDNA libraries of the gynophore, roots, leaves and seedlings. A total of 3,187 primer pairs were designed on flanking regions of SSRs, some of which allowed one and two base mismatches. Among the 3,187 markers generated, 2,540 (80%) were trinucleotide repeats, 302 (9%) were dinucleotide repeats, and 345 (11%) were tetranucleotide repeats. Pre-polymorphic analyses of 24 Arachis accessions were performed using 10% polyacrylamide gels. A total of 1,571 EST-SSR markers showing clear polymorphisms were selected for further polymorphic analysis with a Fluorofragment Analyzer. The 16 Arachis accessions examined included cultivated groundnut varieties as well as diploid species with the A or B genome. Altogether 1,281 (81.5%) of the 1,571 markers were polymorphic among the 16 accessions, and 366 (23.3%) were polymorphic among the 12 cultivated varieties. Diversity
analysis was performed and the genotypes of all 16 *Arachis* accessions showed similarity coefficients ranging from 0.37 to 0.97.

**Manish K. Pandey et al. (2012)** studied on highly informative genic and genomic SSR markers to facilitate molecular breeding in cultivated groundnut (*Arachis hypogaea*). With an objective of identification of highly informative set of SSR markers in cultivated groundnut (*Arachis hypogaea* L.), a total of 4485 markers were used for screening using a set of 20 parental genotypes of 15 mapping populations. Although 3582 (79.9%) markers provided scorable amplification, only 1351 (37.3%) markers showed polymorphism. Polymorphism information content (PIC) value ranged from 0.10 (GM742) to 0.89 (S009) with an average of 0.31. Similarly, number of alleles ranged from 2 to 14 with an average of 3.2 alleles. In general, the SSR markers based on dinucleotide repeats displayed higher PIC value and number of alleles. Based on these polymorphism features, 199 markers with >0.50 PIC values have been identified. Polymorphism features of these markers along with the primer sequences, for the first time, for a total of 946 SSR markers have been provided. It is anticipated that the identified set of highly informative markers, instead of starting from the random set of SSR markers, should be very useful to initiate molecular genetics and breeding studies in cultivated groundnut.

**Marcio C. Moretzsohn et al. (2013)** evaluated the relationships of cultivated peanut (*Arachis hypogaea*) and its most closely related wild species using intron sequences and microsatellite markers. The genus *Arachis* contains 80 described species. Section *Arachis* is of particular interest because it includes
cultivated peanut, an allotetraploid, and closely related wild species, most of which are diploids. This study aimed to analyse the genetic relationships of multiple accessions of section *Arachis* species using two complementary methods. Microsatellites allowed the analysis of inter- and intraspecific variability. Intron sequences from single-copy genes allowed phylogenetic analysis including the separation of the allotetraploid genome components. Intron sequences and microsatellite markers were used to reconstruct phylogenetic relationships in section *Arachis* through maximum parsimony and genetic distance analyses. Although high intraspecific variability was evident, there was good support for most species. However, some problems were revealed, notably a probable polyphyletic origin for *A. kuhlmannii*. The validity of the genome groups was well supported. The F, K and D genomes grouped close to the A genome group. The 2n = 18 species grouped closer to the B genome group. The phylogenetic tree based on the intron data strongly indicated that *A. duranensis* and *A. ipaensis* are the ancestors of *A. hypogaea* and *A. monticola*. Intron nucleotide substitutions allowed the ages of divergences of the main genome groups to be estimated at a relatively recent 2.3–2.9 million years ago. This age and the number of species described indicate a much higher speciation rate for section *Arachis* than for legumes in general. The analyses revealed relationships between the species and genome groups and showed a generally high level of intraspecific genetic diversity. The improved knowledge of species relationships should facilitate the utilization of wild species for peanut improvement. The estimates of speciation rates in section
Arachis are high, but not unprecedented and suggest these high rates may be linked to the peculiar reproductive biology of Arachis.

2.4.2.4 Primers Designed from Consensus Branch Point Signal Sequences

Faqian Xiong et al. (2011) introduced a novel method for producing molecular markers in plants using single 15- to 18-mer PCR primers designed from the short conserved consensus branch point signal sequences and standard agarose gel electrophoresis. This method was tested on cultivated peanut and verified to give good fingerprinting results in other plant species (mango and banana). These single primers, designed from relatively conserved branch point signal sequences within gene introns, should be universal across other plant species. The method is rapid, simple, and efficient, and it requires no sequence information of the plant genome of interest. It could be used in conjunction with, or as a substitute for, conventional RAPD or ISSR techniques for applications including genetic diversity analysis, phylogenetic tree construction, and quantitative trait locus mapping. This technique provides a new way to develop molecular markers for assessing genetic diversity of germplasm in diverse species based on conserved branch point signal sequences.

2.4.2.5 RAPDs (Random Amplified Polymorphic DNAs)

Due to advances in molecular biology techniques, large numbers of highly informative DNA markers have been developed for the identification of genetic polymorphism. The random amplified polymorphic DNA (RAPD) technique based on the polymerase chain reaction (PCR) has been one of the most commonly used molecular techniques to develop DNA markers. RAPD markers are amplification
products of anonymous DNA sequences using single, short and arbitrary oligonucleotide primers, and thus do not require prior knowledge of a DNA sequence. Low expense, efficiency in developing a large number of DNA markers in a short time and requirement for less sophisticated equipment has made the RAPD technique valuable although reproducibility of the RAPD profile is still the centre of debate. (Fevzi BARDAKCI, 2001)

Subramanian et al. (2000) studied on identification of DNA polymorphism in cultivated groundnut using random amplified polymorphic DNA (RAPD) assay. Construction of a genetic linkage map is necessary to apply marker-assisted selection tools in a crop improvement program. In this study, 70 selected genotypes, representing variability for several morphological, physiological, and other characters, were studied for polymorphism employing random amplified polymorphic DNA (RAPD) assay with 48 oligonucleotide primers. Of the 48 oligonucleotide primers, only 7 (14.6%) yielded polymorphic amplification products. The total number of bands from the 7 primers was 408, of which 27 were polymorphic. Detection of polymorphism in cultivated groundnut opens up the possibility of development of its molecular map by judicious selection of genotypes that show DNA polymorphism. This approach will be useful for developing marker-assisted selection tools for genetic enhancement of groundnut for desirable traits.

Raina S.N. et al. (2001) identified RAPD and ISSR fingerprints as useful genetic markers for analysis of genetic diversity, varietal identification, and phylogenetic relationships in peanut (Arachis hypogaea) cultivars and wild species.
Twenty-one random and 29 SSR primers were used to assess genetic variation and interrelationships among subspecies and botanical varieties of cultivated peanut, *Arachis hypogaea* (2n = 4x = 40), and phylogenetic relationships among cultivated peanut and wild species of the genus *Arachis*. In contrast with the previous generalization that peanut accessions lack genetic variation, both random and SSR primers revealed 42.7 and 54.4% polymorphism, respectively, among 220 and 124 genetic loci amplified from 13 accessions. Moreover, the dendrograms based on RAPD, ISSR, and RAPD + ISSR data precisely organized the five botanical varieties of the two subspecies into five clusters. One SSR primer was identified that could distinguish all the accessions analysed within a variety. Although the polymorphic index content varied from 0.1 to 0.5 for both ISSR and RAPD markers, primer index values were substantially higher for RAPD primers (0.35–4.65) than for SSR primers (0.35–1.73). It was possible to identify accessions, particularly those of divergent origins, by RAPD and (or) ISSR fingerprints. Based on these results, marker-based genetic improvement in *A. hypogaea* appears possible. None of the 486 RAPD and 330 ISSR amplification products were found to be commonly shared among 13 species of section *Arachis* and one species each of sections *Heteranthae*, *Rhizomatosae*, and *Procumbentes*. Dendrograms constructed from RAPD, ISSR, and RAPD + ISSR data showed overall similar topologies. They could be resolved into four groups corresponding to the species grouped in four taxonomic sections. The results strongly support the view that *Arachis monticola* (2n = 4x = 40) and *A. hypogaea* are very closely
related, and indicate that *A. villosa* and *A. ipaensis* are the diploid wild progenitors of these tetraploid species.

**Dwivedi S. L. et al. (2001)** studied on genetic diversity among selected groundnut germplasm by RAPD analysis. Assessment of genetic diversity in a crop species is prerequisite to its improvement. The use of germplasm with distinct DNA profiles will help to generate genetically diversified breeding populations. The aims of this experiment were to study molecular diversity among selected groundnut accessions and identify those with distinct DNA profiles for mapping and genetic enhancement. Twenty-six accessions and eight primers of a 10-mer were selected for random amplified polymorphic DNA assay. The genetic similarity (*S*_ij*) ranged from 59.0% to 98.8%, with an average of 86.2%. Both multidimensional scaling and unweighted pair-group method with arithmetic averages (UPGMA) dendrograms 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 profiles (ICG 1448, 7101, and 1471, and ICGV 99006 and 99014) were identified for mapping and genetic enhancement in groundnut.

**Sergio Emilio Dos Santos et al. (2003)** studied on genetic variation within and among species of five sections of the genus *Arachis* L. (Leguminosae) using RAPDs. RAPDs were used to assay the genetic variation within and among 48 accessions of five sections of the genus *Arachis* and to establish the genetic relationships among these accessions. Ten of 34 primers tested were selected for DNA amplification reactions since they yielded the largest numbers of polymorphic
loci. A dendrogram was constructed based on data from the 10 primers selected. Eighty RAPD polymorphic bands were analyzed among the accessions studied. The relationships among species based on RAPDs were similar to those previously reported based on morphological, cytological and crossability data, demonstrating that RAPDs can be used to determine the genetic relationships among species of the different sections of the genus *Arachis*. In general, wide variation was found among accessions and low variation was found within the accessions that had two or more plants analyzed. However, higher polymorphism was found in the section *Trierectoides* and in one accession of *A. major*, indicating that generalizations should be avoided and each species should be analyzed in order to establish collection and maintenance strategies.

*Wardsson Lustrino Borges et al.* (2007) studied on genetic variability among peanut accessions. The objective of this study was to evaluate the genetic variability among 29 accessions of peanut (*Arachis hypogaea* L.) by means of random molecular markers (Random Amplified Polimorphic DNA – RAPD). The molecular assay was performed with 31 primers, of which 12 (39%) revealed polymorphism. It was observed a total of 145 amplified fragments, of which 35 (24%) were polymorphic, with an average of 4.67 fragments by primer and 1.13 polymorphic fragment by primer. It was observed through the dendrogram that the accessions were separated into two groups with 89% of similarity. This distribution shows the variability among the accessions of the different botanical varieties, since the accessions of subspecie *fastigiata* are present in two principal groups, and the
accessions of subspecie *hypogaea* are distributed in subgroups A and B from dendrogram group II.

**Nguyen Thi Lang et al. (2007)** studied on genetic divergence analysis on groundnut by RAPDs. This study demonstrated the utility of random amplified polymorphic DNAs (RAPDs) to analyse genetic divergence of peanut genotypes in the South Vietnam. Nucleic acid extracts from 29 *Arachis hypogaea* L. cultivars were amplified with five random decamers by PCR. Markers were used as RAPD 2, RAPD 3, RAPD 5, RAPD 6, OPC 11. The distinctive RAPD patterns generated from these cultivars could be used as genomic fingerprint to establish the identity of a given genotype. 29 peanuts were clearly separated in distinct subclusters in a phyllogram obtained by unweighted pair group method analysis (UPGMA) of genetic distances.

**Fanley Bertoti da Cunha et al. (2008)** studied on genetic relationships among *Arachis hypogaea* L. (AABB) and diploid *Arachis* species with AA and BB genomes. The cultivated peanut (*Arachis hypogaea* L.) is an allotetraploid, with two types of genomes, classified as AA and BB, according to cytogenetic characters. Similar genomes to those of *A. hypogaea* are found in the wild diploid species of section *Arachis*, which is one of the nine *Arachis* sections. The wild species have resistances to pests and diseases that affect the cultivated peanut and are a potential source of genes to increase the resistance levels in peanut. The aim of this study was to analyze the genetic variability within AA and BB genome species and to evaluate how they are related to each other and to *A. hypogaea*, using RAPD markers. Eighty-seven polymorphic bands amplified by ten 10-mer primers were
analyzed. The species were divided into two major groups, and the AA and the BB genome species were, in general, separated from each other. The results showed that high variation is available within species that have genomes similar to the AA and the BB genomes of *A. hypogaea*.

Varsha Kumari *et al.* (2009) studied on molecular characterization of induced mutants in groundnut using random amplified polymorphic DNA markers. Twenty-one mutants belonging to different botanical types of groundnut were used to assess molecular diversity using RAPD analysis. All twenty-seven random primers showed polymorphic bands. The number of amplicons varied from six to thirteen with an average of nine per primer, of which 3 were polymorphic. The polymorphism per primer ranged from 9.09 to 71.42 per cent with an average of 30.16 per cent. High genetic similarity values (*Sij*) of 0.88 to 0.98 were obtained for the genotypes, indicating limited genetic diversity. Dendrogram revealed five different clusters at *Sij* 0.95. TMV2 and its mutant NLM were distributed in different clusters indicating diversity at molecular level. DER and its mutants, viz. (SB 3, SB 6, VL 1, VL 3, VB 1, VB 3 and VR 8) also showed high genetic divergence. But VL 1 and its mutants, viz. (M 110 and M 28- 2) clustered together. Although the cluster analysis grouped genotypes into five different clusters, most of the genotypes were grouped in a single cluster, indicating narrow genetic diversity among the genotypes.
MATERIALS AND METHODS

The present investigation on “Studies on Genetic Diversity of Groundnut (Arachis hypogaea L.) using Morphological and Molecular Markers” was taken up under the Department of Plant Breeding and Genetics, and Department of Agricultural Biotechnology, College of Agriculture, OUAT, Bhubaneswar. The field trial was conducted at EB-II section of the Department of Plant Breeding and Genetics during rabi season 2012-13 and molecular work was conducted at laboratory of Department of Agricultural Biotechnology.

3.1 Materials and field plot design for evaluation

Materials for the present study included 27 advance breeding lines and 2 high yielding standard varieties of groundnut. The field trial on 29 entries was conducted in randomized block design (RBD) with three replications. The trial was sown on 01st December, 2012 in rabi season. Each entry was represented by 1 row of 3 m length with a spacing of 30 cm x 10 cm. A fertilizer dose of 20:40:40 kg NPK/ha was applied. Hoeing and hand weeding were done at the time of top dressing and need based plant protection measures were followed. The crop was harvested on 2nd April, 2013 during rabi season.

3.2 Characters studied

Twelve characters related to growth, vigour and yield were recorded. Days to 50% flowering was taken on plot basis. For other characters like plant height (cm), branches per plant, number of pods/plant, number of kernels/plant, 100
kernel weight, sound mature kernel percent, shelling percent, harvest index, kernel yield/plant, Haulm yield per plant and pod yield/plant, observations were recorded on five consecutive plants per genotype in each replication. The observations recorded were as follows.

**Days to 50 % flowering:** Number of days from sowing to the date on which 50 % plants in the plot had started blooming, recorded on plot basis.

**Plant height (cm):** Height of the five consecutive plants from ground to the tip of main stem was recorded.

**Branches per plant:** The branches directly emerging from the base of the main shoot were counted on five consecutive plants during harvest.

**Number of pods/ plant:** The pods on five consecutive plants were counted during harvest to calculate the mean pods per plant.

**Number of kernels/ plant:** The kernels from the pods of five consecutive plants were counted at harvest to calculate the mean kernels per plant.

**100-kernel weight (g):** Hundred sound mature kernels were taken from the harvested bulk at random and weighed to the nearest 0.01 g.
Sound mature kernel (%): The percent of number of sound matured kernel in hundred gram of kernel was recorded.

Shelling(%): The mass of kernels obtained from 100 g. of sound matured pods was recorded.

Harvest index(%): It was deduced by taking the ratio of sound mature pod weight of 5 plants to the biological yield of the same 5 plants and was expressed in %. The biological yield was recorded as the sum total of dry weight of 5 plants and it’s pod yield.

Kernel yield per plant (g): The average kernel weight of five consecutive plant was recorded.

Pod yield per plant (g): The average pod weight of five consecutive plants was recorded.

Haulm yield per plant (g): The average dry weight of five consecutive plants was recorded.

3.3 Statistical methods

Observations on the 12 yield and yield component characters were recorded on the 29 groundnut breeding lines in each of the three replications. Statistical analyses was carried out on the data recorded are outlined in the following paragraphs.
3.3.1 Analysis of variance

Analysis of variance for each character was carried out in RBD with plot means for partitioning of total variance into components (Table 1). The test of significance of difference among lines was done by ‘F’ test.

Table 1. ANOVA for RBD with expectation of mean squares (MS)

<table>
<thead>
<tr>
<th>Source</th>
<th>Df</th>
<th>MS</th>
<th>F</th>
<th>Expectation of MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replication (r)</td>
<td>(r-1)</td>
<td>MSr</td>
<td></td>
<td>$\sigma_r^2 + g\sigma_g^2$</td>
</tr>
<tr>
<td>Genotype (g)</td>
<td>(g-1)</td>
<td>MSg</td>
<td></td>
<td>$\sigma_g^2 + r\sigma_g^2$</td>
</tr>
<tr>
<td>Error (e)</td>
<td>(r-1) (g-1)</td>
<td>MSe</td>
<td></td>
<td>$\sigma_e^2$</td>
</tr>
</tbody>
</table>

The test of significance of difference between means of two lines (genotypes) were done by ‘t’ test and critical difference (CD at 5 %) was calculated as follows.

$$\text{CD (at 5 %)} = (2\text{MSe}/r)^{1/2} \times t_{0.05} \text{ at error df}$$

3.3.2 Estimation of genetic parameters:

**Variance components**: The phenotypic, genotypic and environmental variance components for different characters were estimated from the mean squares in ANOVA according to Al-Jibouri *et al.* (1958) as follows.

- Environmental variance: $$(\sigma_e^2) = \text{MS}_e$$

- Genotypic variance: $$(\sigma_g^2) = \frac{\text{MS}_g - \text{MS}_e}{r}$$

- Phenotypic variance: $$(\sigma_p^2) = \sigma_g^2 + \sigma_e^2$$
Where, $MS_g$ and $MS_e$ are mean squares due to genotype and error, respectively, and ‘r’ is the number of replication.

**Coefficient of variation:** The phenotypic and genotypic coefficients of variation for different characters were estimated as follows.

\[
\text{Phenotypic coefficient of variation (PCV)} = \frac{\sigma_p}{\bar{X}} \times 100
\]

\[
\text{Genotypic coefficient of variation (GCV)} = \frac{\sigma_g}{\bar{X}} \times 100
\]

Where, $\sigma_p$ & $\sigma_g$ are square root of phenotypic and genotypic variance, respectively and $\bar{X}$ is grand mean for the character.

**Heritability:** Estimation of heritability (in broad sense) of different characters was done by the following formula using the components of variance as follows.

\[
\text{Heritability } (h^2) = \frac{\sigma_g^2}{\sigma_p^2}
\]

**Genetic advance (GA):** The expected genetic advance or genetic gain as a result of selection at 5 % selection intensity among the genotypes for different characters was calculated as follows:

\[
\text{GA} = k \cdot h \cdot \sigma_g = k \cdot h^2 \cdot \sigma_p
\]

Where, $k = \text{standardized selection differential for specified selection intensity (k = 2.06 at 5 % selection intensity)}$
h = heritability coefficient = $\sigma_g / \sigma_p$

$\sigma_g =$ square root of genotypic variance

$\sigma_p =$ square root of phenotypic variance

For comparison of GA of different characters, GA was expressed as percentage of mean of the characters.

$$GA \text{ (as } % \text{ of mean)} = \frac{GA}{Mean} \times 100$$

3.3.3 Estimation of genotypic and phenotypic correlation

The analyses of co-variance between all possible pair of 12 characters were done with plot means as in Table 2. The symbols and contents with respect to the components of co-variance in mean sum of products are analogous to that of mean squares and the components of variance as described earlier.

**Table 2. Analysis of co-variance in RBD with expectation of mean sum of products (MSP)**

<table>
<thead>
<tr>
<th>Source</th>
<th>Df</th>
<th>MSP</th>
<th>Expectation of MSP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replication</td>
<td>(r-1)</td>
<td>MSP_r</td>
<td>$\sigma_e(x,y) + g \sigma_r(x,y)$</td>
</tr>
<tr>
<td>Genotype</td>
<td>(g-1)</td>
<td>MSP_g</td>
<td>$\sigma_e(x,y) + r \sigma_{gxy}$</td>
</tr>
<tr>
<td>Error</td>
<td>(r-1)(g-1)</td>
<td>MSP_e</td>
<td>$\sigma_e(x,y)$</td>
</tr>
</tbody>
</table>
The phenotypic, genotypic and error component of co-variance between two characters were estimated according to Al-Jibouri et al. (1958) in similar manner as described under the components of variance.

The variance and co-variance analysis were made for the characters based on the 29 genotypes as described earlier. Utilizing the various components of variance and co-variance, the genotypic and phenotypic correlations were computed according to Al-Jibouri et al. (1958) by following formula.

Genotypic correlation \( r_g \) =

\[
\frac{\sigma_{g(xy)}}{\sqrt{\sigma_{g(x)}^2 \sigma_{g(y)}^2}}
\]

Where, \( \sigma_{g(xy)} \) is the genotypic co-variance between x and y and \( \sigma_{g(x)}^2 \) and \( \sigma_{g(y)}^2 \) are the genotypic variance for the characters x and y, respectively.

Phenotypic correlation \( r_p \) =

\[
\frac{\sigma_{p(xy)}}{\sqrt{\sigma_{g(x)}^2 \sigma_{g(y)}^2}}
\]

Where, \( \sigma_{p(xy)} \) were phenotypic co-variance between x and y and \( \sigma_{p(x)}^2 \) and \( \sigma_{p(y)}^2 \) are phenotypic variance of x and y, respectively.

Significance of correlation co-efficient was tested by t-test with \((n - 2)\) degrees of freedom by the formula.

\[
t = \frac{r}{\left[\left(1 - r^2\right)/(n - 2)\right]^{1/2}}
\]

Where, ‘r’ is the correlation co-efficient and ‘n’ us the number of genotypes.
3.3.4 Path co-efficient analysis

The path co-efficient analysis gives cause and effect relationship among the various correlated characters. Path co-efficient are standardized partial regression co-efficient which individually provide measures of direct effect of each causal factor on the effect variable. It permits partitioning of the correlations between the causal factors and the effect variable into components of direct and indirect effects and thus, gives a better picture of the association of causal factors with the effect variable.

In the present investigation pod yield is taken as the ‘effect’ and 11 growth component characters related to yield as ‘causal factors’. Path co-efficient are obtained by solving simultaneous equations, which gives the basic relationship between correlations (Wright, 1921; Dewey and Lu, 1959).

\[
\begin{align*}
P_{1.12} + r_{1.2} P_{2.12} + r_{1.3} P_{3.12} + \ldots &+ r_{1.11} P_{11.12} = r_{1.12} \\
\end{align*}
\]

\[
\begin{align*}
r_{2.1} P_{1.12} + P_{2.12} + r_{2.3} P_{3.12} + \ldots &+ r_{2.11} P_{11.12} = r_{2.12} \\
\end{align*}
\]

\[
\begin{align*}
r_{11.1} P_{1.12} + r_{11.2} P_{2.12} + r_{11.3} P_{3.12} + \ldots &+ P_{11.12} = r_{11.12} \\
\end{align*}
\]

Where, \( r_{ij} \) is the correlation co-efficient between \( i^{th} \) and \( j^{th} \) character and \( P_{i.12} \) is the path co-efficient (direct effect) of \( i^{th} \) character on yield (\( 12^{th} \) character).
3.3.5 Analysis of genetic diversity among genotypes:

Genetic divergence analysis with regard to twelve quantitative traits among 29 groundnut genotypes was done by following two methods.

(i) \( D^2 \) analysis of genetic divergence

(ii) Canonical analysis

(i) \( D^2 \) analysis for genetic divergence

Twelve morpho-metric observations were recorded on five consecutive plants in each entry and replications and the mean values of 29 entries were used for the analysis of genetic divergence using Mahalonobis’s \( D^2 \) statistics.

Mahalanobis’ \( D^2 \) statistic (Rao, 1952) was used for estimation of genetic divergence among the 29 genotypes of groundnut for eleven characters. Genetic divergence \( (D^2) \) between any two genotypes is given by the formula.

\[
D_p^2 = \sum_{i=1}^{p} \sum_{j=1}^{p} w_{ij} d_i d_j
\]

where, \( w_{ij} \) is the inverse of the common dispersion matrix \( (w_{ij}) \), \( d_i \) and \( d_j \) are the difference in the means of the 2 genotypes for \( i^{th} \) and \( j^{th} \) characters.

The computation of \( D^2 \) using this formula is complicated and laborious when more number of mutually correlated characters is involved in the divergence analysis. So the characters means were transformed into set of uncorrelated variable using
pivotal condensation of common dispersion matrix following Rao (1952). After this transformation, the formula for genetic divergence becomes:

\[ D_p^2 = \sum_{i=1}^{n} d_i^2 \]

where, \(d_i\) is the difference between the transformed means of any two genotypes for the \(i^{th}\) character.

All possible \(D^2\) among the 29 genotypes were computed, the relative contribution of individual characters to divergence was assessed by (a) ranking of components \(D^2\) as well as by (b) percentage contribution to total \(D^2\) over all combinations.

(a) **Rank average:** In all the \(D^2\) combinations, the characters were ranked 1 to 11 on the basis of their contribution to the \(D^2\). Then ranks of each character are summed over all the \(D^2\) combinations to get rank total and then rank average is estimated.

(b) **Average \(D^2\):** Average contribution of each character to all the \(D^2\) combinations is worked out.

**Grouping of genotypes into different clusters**

**Tocher’s method:** Usually a cluster is defined as a group of genotypes or varieties or lines such that any two genotypes belonging to the same cluster, on an average, show a smaller \(D^2\) than those belonging to the different clusters. A simple device suggested by Tocher (Rao, 1952) for construction of clusters is to start with two most closely related genotypes (having the smallest \(D^2\)) and then find a third one
which has smaller average $D^2$ from the first two and so on. At certain stage when it is felt that after adding a particular population, there is a disrupt increase in the average $D^2$, this population is not added to cluster. Similarly, construction of 2nd and 3rd and other clusters are formed till all the genotypes are included in one or the other cluster. Singh and Choudhari (1977) suggested a method for determining cut off value for addition of a genotype/population to a cluster. In that the $D^2$ values of each genotype with all others are to be arranged from lowest to highest values in matrix form. The highest value of the lowest column is taken as cut off value for deciding on inclusion a genotype in the cluster. After construction of clusters, average intra-cluster and inter-cluster $D^2$ value were estimated.

(ii) Canonical analysis for genetic divergence:

Canonical analysis was done according to Anderson (1958). The divergences of 29 groundnut genotypes were represented in two – dimensional graph using first two canonical vectors ($Z_1$ and $Z_2$) as coordinates.

3.4 Molecular Technique-

A total of fourteen accessions of Groundnut ($Arachis hypogaea$ L.) collected from different parts of Odisha and were used for genetic diversity analysis in the present study.
3.4.1 Sample collection and crushing of leaves

Four to five young, fresh leaves of each accessions were collected, wrapped in aluminium foil and brought to the Department of Agricultural Biotechnology, OUAT, Bhubaneswar. These leaves were gently washed, and 2 gm. of leaves were weighed.

Crushing of leaves was done in a sterilized pestle and mortar in liquid nitrogen and care was taken to avoid thawing of the material. Before thawing, the material was transferred into 50 ml centrifuge tube and kept at -20ºC.

3.4.2 DNA extraction and purification

Extraction of total genomic DNA was carried out as described by Saghai-Maroof et al., (1984) with minor modifications to suit the material under consideration.

Extraction buffer (Annexure-II) in 2:1 ratio of plant material was added, which already maintained at 60ºC in a water bath. The contents were mixed vigorously and vortexed. Then it was incubated at 60ºC for 1 h in hot water bath. In the meanwhile 6-7 intermittent shaking were given for complete mixing and formation of emulsion. After the adding of equal volume of choloform : isoamyl alcohol (24:1) mixture was inverted for 8-10 min. After complete emulsion formation, centrifugation was done at 10,000 rpm for 20 min at 25ºC.

The aqueous phase was transferred to a fresh centrifuge tube and then double amount of isopropanol was added along with 300µl of 3M sodium acetate and allowed the DNA to settle down for overnight at -20ºC. The DNA was spooled out
with the help of sterile glass hook and kept in a sterile eppendorf tube. The excess amount of chemicals were drained out with a pipette. Ethyl alcohol (0.5 ml, 70% concentration) was added to wash the salt impurities and some other residues. The contents were mixed gently and incubated for 30 min. The contents were decanted by gentle spinning and then 70% ethyl alcohol was added and kept for 2 hours. The alcohol was decanted and the pellets were dried under vacuum dryer (layophilizer) at -40°C for 45 min. The DNA was dissolved in 300 µl of TE (10:1) buffer (pH 8.0) and kept overnight in refrigerator at 18°C for complete dissolution.

Further purification of DNA was made by adding 7µl RNase, and mixed it well subsequently and incubated at 37°C for 1 hr. Equal volume of phenol: chloroform: isoamyl alcohol (25:24:1) was added and mixed properly for 2 min and spin for 5 min at 8000 rpm. The DNA supernatant was taken out and again added equal volume of chloroform: isoamyl alcohol and mixed well and centrifuged for 10 min. The aqueous layer was removed and repeated the procedure to produce creamy colour solution. Then 1/10th amount of 3M sodium acetate and 2.5 volume of absolute chilled alcohol were added and mixed it gently so that DNA could precipitate down and kept overnight or for an hour at -20°C when precipitation was not observed.

The solution was then centrifuged at 8000 rpm for 5 min and the supernatant was decanted off. Extra salts were removed by two washing with 70% ethanol. Then the DNA was dried under vacuum, dissolved in TE (10:1) buffer at room temperature and stored frozen at -20°C.
3.4.3 Measurement of DNA concentration

The concentration of DNA was estimated by the measurement of the UV irradiation absorbed by nucleic acid bases. First the spectrophotometer was calibrated using 2000 µl of TE in a quartz cuvette at 260 nm and 280 nm. Then five µl of DNA sample was added to 1995 µl of TE, mixed well and absorbance (OD) was taken at 260 nm and 280 nm. The concentration of the DNA in the sample was estimated as follows:

\[
\text{Concentration of DNA (µg/ml) } = \text{OD at 260} \times \text{Dilution factor} \times 50
\]

3.4.4 DNA quality checking

The ratio between readings at 260 nm and 280 nm (OD\textsubscript{260} / OD\textsubscript{280}) provided an estimate for the purity of nucleic acid. Any sample showing the ratio below 1.8 or above 2.0 was further subjected to purification.

3.4.5 DNA Quantification

In order to know the contamination of genomic DNA with cellular proteins and/or RNA, an aliquot (2 µl) of each sample was subjected to agarose gel (0.8 % w/v) electrophores for about 2 h along with 500 ng of molecular weight marker (Lambda / EcoRI digest). The gel was stained with ethidium bromide (0.5µg/ml), viewed under UV Transilluminator and photographed immediately for further interpretation using a Gel-Doc system (UVITECH, UK). By comparing the fluorescent intensity of the bands with the standard, DNA concentration was estimated following the method described by Sambrook \textit{et al.}, (1989).
Further DNA concentration was estimated by using DNA quant 240 Flourimeter (BIORAD, Versafluor) using Hoechst 33258 (Bisbenzamide) solution as fluorescent dye and calf thymus DNA as the standard.

**Procedure of quantification**

I. Put out all DNA samples in water bath at 60ºC for 10 min, and remove from water bath and cool it at room temperature.

II. Turn on the flourimeter.

III. After 15 minutes set out the standard of 100ng/µl of calf thymus by adding 2µl of standard calf thymus DNA in 2ml of TNE buffer (Annexure - II).

IV. Recheck the standard by adding 2µl of calf thymus 100±5 reading indicate correct measurement of quantity of DNA in sample.

V. Then repeat the procedure by adding unknown DNA sample which gives directly concentration of DNA sample in ng/µl. Care should be taken as Hoechst-dye is carcinogen so better to use gloves.

**3.4.6 Dilution of DNA**

Part of the stock DNA samples were diluted with appropriate amount of TE buffer yield a working concentration of 10ng/µl and stored at 4ºC.

**3.4.7 Optimization of condition for PCR**

The procedure described by Williams *et al.* (1990) was used for carrying out polymerase chain reaction (PCR) with minor modification to produce RAPD profiles. The PCR mixture consisted of *Taq* DNA polymerase, PCR buffer, dNTPs,
MgCl₂, oligonucleotide primers and genomic DNA. Optimization of concentration of PCR components was carried out for MgCl₂, Taq DNA polymerase and genomic DNA concentration. To determine optimal amplification reaction conditions, a factorial experiment was carried out at three concentrations of MgCl₂ (2.0 mM, 2.5 mM and 3.0 mM), three concentrations of Taq DNA polymerase (0.5 U, 1.0 U, 1.5 U), three concentrations of template DNA (10 ng, 25 ng, 50 ng) and 10 pmole primer in a volume of 25 µl. PCR was carried out using Thermal Cycler (Bio-Rad, USA), PCR conditions that gave better amplified DNA profile were determined and presented in Table 3.

For RAPD analysis, all PCR were carried out in a final volume of 25 ml reaction mixture containing 50 ng template DNA, 200 µM each dNTPs, 2 mM MgCl₂, 10 pmoles primer, 1X Taq polymerase buffer and 1 unit of Taq DNA polymerase. The Thermal Cycler was programmed to include a pre-denaturation step at 94 °C for 3 min, followed by 45 cycles of denaturation at 94 °C for 1 min annealing at 37 °C for 1 min and extension at 72 °C for 2 min. The final extension was made for 7 min at 72 °C.
Table 3. PCR constituents optimized for RAPD analysis.

<table>
<thead>
<tr>
<th>Component</th>
<th>Stock</th>
<th>Quantity in µl</th>
<th>Final concentration in the reaction mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR Buffer with MgCl₂ (15mM)</td>
<td>10 x</td>
<td>2.5</td>
<td>1 x</td>
</tr>
<tr>
<td>dNTPs mix</td>
<td>10 mM</td>
<td>2.0</td>
<td>200 µM each</td>
</tr>
<tr>
<td>Taq DNA polymerase</td>
<td>I U/µl</td>
<td>1.0</td>
<td>1 unit</td>
</tr>
<tr>
<td>RAPD Primers</td>
<td>250 pM</td>
<td>4.0</td>
<td>10 pmoles</td>
</tr>
<tr>
<td>Sterile DNase, RNase free water</td>
<td>-</td>
<td>13.5</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>-</td>
<td>23.0</td>
<td>-</td>
</tr>
<tr>
<td>Template DNA</td>
<td>-</td>
<td>2.0</td>
<td>30 ng</td>
</tr>
</tbody>
</table>

3.4.8 Thermocycler conditions for RAPD

I. Pre-denaturation at 94°C for 5min.

II. Fifty Five cycles of denaturation, annealing and extension was as follow

<table>
<thead>
<tr>
<th>55 cycles</th>
<th>Temp °C</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Denaturation</td>
<td>94°C</td>
<td>1min</td>
</tr>
<tr>
<td>Annealing</td>
<td>37°C</td>
<td>1min</td>
</tr>
<tr>
<td>Extension</td>
<td>72°C</td>
<td>2min</td>
</tr>
</tbody>
</table>
III. Followed by final extension at 72°C for 15 min.

3.4.9 PCR Reaction mix (Master Mix)

Total volume of PCR reaction mix was made to 25µl of which 23.0µl was master and remaining 2µl was the individual DNA of the genotype.

3.4.10 Primer screening for RAPD

RAPD primers used in the present investigation were purchased from Operon Technology Inc (Alameda, USA) as well as Bangalore Genei Pvt. Ltd (Bangalore, India). Initially, RAPD primers were screened for RAPD reactions with the DNA of 15 selected groundnut genotypes. On the basis of screening, primers that resulted in discrete, reproducible well separated bands on agarose gel were selected for final amplification for all genotypes.

3.4.11 Agarose gel electrophoresis

Agarose gel (1.5%) was prepared by mixing 4.5 g of agarose in 300 ml of 1X TBE buffer. The content was boiled in microwave oven till it completely dissolved. During warming intermittent shaking was made (4-5 times) to prevent formation of clumps of agarose. The molten agarose was kept for cooling up to 50-60 °C and then ethidium bromide (1 µg/ml) was added. The molten agarose was poured into the clean, leveled casting plate containing 20 well combs and was kept for solidification. The gel was transferred to the electrophoresis unit containing 1X TBE buffer. The PCR reaction products were first mixed with 2.0 µl of loading dye (Appendix II) and spun for a while before loading into the wells of the gels. The medium range ruler (Bangalore Genei) was also loaded in first and/or last well of
the gel to serve as standard molecular weight marker for determining the size of the amplified DNA fragments. The gel was run at 80 volts for 4 h. The run was stopped when bromophenol blue dye had travelled 2/3rd length of the gel.

**Table 4. Primers used for RAPD analysis.**

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Primer</th>
<th>Sequence 5’-3’</th>
<th>GC content (%)</th>
<th>*Ta (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>OPW-1</td>
<td>CTCAGTGTCCTCC</td>
<td>60</td>
<td>37 °C</td>
</tr>
<tr>
<td>2</td>
<td>OPE-19</td>
<td>ACGGCCTATG</td>
<td>60</td>
<td>37 °C</td>
</tr>
<tr>
<td>3</td>
<td>OPW-8</td>
<td>GACTGCCTCT</td>
<td>60</td>
<td>37 °C</td>
</tr>
</tbody>
</table>

**3.4.12 Staining DNA**

After completion of electrophoresis, the gel was transferred to the staining tray which contained 10µl EtBr per 100 ml distilled water. Then required amount of water was added and kept for 30-40 min on the shaker for staining. To remove the excess stain from the gel, destaining was done using distilled water.

**3.4.13 Gel photography**

After electrophoresis, the stained DNA gel was visualized under UV light in a Gel-Doc system (Bio-Rad, USA) and photographed. Fragment size of all amplification products were estimated from the gel by comparison with standard
molecular weight marker (Medium Range Ruler). A pair-wise matrix of similarity was determined from the band data using Jaccard's similarity coefficient.

### 3.4.14 Scoring of gel data and its analysis

Scoring of amplification product was done by ‘1’ if band is present and ‘0’ if band is absent. Homology of bands was based on the distance of migration of amplified DNA fragments according to their molecular weights in the gel. Each amplification product was considered as single molecular marker and was scored across all samples.

### 3.4.15 Percent of polymorphic Loci

A locus was defined as polymorphic when the frequency of marker (allele) was < 1.0.

\[
\text{Percent polymorphic Loci} = \frac{\text{Number of polymorphic bands}}{\text{Total number of bands compared}} \times 100
\]

### 3.4.16 Similarity coefficient

For similarity coefficient selected genotype was compared with the rest of genotypes. Greater the value of coefficient, compared variety will be more similar to selected variety. In addition to this the value of coefficient also signifies about the extent of similarity between the two varieties. Jaccard's similarity coefficient was calculated according to Jaccard (1908) as follows;

\[
\text{Jaccard's similarity Coefficient} = \frac{n_{xy}}{n_1 - n_2}
\]

\(n_{xy} = \text{Number of bands common in sample a and b}\)
\[ n_1 = \text{Total number of bands present in all samples} \]

\[ n_2 = \text{Number of bands not present in sample a or b but found in other samples} \]

The similarity matrix was subjected to generate a dendrogram using software programme NTSYS pc Ver 2.1. Exeter Software, N.Y. (Rohlf, 2005).

### 3.4.17 Principal Component Analysis (PCA)

The Jaccard’s Similarity matrix was subjected to principal component analysis. It is an ordination technique that produces in visual representation of the relative position of genotypes in a space of reduced dimensions, thus indicating spatial relationships among genotypes. PCA entails transforming original variables \( X_1, X_2, \ldots, X_p \) into new variables \( Y_1, Y_2, \ldots, Y_p \) such that the new variables are “uncorrelated with each other and account for decreasing portions of the variance of the original variables”. The new variables are called ‘principal components’ and are estimated from the eigenvectors of covariance or correlation matrices of the original variable of the similarity matrix by using software programme NTSYS pc Ver 2.1. Exeter Software, N.Y. (Rohlf, 2005). The coordination method makes the use of multidimensional solution of the observed relationship.
3.4.18 Primer Efficiency

Polymorphic information content (PIC)

Most informative primers were selected based on the extent of polymorphism. The polymorphic information content (PIC) was calculated by applying the formula given by Powell et al. (1997).

\[
\text{PIC} = 1 - \sum_{i=1}^{n} f_i^2
\]

Where \( f_i \) is the frequency of the \( i^{th} \) allele and the summation extends over \( n \) alleles.
RESULTS AND DISCUSSION

The present investigation on “Studies on genetic diversity of groundnut (Arachis hypogaea L.) using morphological and molecular markers” was undertaken in the Department of Plant Breeding and Genetics, College of Agriculture, Orissa University of Agriculture and Technology, Bhubaneswar. The twenty nine genotypes were evaluated comprised of 27 advance breeding lines and 2 high yielding standard varieties. Field trial was conducted in RBD with three replications at EB-II during Rabi season of 2012-13.

Observations on twelve quantitative characters like days to 50 % flowering, plant height (cm), number of branches per plant, number of pods per plant, number of kernels per plant, 100 kernel weight (g), sound mature kernel (%), shelling percentage, harvest index (%), haulm yield per plant (g), kernel yield per plant (g) and pod yield per plant (g) were recorded from the field trial. The data were analyzed to get information on genetic parameters of yield and its components. Correlations among traits, direct and indirect effects of component traits on yield were estimated for selection of promising lines. Based on the genetic divergence the genotypes were grouped into different clusters. It would help in selection of parents for hybridization programme.

Moreover, assessment of genetic diversity of fourteen accessions of groundnut was carried out on the basis of polymorphic banding pattern at Department of Agricultural Biotechnology, OUAT, Bhubaneswar.
Table 5. Analysis of variance of twelve characters of groundnut in *rabi* season

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Character</th>
<th>Source</th>
<th>MSS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Days to 50% flowering</td>
<td>Replication</td>
<td>1.794</td>
<td>0.371</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Genotype</td>
<td>21.431**</td>
<td>4.438</td>
</tr>
<tr>
<td>2</td>
<td>Plant height (cm)</td>
<td>Replication</td>
<td>17.444</td>
<td>1.945</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Genotype</td>
<td>25.894**</td>
<td>2.887</td>
</tr>
<tr>
<td>3</td>
<td>No of branches/ plant</td>
<td>Replication</td>
<td>8.985**</td>
<td>25.405</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Genotype</td>
<td>1.194**</td>
<td>3.375</td>
</tr>
<tr>
<td>4</td>
<td>No. of pods/ plant</td>
<td>Replication</td>
<td>2.417</td>
<td>1.626</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Genotype</td>
<td>8.643**</td>
<td>5.816</td>
</tr>
<tr>
<td>5</td>
<td>Sound mature kernel(%)</td>
<td>Replication</td>
<td>3.597</td>
<td>0.152</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Genotype</td>
<td>41.516*</td>
<td>1.750</td>
</tr>
<tr>
<td>6</td>
<td>100 kernel weight(g)</td>
<td>Replication</td>
<td>0.641</td>
<td>0.178</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Genotype</td>
<td>83.797**</td>
<td>23.286</td>
</tr>
<tr>
<td>7</td>
<td>No. of kernels/ plant</td>
<td>Replication</td>
<td>5.454</td>
<td>1.409</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Genotype</td>
<td>16.346**</td>
<td>4.223</td>
</tr>
<tr>
<td>8</td>
<td>Kernel yield/ plant (g)</td>
<td>Replication</td>
<td>1.853</td>
<td>1.148</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Genotype</td>
<td>4.683**</td>
<td>2.902</td>
</tr>
<tr>
<td>9</td>
<td>Shelling (%)</td>
<td>Replication</td>
<td>1.048</td>
<td>0.151</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Genotype</td>
<td>105.467**</td>
<td>15.226</td>
</tr>
<tr>
<td>10</td>
<td>Haulm yield/ plant (g)</td>
<td>Replication</td>
<td>0.591</td>
<td>0.100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Genotype</td>
<td>9.614</td>
<td>1.635</td>
</tr>
<tr>
<td>11</td>
<td>Harvest index (%)</td>
<td>Replication</td>
<td>5.709</td>
<td>1.323</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Genotype</td>
<td>38.220**</td>
<td>8.859</td>
</tr>
<tr>
<td>12</td>
<td>Pod yield/ plant (g)</td>
<td>Replication</td>
<td>1.615</td>
<td>1.994</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Genotype</td>
<td>10.001**</td>
<td>12.349</td>
</tr>
</tbody>
</table>

*Significant at 5 % level, **Significant at 1 % level
The 27 breeding lines with 2 released varieties showed wide variation in all the 12 traits including yield. The genotypic source of variations for all the twelve characters were highly significant except haulm yield per plant at 1% level of significance (Table 5). The replication source of variation was highly significant for only number of branches per plant at 1% level of significance.

4.1 Mean performance of the genotypes

Mean performance of 29 genotypes for all the twelve morphometric traits is presented in the table 6 for rabi season. The accessions GPBD 5 and DhS 102 were late where as UG 5 and AG 2240 were early, exhibiting 36.67-37.33 and 27.67 days respectively in days to 50% flowering. ICGV 9921 was the tallest plant with 26.53 cm in plant height. K 1371 was observed to be short in plant height of 13.53 cm. The number of branches per plant varied from 3.47 in DRT 53 to 7.0 in TCGS 159. Highest number of pods per plant and number of kernels per plant were observed in UG 3. DRT 53 exhibited lowest number of pods per plant (9.07) and JL 575 exhibited lowest number of kernels per plant (15.20). The hundred kernel weight of test entries ranged from 34.87 g in JNDB 14 to 54.85 g in Dh 206. The test genotypes exhibited sound matured kernel per cent minimum of 82.98 % in UG 3 and maximum of 98.84% in CSMG 2014. Highest shelling percentage (84.00 %) was observed in KGN 34 and UG 5 and the lowest 63.67 % was observed in R 8892. Similar extent of variation was observed in 32 genotypes of both spreading and bunch type by Singh et al. (2010), 53-87% in shelling, 11-27% in harvest index and 245-594 gm. in thousand seed weight. The harvest index ranged from 25.66% (AG 2006-15) to 36.65 % (Dh 108). The lowest haulm yield per plant (19.57 g) was
observed in K 1371. The haulm yield per plant was highest (28.87 g) in JL 575. Dh 108 was highest in both kernel yield per plant (10.49 g) and pod yield per plant (14.74 g) per plant. Both the lowest kernel yield (5.86 g) and lowest pod yield (7.54 g) per plant was observed in K1371. This degree of variation in seed yield and its component characters provide an interesting source which could perhaps be exploited to develop improved varieties.
Table 6. Mean performance of the groundnut genotypes for twelve characters in *rabi* season

<table>
<thead>
<tr>
<th>Characters</th>
<th>Days to 50% flowering</th>
<th>Plant height (cm)</th>
<th>No. of branches/plant</th>
<th>No. of pods/plant</th>
<th>Sound mature kernel (%)</th>
<th>100 kernel weight (g)</th>
<th>No. of kernels/plant</th>
<th>Kernel yield/plant (g)</th>
<th>Shelling (%)</th>
<th>Haulm yield/plant (%)</th>
<th>Harvest index (%)</th>
<th>Pod yield/plant (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DhS-102</td>
<td>37.33</td>
<td>21.93</td>
<td>5.13</td>
<td>15.80</td>
<td>92.96</td>
<td>37.36</td>
<td>20.60</td>
<td>7.69</td>
<td>76.00</td>
<td>27.87</td>
<td>26.00</td>
<td>9.84</td>
</tr>
<tr>
<td>Dh-8</td>
<td>33.33</td>
<td>26.23</td>
<td>4.80</td>
<td>12.73</td>
<td>93.51</td>
<td>47.19</td>
<td>21.33</td>
<td>10.04</td>
<td>75.67</td>
<td>25.00</td>
<td>35.30</td>
<td>13.60</td>
</tr>
<tr>
<td>Dh-216-1</td>
<td>35.33</td>
<td>20.87</td>
<td>4.67</td>
<td>11.93</td>
<td>96.36</td>
<td>39.19</td>
<td>20.40</td>
<td>8.11</td>
<td>75.33</td>
<td>26.23</td>
<td>28.20</td>
<td>10.30</td>
</tr>
<tr>
<td>Dh-209</td>
<td>33.00</td>
<td>24.40</td>
<td>5.33</td>
<td>14.80</td>
<td>93.69</td>
<td>41.37</td>
<td>23.40</td>
<td>9.70</td>
<td>76.33</td>
<td>23.67</td>
<td>35.01</td>
<td>12.75</td>
</tr>
<tr>
<td>Dh-206</td>
<td>34.33</td>
<td>23.27</td>
<td>4.27</td>
<td>11.20</td>
<td>94.68</td>
<td>54.85</td>
<td>17.47</td>
<td>9.59</td>
<td>73.33</td>
<td>23.80</td>
<td>35.43</td>
<td>13.05</td>
</tr>
<tr>
<td>Dh-204</td>
<td>29.67</td>
<td>20.80</td>
<td>5.00</td>
<td>11.53</td>
<td>94.75</td>
<td>45.37</td>
<td>18.20</td>
<td>8.31</td>
<td>80.00</td>
<td>24.53</td>
<td>30.51</td>
<td>10.76</td>
</tr>
<tr>
<td>TCGS-159</td>
<td>32.68</td>
<td>22.53</td>
<td>7.00</td>
<td>13.60</td>
<td>88.79</td>
<td>49.64</td>
<td>21.33</td>
<td>10.30</td>
<td>78.67</td>
<td>25.00</td>
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<th>100 kernel weight (g)</th>
<th>No. of kernels/plant</th>
<th>Kernel yield/plant (g)</th>
<th>Shelling (%)</th>
<th>Haulm yield/plant (g)</th>
<th>Harvest index (%)</th>
<th>Pod yield/plant (g)</th>
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4.2 Variability, heritability and genetic advance

The genetic parameters of morphological characters in groundnut during *rabi* seasons are presented in table 7. Wide range of variation was recorded for all the characters. The P.C.V. and G.C.V. estimates were high for plant height, number of branches per plant, number of pods per plant, number of kernels per plant, hundred kernel weight, harvest index percentage, kernel yield per plant and pod yield per plant. John (2005) reported high genotypic and phenotypic coefficients of variation for pod yield per plant in 22 genotypes of groundnut grown during *rabi* season. In a study of three F₂ cross derivatives and their four parents, Shoba *et al.* (2009) reported high mean performance, G.C.V. and P.C.V. values for yield and its contributing characters in the cross TMV 2 X COG 0437. High magnitude of G.C.V. and P.C.V for pods per plant, kernel yield per plant and pod yield per plant was reported in fifty elite genotypes of bunch groundnut evaluated in summer season by Meta and Monpara (2010). The high magnitude of genotypic coefficient of variation revealed greater extent of variability present in these characters, there by suggesting ample scope for improvement through selection among these advance breeding lines. However, Sudhir kumar *et al.* (2008) reported moderate estimates of P.C.V. and G.C.V. for plant height, pods per plant, pod yield per plant, kernel yield per plant and harvest index.
Table 7. Genetic parameters of the morphological characters in groundnut during *rabi* season

<table>
<thead>
<tr>
<th>Character</th>
<th>Range</th>
<th>Mean</th>
<th>PCV (%)</th>
<th>GCV (%)</th>
<th>$h^2$ (%)</th>
<th>GA (% of mean)</th>
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<td>Days to 50% flowering</td>
<td>27.67-37.33</td>
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<td>17.70</td>
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<td>38.61</td>
<td>12.03</td>
</tr>
<tr>
<td>No of branches/plant</td>
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<td>4.653</td>
<td>17.11</td>
<td>11.37</td>
<td>44.19</td>
<td>13.31</td>
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<tr>
<td>Sound mature kernel(%)</td>
<td>82.98-98.84</td>
<td>92.383</td>
<td>5.89</td>
<td>2.64</td>
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<td>100 kernel weight (g)</td>
<td>34.87-54.85</td>
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<td>88.14</td>
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<td>No. of kernels/plant</td>
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<td>Kernel yield/plant (g)</td>
<td>5.86-10.49</td>
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<td>18.95</td>
<td>11.80</td>
<td>38.81</td>
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<tr>
<td>Shelling (%)</td>
<td>63.67-84.00</td>
<td>74.184</td>
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<td>7.73</td>
<td>82.58</td>
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<td>Haulm yield/plant (g)</td>
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<td>24.489</td>
<td>10.90</td>
<td>4.56</td>
<td>17.47</td>
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<tr>
<td>Harvest index(%)</td>
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<td>31.710</td>
<td>12.46</td>
<td>10.60</td>
<td>72.37</td>
<td>15.87</td>
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<tr>
<td>Pod yield/plant (g)</td>
<td>7.54-14.74</td>
<td>11.412</td>
<td>17.25</td>
<td>15.34</td>
<td>79.09</td>
<td>24.01</td>
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Low values of G.C.V. was observed in days to 50% flowering, sound mature kernel percentage, shelling percentage and haulm yield per plant indicated the need to create variability either by hybridization or mutation followed by selection. Low G.C.V. estimates for shelling percent was also reported by Pradhan and Patra (2011) in a study taking 460 genotypes of groundnut germplasm in four different seasons for pod yield and yield component characters.

In the current study, high heritability estimates were observed for 100 kernel weight, shelling percentage, pod yield per plant, harvest index and number of pods per plant indicating less influence of environment on these characters. Johnson et al. (1955) reported that heritability estimates along with genetic gain would be more useful than the former alone in predicting the effectiveness of selecting the best individual. Therefore it is essential to consider the predicted genetic advance along with heritability estimate as a tool in the selection programme for better efficiency. In the present study, along with high heritability, high genetic advance as percentage of mean has been noticed for pod yield per plant, hundred kernel weight, number of pods per plant and harvest index. Therefore it was clear that these four traits were less influenced by the environmental changes due to the presence of additive gene action in their expressions. Hence improvement of these traits would be more effective through the selection. High heritability coupled with high genetic advance was noticed by Sudhir Kumar et al. (2008) for all yield and its component characters except days to fifty percent flowering and sound mature kernel percent. In sixty genotypes of
groundnut received from ICRISAT, Hyderabad, John et al. (2009) reported high heritability along with high genetic advance as percent of mean for shelling percent, 100 kernel weight, total number of pods and pod yield. This showed additive type of gene action and indicated phenotypic selection to be effective for pod yield per plant, hundred kernel weight number of pods per plant and harvest index. Heritability and genetic advance as percent of mean were moderate for days to 50% flowering, number of kernels per plant, number of branches per plant, kernel yield per plant and plant height. High heritability along with high genetic advance as percent of mean for number of branches per plant was observed by Korat et al. (2009). For number of branches per plant, John K. et al. (2012) reported low heritability and moderate genetic advance as per cent of mean whereas John et al. (2009) and Mohan Vishnuwardhan K. et al. (2013) observed high GCV accompanied by high heritability and high genetic advance as per cent of mean. This indicated predominant role of additive gene action and amenability for phenotypic selection of these traits. Low genetic advance along with low heritability estimate for sound mature kernel percent, and haulm yield per plant was observed in present study. Similar result was also reported by Mohan Vishnuwardhan K. et al. (2013) for shelling percentage and harvest index. This indicated the additive and non additive gene actions that have a role in their inheritance and phenotypic selection would be effective to some extent.

In a field study conducted for two seasons under irrigated conditions using forty nine varieties released during 1905-2002, Rathnakumar et al. (2010) reported the annual increments of 9.4 kg/ha and 6.2 kg/ha in pod and kernel
yields respectively. The enhanced pod yield has resulted mainly from improvements in number of pods per plant, pod weight and seed weight. Improvement in shelling and sound mature kernel percent was not significant. This may be due to low genetic advance along with low heritability estimate for sound mature kernel percent and haulm yield per plant as observed in present study.

4.3. Phenotypic and genotypic correlation among traits

Out of the 66 correlation coefficients among the 12 traits, 24 correlation coefficients were significant at phenotypic level (table 8) whereas 25 correlation coefficients were significant at genotypic level. In general, the values of genotypic correlation ($r_g$) were higher than their corresponding phenotypic correlation ($r_p$) indicating that there was high degree of association between two variables at genotypic level. Its phenotypic expression was deflated by the influence of environment, pointing out the possibilities of effective phenotypic selection. Pod yield per plant and kernel yield per plant exhibited significant positive correlation and both these traits also exhibited significant positive correlation with plant height, number of pods per plant, 100 kernel weight, number of kernels per plant and harvest index at both phenotypic and genotypic level. This indicated the strong association of harvest index, number of kernel per plant and number of pods per plant with pod yield and kernel yield per plant in rabi season. Babariya and Dobariya (2012) observed similar association of pod yield per plant with other yield contributing characters. Both phenotypic and
Table 8. Phenotypic correlation coefficients ($r_p$) among the characters in groundnut in *rabi* season

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</table>


*Significance at 5 % level, **Significant at 1 % level
genotypic correlation coefficient for number of kernels per plant were highly significant and positively correlated with plant height, number of pods per plant, kernel yield per plant, harvest index and pod yield per plant. Sound mature kernel percentage was significant and negatively correlated with number of kernels per plant, kernel yield per plant, harvest index percentage and pod yield per plant only genotypic levels. Number of pods per plant was significant and positively correlated with plant height, number of branches per plant, number of kernels per plant, kernel yield per plant and pod yield per plant both at phenotypic and genotypic levels. Number of branches per plant exhibited significant positive correlation with number of pods per plant, number of kernels per plant, kernel yield per plant and pod yield per plant at phenotypic level. Plant height was significant and positively correlated with number of pods per plant, number of kernels per plant, kernel yield per plant and pod yield per plant both at phenotypic and genotypic level. Hundred kernel weight was significant and positively correlated with kernel yield per plant, harvest index percentage and pod yield per plant both at phenotypic and genotypic level. Sumathi et al. (2007) and Dhaliwal et al. (2010) reported significant positive association for pod yield per plant with kernel yield and 100 seed weight both at genotypic and phenotypic level that supports the present finding. Both kernel yield and 100 seed weight were also correlated at both phenotypic and genotypic level. Positive and significant association between 100 kernel weight and pod yield per plant was reported by Korat et al. (2010) at phenotypic level. In contrast to this, days to 50% flowering did not show any significant correlation with pod yield and other
yield contributing characters. From this studies, it can be concluded that number of pods per plant and number of kernels per plant are two most important morphological traits for selecting better genotypes with high kernel yield and pod yield per plant. The highest phenotypic correlation as well as genotypic correlation was observed between kernel yield per plant and pod yield per plant. But the sound mature kernel percent is negatively correlated with number of kernels per plant, harvest index and both pod and kernel yield per plant.

4.4 Path co-efficient analysis

The correlation of pod yield per plant was further analysed by the method of path coefficient analysis based on phenotypic correlation. Correlation of yield with other characters were partitioned into components of direct and indirect effects to know the nature and relative importance of the components in determining pod yield (table 9). The present path coefficient analysis showed low residual effect (0.089) during rabi indicating that most of the major yield components were included in the study. Harvest index percentage had the highest direct positive effects on pod yield per plant followed by haulm yield per plant. Also kernel yield per plant, hundred kernel weight, number of pods per plant and plant height were observed to be the major indirect contributor towards pod yield through harvest index percentage. Present study thus indicated that prime emphasis should be given to harvest index percentage followed by number of kernels per plant and kernel yield per plant. Dhaliwal et al. (2010) also observed
Table 9. Direct and indirect effects of component traits on pod yield in groundnut

<table>
<thead>
<tr>
<th>Characters</th>
<th>Days to 50% flowering</th>
<th>Plant height (cm)</th>
<th>No of branches/plant</th>
<th>No. of pods/plant</th>
<th>Sound mature kernel (%)</th>
<th>100 kernel weight (g)</th>
<th>No. of kernels/plant</th>
<th>Kernel yield/plant (g)</th>
<th>Shelling (%)</th>
<th>Haulm yield/plant(g)</th>
<th>Harvest index (%)</th>
<th>Pod yield/plant(g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days to 50% flowering</td>
<td>0.015</td>
<td>-0.001</td>
<td>-0.002</td>
<td>0.000</td>
<td>0.005</td>
<td>0.002</td>
<td>0.002</td>
<td>0.010</td>
<td>-0.001</td>
<td>0.078</td>
<td>0.055</td>
<td>0.163</td>
</tr>
<tr>
<td>Plant height (cm)</td>
<td>-0.001</td>
<td><strong>0.027</strong></td>
<td>-0.005</td>
<td>0.000</td>
<td>0.001</td>
<td>0.003</td>
<td>0.010</td>
<td>0.041</td>
<td>0.005</td>
<td>0.185</td>
<td>0.313</td>
<td>0.580</td>
</tr>
<tr>
<td>No. of branches/plant</td>
<td>0.001</td>
<td>0.006</td>
<td><strong>-0.020</strong></td>
<td>0.000</td>
<td>0.004</td>
<td>0.001</td>
<td>0.009</td>
<td>0.034</td>
<td>0.006</td>
<td>0.128</td>
<td>0.206</td>
<td>0.377</td>
</tr>
<tr>
<td>No. of pods/plant</td>
<td>0.002</td>
<td>0.013</td>
<td>-0.009</td>
<td><strong>0.001</strong></td>
<td>0.006</td>
<td>-0.004</td>
<td>0.016</td>
<td>0.043</td>
<td>0.007</td>
<td>0.148</td>
<td>0.331</td>
<td>0.553</td>
</tr>
<tr>
<td>Sound mature kernel (%)</td>
<td>-0.002</td>
<td>-0.001</td>
<td>0.003</td>
<td>0.000</td>
<td><strong>-0.029</strong></td>
<td>0.000</td>
<td>-0.003</td>
<td>-0.009</td>
<td>0.003</td>
<td>-0.102</td>
<td>-0.068</td>
<td>-0.209</td>
</tr>
<tr>
<td>100 kernel weight (g)</td>
<td>0.002</td>
<td>0.005</td>
<td>-0.001</td>
<td>0.000</td>
<td>0.000</td>
<td><strong>0.018</strong></td>
<td>-0.003</td>
<td>0.033</td>
<td>0.001</td>
<td>-0.031</td>
<td>0.568</td>
<td>0.592</td>
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<tr>
<td>No. of kernels/plant</td>
<td>0.001</td>
<td>0.014</td>
<td>-0.010</td>
<td>0.001</td>
<td>0.004</td>
<td>-0.003</td>
<td><strong>0.019</strong></td>
<td>0.055</td>
<td>0.009</td>
<td>0.141</td>
<td>0.388</td>
<td>0.620</td>
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<tr>
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<td>0.002</td>
<td>0.015</td>
<td>-0.009</td>
<td>0.000</td>
<td>0.004</td>
<td>0.008</td>
<td>0.014</td>
<td><strong>0.074</strong></td>
<td>0.009</td>
<td>0.136</td>
<td>0.629</td>
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<tr>
<td><strong>Kernel yield/plant (g)</strong></td>
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</tr>
<tr>
<td>Shelling (%)</td>
<td>-0.001</td>
<td>0.007</td>
<td>-0.007</td>
<td>0.000</td>
<td>-0.004</td>
<td>0.001</td>
<td>0.009</td>
<td>0.036</td>
<td><strong>0.018</strong></td>
<td>0.144</td>
<td>0.183</td>
<td>0.389</td>
</tr>
<tr>
<td>Haulm yield/plant(g)</td>
<td>0.002</td>
<td>0.010</td>
<td>-0.005</td>
<td>0.000</td>
<td>0.006</td>
<td>-0.001</td>
<td>0.006</td>
<td>0.021</td>
<td>0.005</td>
<td><strong>0.484</strong></td>
<td>-0.325</td>
<td>0.204</td>
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<tr>
<td>Harvest index (%)</td>
<td>0.001</td>
<td>0.009</td>
<td>-0.004</td>
<td>0.000</td>
<td>0.002</td>
<td>0.011</td>
<td>0.008</td>
<td>0.050</td>
<td>0.004</td>
<td>-0.169</td>
<td><strong>0.930</strong></td>
<td>0.841</td>
</tr>
</tbody>
</table>

**Residual effect = 0.089**

The bold values in diagonal indicates direct effects.
high positive direct contribution of kernel yield per plant to the pod yield. This finding supports the work of Bera et al. (2010) reported positive direct contribution of harvest index to the seed yield irrespective of location and years effect.

4.5 $D^2$ analysis or genetic divergence

The analysis of variance revealed significant differences among the genotypes for all the characters under study there by indicating the presence of ample variability among the genotypes. On the basis of magnitude of $D^2$ values, all the 29 genotypes of groundnut for 12 characters, showed that the generalized distance ($D^2$) between two populations varied from 9.044 (ALG 234 and AG 2006-15) to 640.258 (ALG 234 and GPBD 5) which were indicators of considerable diversity available in the material evaluated. The smallest $D^2$ estimate (9.044) was observed between ALG 234 and AG 2006-15, so these genotypes were much similar in many traits. The largest $D^2$ estimate (640.258) was obtained between ALG 234 and GPBD 5, which indicated the maximum diversity. In the present study the advance breeding lines showed considerable amount of diversity for the morphological traits. The genetic diversity study among the cultivated groundnut accessions based on morphological trial in one or more seasons was studied by Upadhyaya (2003), Mahalaxmi et al. (2005), Upadhyaya et al. (2006), Laxmidevamma et al. (2006), Aalami et al. (2007), Khote et al. (2010), Dolma et al. (2010), Venkataravana et al. (2010), Sudhir Kumar et al. (2010), Upadhyaya et al. (2011) and Sonone et al. (2011). It revealed wide range of $D^2$ values suggesting the presence of considerable amount
of genetic diversity in the genotypes studied which were grouped into several clusters. Genetic relationships among cultivated and wild accessions of groundnut were studied based on microsatellite markers or SSR (Simple Sequence Repeat) revealed that cultivated groundnut presents a relatively reduced variation at the DNA level (Moretzsohn, 2004 and Tang et al. 2007). Similar little variation has also been detected at the DNA level using techniques such as RAPDs, AFLPs and RFLPs (Subramanian et al. 2000).

Relative contributions of 12 characters to $D^2$ among the genotypes were estimated by average $D^2$ (table 10). On the basis of average $D^2$, shelling percentage contributed maximum divergence followed by harvest index, kernel yield per plant, hundred kernel weight and all other characters. Sound mature kernel percent, Plant height, pod yield per plant, days to 50% flowering, number of branches per plant, haulm yield per plant, number of pods per plant and number of kernels per plant contributed least to $D^2$ estimates. Days to first flowering followed by shelling percentage contributed maximum to the total divergence as reported by Mahalaxmi et al. (2005). Laxmidevamma et al. (2006) observed test weight, days to maturity and oil content as the most potential traits contributing towards the total divergence. Sonone et al. (2011) observed contribution of various characters towards the expression of genetic divergence on pooled performance in twelve different environments indicated that pod length (25.6 %), plant height (15.1 %), shelling percent (11.7 %), seed weight (8 %) and number of kernels per plant (7.2 %) contributed maximum (67.5 %) towards total divergence in the material.
Table 10. Relative contribution of each character to genetic divergence in *rabi* seasons

<table>
<thead>
<tr>
<th>Character</th>
<th>Average $D^2$ value</th>
<th>% Contribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days to 50% flowering</td>
<td>2.959</td>
<td>1.711</td>
</tr>
<tr>
<td>Plant height (cm)</td>
<td>2.268</td>
<td>1.312</td>
</tr>
<tr>
<td>No of branches/plant</td>
<td>2.968</td>
<td>1.716</td>
</tr>
<tr>
<td>No of pods/plant</td>
<td>4.190</td>
<td>2.423</td>
</tr>
<tr>
<td>Sound mature kernel (%)</td>
<td>1.558</td>
<td>0.901</td>
</tr>
<tr>
<td>100 kernel weight (g)</td>
<td>18.503</td>
<td>10.700</td>
</tr>
<tr>
<td>No of kernels/plant</td>
<td>5.141</td>
<td>2.973</td>
</tr>
<tr>
<td>Kernel yield/plant (g)</td>
<td>22.065</td>
<td>12.759</td>
</tr>
<tr>
<td>Shelling %</td>
<td>73.455</td>
<td>42.476</td>
</tr>
<tr>
<td>Haulm yield/plant (g)</td>
<td>3.452</td>
<td>1.996</td>
</tr>
<tr>
<td>Harvest index (%)</td>
<td>33.787</td>
<td>19.538</td>
</tr>
<tr>
<td>Pod yield/plant (g)</td>
<td>2.589</td>
<td>1.497</td>
</tr>
</tbody>
</table>

N.B.: Figures in parentheses indicate the order of contribution to divergence
Table 11. Composition of genetic clusters using $D^2$ value

<table>
<thead>
<tr>
<th>Cluster</th>
<th>No. of genotype</th>
<th>Name of genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>6</td>
<td>ALG-234, AG-2006-15, KGN-34, Dh-204, UG-5, Dh-107.</td>
</tr>
<tr>
<td>2.</td>
<td>5</td>
<td>K-1371, Dh-216-2, DRT-53, Dh-209, GG-6.</td>
</tr>
<tr>
<td>4.</td>
<td>2</td>
<td>R-8892, GPBD-5.</td>
</tr>
<tr>
<td>5.</td>
<td>2</td>
<td>K-1336, Dh-216-1.</td>
</tr>
<tr>
<td>6.</td>
<td>2</td>
<td>OG-86-5-2, JNDB-14.</td>
</tr>
<tr>
<td>7.</td>
<td>1</td>
<td>UG-3.</td>
</tr>
<tr>
<td>8.</td>
<td>1</td>
<td>DhS-102.</td>
</tr>
</tbody>
</table>

On the basis of critical $D^2$ values (83.614), 29 genotypes were classified into 8 clusters (table 11). In the present study, advance breeding lines of major groundnut growing states of India were grouped into eight different clusters. This indicated the large diversity existing in the advance breeding lines of groundnut giving the opportunity for further improvement in groundnut. Thus recent
released varieties contain sufficient diversity though groundnut itself has narrow genetic base. Cluster III was the largest, accommodating as many as 10 genotypes and cluster VII and VIII was smallest with one genotype each. Cluster IV, V and VI possessing two genotypes each. Cluster I and II possessing six and five genotypes, respectively. Two genotypes (UG 3 and DhS 102) remained in isolated clusters. The clustering pattern of genotypes showed that the genotypes of different origins were clubbed into one cluster where as the genotypes belonging to same state or origin were grouped into different clusters indicating that the geographic distribution was not the sole criterion of genetic diversity. Murthy and Arunachalam (1966) also stated that the genetic drift and selection in different environments could cause greater diversity than geographic distance. Further the free exchange of genetic materials among the different regions consequently causes characters constellation because of the human interference and materials may lose its individuality. Intra cluster distance values were lower than the inter cluster distances indicating the genotypes included within the cluster tended to diverge less from each other.

Lowest intra-cluster distance was observed between cluster IV (24.933) (table 12). Highest intra-cluster distance was observed within cluster VI followed by cluster V. Inter cluster distance is the main criterion for selection of genotypes. Maximum inter-cluster distance was observed between cluster VI and cluster VIII followed by cluster VI and cluster VII. In this contest, the genotypes from cluster VI and VIII or cluster VI and VII could be selected as parents for hybridization. Cluster VIII exhibited high value of days to 50 % flowering,
number of branches per plant and haulm yield per plant (table 13). Cluster VII exhibited high value of plant height, number of pods per plant, number of kernels per plant, harvest index percentage and pod yield per plant. Cluster IV exhibited high value of hundred kernel weight. Cluster V exhibited high value of sound mature kernel percent. Cluster I exhibited high value of shelling percent. Cluster VI and IV exhibited high value of kernel yield per plant. Thus the advance breeding line ALG 234 from cluster I and GPBD 5 from cluster IV, exhibiting highest genetic diversity of 640.258 may be selected as parents in the future hybridization programme to give maximum high yielding segregates with desirable features.

4.6 Canonical analysis:

The two canonical roots accounted for 83.6% of the total variability, thus qualifying for graphic presentation (Table14). The values of the first two canonical vectors $Z_1$ and $Z_2$ were used as coordinates in plotting a two dimensional dispersion complex. The grouping obtained through $D^2$ analysis are super imposed on the two dimensional representation of the genotypes by canonical analysis (fig.2). The scattered points on the $Z_1$–$Z_2$ graph were broadly in agreement with the magnitude of divergence measured by $D^2$ statistic, thus very well corroborating the grouping by Tocher's method.
Table 12. Average intra and inter cluster $D^2$ values among clusters of groundnut genotypes

<table>
<thead>
<tr>
<th>Clusters</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
<th>VII</th>
<th>VIII</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>54.344</td>
<td>237.355</td>
<td>143.007</td>
<td>441.085</td>
<td>89.129</td>
<td>271.645</td>
<td>367.679</td>
<td>168.340</td>
</tr>
<tr>
<td>II</td>
<td>64.398</td>
<td>197.068</td>
<td>159.571</td>
<td>104.041</td>
<td>332.160</td>
<td>94.978</td>
<td>136.397</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td></td>
<td>63.218</td>
<td>192.986</td>
<td>105.234</td>
<td>89.534</td>
<td>308.857</td>
<td>280.113</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td></td>
<td></td>
<td>24.933</td>
<td>238.005</td>
<td>201.550</td>
<td>196.067</td>
<td>391.382</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td></td>
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<td>73.010</td>
<td>227.570</td>
<td>211.113</td>
<td>95.664</td>
<td></td>
</tr>
<tr>
<td>VI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>79.633</td>
<td>465.498</td>
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<tr>
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<td>0.000</td>
</tr>
</tbody>
</table>
Table 13. Cluster means of different characters of groundnut in *rabi* season.

<table>
<thead>
<tr>
<th>Clusters</th>
<th>Days to 50% flowering</th>
<th>Plant height (cm)</th>
<th>No. of branches/plant</th>
<th>No. of pods/plant</th>
<th>Sound mature kernel (%)</th>
<th>100 kernel weight (g)</th>
<th>No. of kernels/plant</th>
<th>Kernel yield/plant (g)</th>
<th>Shelling (%)</th>
<th>Haulm yield/plant (g)</th>
<th>Harvest index (%)</th>
<th>Pod yield/plant (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>30.45</td>
<td>21.00</td>
<td>4.67</td>
<td>12.20</td>
<td>93.65</td>
<td>42.00</td>
<td>19.66</td>
<td>8.30</td>
<td>80.72</td>
<td>24.94</td>
<td>29.74</td>
<td>10.64</td>
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<tr>
<td>II</td>
<td>30.93</td>
<td>19.22</td>
<td>4.41</td>
<td>12.00</td>
<td>91.91</td>
<td>40.40</td>
<td>18.72</td>
<td>7.56</td>
<td>67.47</td>
<td>23.21</td>
<td>29.65</td>
<td>9.86</td>
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<td>III</td>
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<td>48.49</td>
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<td>12.20</td>
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<td>92.96</td>
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<td>9.32</td>
<td>9.16</td>
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<td>74.00</td>
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<td>25.02</td>
<td>23.54</td>
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<td>29.48</td>
<td>35.28</td>
<td>35.57</td>
<td>26.10</td>
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<td></td>
<td>10.44</td>
<td>12.81</td>
<td>13.03</td>
<td>9.84</td>
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</table>
Table 14. The coefficient of first two canonical vectors (Z1 and Z2) for 12 characters of 29 groundnut genotypes

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Character</th>
<th>Z1</th>
<th>Z2</th>
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<tr>
<td>1</td>
<td>Days to 50% flowering</td>
<td>-0.0209</td>
<td>0.0592</td>
</tr>
<tr>
<td>2</td>
<td>Plant height (cm)</td>
<td>0.0031</td>
<td>0.0571</td>
</tr>
<tr>
<td>3</td>
<td>No of branches/plant</td>
<td>0.0089</td>
<td>-0.0135</td>
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<tr>
<td>4</td>
<td>No of pods/plant</td>
<td>-0.0850</td>
<td>-0.0897</td>
</tr>
<tr>
<td>5</td>
<td>Sound mature kernel (%)</td>
<td>0.0428</td>
<td>-0.0349</td>
</tr>
<tr>
<td>6</td>
<td>100 kernel weight (g)</td>
<td>0.1946</td>
<td>0.04733</td>
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<tr>
<td>7</td>
<td>No of kernels/plant</td>
<td>0.0291</td>
<td>-0.0012</td>
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<tr>
<td>8</td>
<td>Kernel yield/plant (g)</td>
<td>0.2415</td>
<td>0.4709</td>
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<tr>
<td>9</td>
<td>Shelling %</td>
<td>0.9433</td>
<td>-0.2650</td>
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<td>10</td>
<td>Haulm yield/plant (g)</td>
<td>0.0029</td>
<td>-0.0620</td>
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<tr>
<td>11</td>
<td>Harvest index (%)</td>
<td>0.0559</td>
<td>0.6801</td>
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<td>12</td>
<td>Pod yield/plant (g)</td>
<td>-0.0186</td>
<td>-0.0362</td>
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<tr>
<td></td>
<td>Variances accounted for (%)</td>
<td>44.5%</td>
<td>39.1%</td>
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Table 15. Mean canonical values of 29 groundnut genotypes.

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<th>Genotype name</th>
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<tr>
<td>1</td>
<td>53.170</td>
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<td>57.441</td>
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<td>61.347</td>
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<td>6</td>
<td>64.705</td>
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<td>24</td>
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<td>25</td>
<td>48.202</td>
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<td>27</td>
<td>59.208</td>
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<td>28</td>
<td>58.260</td>
<td>28.971</td>
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<tr>
<td>29</td>
<td>45.700</td>
<td>17.174</td>
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</tbody>
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4.6.1 Contribution of characters to genetic divergence:

The coefficients of the first two canonical vectors ($Z_1$ and $Z_2$) presented in Table 14 reflects relative importance of the characters contributing towards divergence. The canonical analysis revealed that values in both vectors (Vector I and II) for plant height, 100 kernel weight, kernel yield per plant and harvest index were positive. It was observed that the important characters responsible for genetic divergence were shelling percentage and kernel yield per plant in the first axis and harvest index and kernel yield per plant in the second axis. Such results indicated that these characters contributed maximum towards total divergence of the genotypes and it was also suggested that attention should be given for these five characters for yield improvement of groundnut. Similar finding was recorded by Reddy and Reddy (1993). Hossain et al., (2003) also recorded the similar results in groundnut where the harvest index contributed maximum towards the total divergence of the genotypes. Zaman et al., (2010) also recorded the similar results in groundnut. In his finding, the canonical analysis revealed that values in both vectors (Vector I and II) for days to flowering and days to maturity were positive, values in one vectors for branches per plant, mature nuts
per plant and 100 kernel weight was positive. Such results indicated that these characters contributed maximum towards total divergence of the genotypes and it was also suggested that attention should be given for these five characters for yield improvement of groundnut.
Fig.1 Two dimensional representation of divergence of 29 groundnut genotypes, using the first two canonical vectors ($Z_1$ and $Z_2$) as coordinates, the grouping by $D^2$ super imposed.
4.7 Molecular characterization

DNA extraction and purification as detailed in Materials and Methods section yielded good quality DNA which was amenable to PCR amplification.

4.7.1 Primer screening

Fourteen genotypes were used for screening the primers in order to identify the suitable ones for detailed analysis. There are 15 RAPD were screened, three RAPD were finally chosen that gave satisfactory amplification and band resolution.

4.7.2 Band statistics

DNA profile data for each of the marker had been presented in Fig.2. The frequency of polymorphism between selected accessions of groundnut for each type of marker was calculated based on presence (taken as 1) or absence (taken as 0) of common alleles. Only clear and apparently unambiguous bands were scored. Genetic similarities between the genotypes were measured by the Jaccard’s similarity coefficient based on the proportion of shared bands/alleles using “Simqual” subprogram of NTSYS-PC 2.1 (Exeter software, Setauket, NY, USA) software package (Rohlf, 2005)). The resultant similarity matrix data was used to construct the dendrogram by using un-weighted pair-group method with an arithmetic average (UPGMA) subprogram of NTSYS-PC (Rohlf, 2005). Polymorphism Information Content (PIC) was calculated for each primer. Similarly, rare band and low frequency band were determined.
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<td>0 0 0 0 0 0 0 0 0 0 0 0 1 1 1</td>
<td>0 0 0 0 0 0 0 0 0 0 0 0 1 1 1</td>
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</tbody>
</table>

**Fig. 2** The frequency of polymorphism between selected accessions of groundnut for each type of marker
Plate 3 Amplification profile of Groundnut genotypes employing RAPD primer OPW-1, M- Medium range DNA ladder. Numbers on the left margin represents molecular weight of ladder DNA in base pairs (bp).

Plate 4 Amplification profile of Groundnut genotypes employing RAPD primer OPE-19, M- 100 bp DNA ruler. Numbers on the left margin represents molecular weight of ruler DNA in base pairs (bp).

Plate 5 Amplification profile of Groundnut genotypes employing RAPD primer OPW-8, M- 100 bp DNA ruler. Numbers on the left margin represents molecular weight of ruler DNA in base pairs (bp).
Table 16  RAPD bands and their characteristics generated by three RAPD primers in groundnut

<table>
<thead>
<tr>
<th>Primer</th>
<th>Total no. of bands</th>
<th>Total monomorphic bands</th>
<th>Total polymorphic bands</th>
<th>Percentage polymorphism</th>
<th>Average PIC value</th>
<th>Size range*</th>
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<tr>
<td>OPW-1</td>
<td>10</td>
<td>0</td>
<td>10</td>
<td>100</td>
<td>0.766</td>
<td>200-2000</td>
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<tr>
<td>OPE-19</td>
<td>9</td>
<td>0</td>
<td>9</td>
<td>100</td>
<td>0.654</td>
<td>300-1800</td>
</tr>
<tr>
<td>OPW-8</td>
<td>8</td>
<td>0</td>
<td>8</td>
<td>100</td>
<td>0.578</td>
<td>250-2250</td>
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</table>

4.7.3 RAPD Analysis

A total of 27 bands were detected ranging in size from 200 bp to 2250 bp among the 14 genotypes. The number of bands produced with primer, OPW-1 is 10, OPE-19 is 9 and OPW-8 is 8 with a mean of 9 bands per primer. Of a total 27 RAPD bands, all 27 (100%) were found to be polymorphic. The maximum number of polymorphic bands (10) was obtained using OPW-1 primer whereas the minimum number of polymorphic bands(8) was obtained in Primer OPW-8. Polymorphism information content (PIC) have been used by the several workers (Provost and Wilkinson, 1999; Powell et al, 1997 and Botstein et al., 1980) to compare primers for their efficiency to distinguish genotypes. Subramanian et al. (2001) selected 70 genotypes and studied for polymorphism employing random amplified polymorphic DNA (RAPD) assay with 48 oligonucleotide primers. Of the 48 oligonucleotide primers, only 7 (14.6%) yielded polymorphic amplification products. The total number of bands from the 7 primers was 408, of which 27 were polymorphic. Thus he concluded that detection of polymorphism in cultivated groundnut opens up the possibility of development
of its molecular map by judicious selection of genotypes that show DNA polymorphism. This approach will be useful for developing marker-assisted selection tools for genetic enhancement of groundnut for desirable traits. Sergio Emilio Dos Santos et al. (2003) selected ten of 34 primers for DNA amplification reactions since they yielded the largest numbers of polymorphic loci. A dendrogram was constructed based on data from the 10 primers selected. Eighty RAPD polymorphic bands were analyzed among the accessions studied and he concluded that higher polymorphism was found in the section Trierectoides and in one accession of A. major, indicating that generalizations should be avoided and each species should be analyzed in order to establish collection and maintenance strategies. Wardsson Lustrino Borges et al. (2007) performed with 31 primers, of which 12 (39%) revealed polymorphism. It was observed a total of 145 amplified fragments, of which 35 (24%) were polymorphic, with an average of 4.67 fragments by primer and 1.13 polymorphic fragment by primer. It was observed through the dendrogram that the accessions were separated into two groups with 89% of similarity. This distribution shows the variability among the accessions of the different botanical varieties, since the accessions of subspecie fastigiata are present in two principal groups, and the accessions of subspecie hypogaea are distributed in subgroups A and B from dendrogram group II. Fanley Bertoti da Cunha et al. (2008) analyzed the genetic variability within AA and BB genome species and to evaluate how they are related to each other and to A. hypogaea, using RAPD markers. Eighty-seven polymorphic bands amplified by ten decamer primers were analyzed. The species
were divided into two major groups, and the AA and the BB genome species were, in general, separated from each other. The results showed that high variation is available within species that have genomes similar to the AA and the BB genomes of *A. hypogaea*.

4.8 Genetic diversity and relationships

Genetic relationship between groundnut genotypes were determined on the basis of Jaccard’s pair wise similarity coefficients. The range of pair wise similarity varies from 0.00 to 0.833 as shown in (Table 17). Highest similarity coefficient was observed between ‘Dh-204’ and ‘Dh-209’ (0.833) followed by ‘Dh-108’ and ‘TCGS-159’ (0.692), while minimum similarity was observed in between ‘ICGV-95401’ and ‘DhS-102’ (0.067), whereas no similarity was observed in between ‘Dh-216-1’ and ‘DhS-102’ (0.00). Accessions ‘Dh-204’ and ‘Dh-209’ showed highest similarity coefficient, indicating that they are belonging to similar genetic background. Dwivedi *et al.* (2001) studied molecular diversity among selected groundnut accessions and identify those with distinct DNA profiles for mapping and genetic enhancement. Twenty-six accessions and eight decamer primers were selected for random amplified polymorphic DNA assay. The genetic similarity ($S_{ij}$) ranged from 59.0% to 98.8%, with an average of 86.2%. Both multidimensional scaling and unweighted pair-group method with arithmetic averages (UPGMA) dendrograms revealed the existence of five distinct clusters. Varsha *et al.* (2009) used twenty-one mutants belonging to different botanical types of groundnut to assess molecular diversity using RAPD analysis. High genetic similarity values ($S_{ij}$) of
0.88 to 0.98 were obtained for the genotypes, indicating limited genetic diversity. Dendrogram revealed five different clusters at Sij 0.95.

4.8.1 Cluster Analysis

Following data analysis based on Jaccard’s similarity coefficient, a dendrogram was plotted for the data set that has been shown in Fig. 3. It depicted that, at genetic distance 0.00, two distinct clusters were formed having single root. Two major clusters (I-II) were identified from the dendrogram, which were found distinct from each other having only 0% similarity. The highest number of genotypes (13) was included in the Cluster I whereas cluster II was represented by only one genotype. Cluster I was further subdivided into two sub-clusters (IA and IB). All the genotypes re-presented in Cluster I were similar with respect to most of the morphological characters. The sub-cluster IA was represented by 8 genotypes. Cluster IA further classified into two minor sub clusters AI and AII. AI was represented by one genotypes and AII was represented by 7 genotypes. Similarly cluster IB is divided into two minor subclusters BI and BII. Sub cluster BI represented by 4 genotypes. Sub cluster BII represented by only one genotype. The genotypes namely ‘Dh-204’ and ‘Dh-209’ exhibited the maximum similarity (83.3%). From the clustering pattern it showed that distinct genome specific group exists among the studied 14 accessions in groundnut. These genetic distances are maintained as per geographical distribution of the species which can be exploited for further breeding programme between out
groups and core groups. Nguyen et al. (2007) reported distinctive RAPD patterns generated from 29 cultivars of groundnut used as genomic fingerprint to
Table 17: Jaccard’s similarity estimates for groundnut genotypes

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<tr>
<th></th>
<th>Dhs 102</th>
<th>Dh 8</th>
<th>Dh 216-1</th>
<th>Dh 209</th>
<th>Dh 206</th>
<th>Dh 204</th>
<th>TCGS 159</th>
<th>Dh 108</th>
<th>CSMG 2014</th>
<th>ALG 234</th>
<th>AG 2006-15</th>
<th>ICGV 95401</th>
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<td>DRT 53</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.368</td>
<td>0.000</td>
<td>0.250</td>
<td>0.250</td>
<td>0.267</td>
<td>0.333</td>
<td>0.000</td>
<td>0.000</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KGN 34</td>
<td>0.136</td>
<td>0.143</td>
<td>0.000</td>
<td>0.200</td>
<td>0.450</td>
<td>0.214</td>
<td>0.278</td>
<td>0.438</td>
<td>0.222</td>
<td>0.154</td>
<td>0.308</td>
<td>0.154</td>
<td>0.500</td>
<td>1.000</td>
</tr>
</tbody>
</table>
Fig. 3. Dendrogram depicting genetic relationship among 14 groundnut genotypes based on RAPD profile.
establish the identity of a given genotype. Twenty-nine groundnut cultivars were clearly separated in distinct subclusters in a phyllogram obtained by unweighted pair group method analysis (UPGMA) of genetic distances. Varsha et al. (2009) used twenty-one mutants belonging to different botanical types of groundnut to assess molecular diversity using RAPD analysis. The cluster analysis grouped 21 genotypes of groundnut into five different clusters, most of the genotypes were grouped in a single cluster, indicating narrow genetic diversity among the genotypes. Raina et al. (2001) used twenty-one random and 29 SSR primers to assess genetic variation and interrelationships among subspecies and botanical varieties of cultivated peanut and phylogenetic relationships among cultivated peanut and wild species of the genus Arachis. The dendrograms based on RAPD, ISSR, and RAPD + ISSR data precisely organized the five botanical varieties of the two subspecies into five clusters. Dendrograms constructed from RAPD, ISSR, and RAPD + ISSR data showed overall similar topologies. They could be resolved into four groups corresponding to the species grouped in four taxonomic sections. The results strongly support the view that Arachis monticola and A. hypogaea are very closely related, and indicate that A. villosa and A. ipaensis are the diploid wild progenitors of these tetraploid species.
Fig. 4 Two Dimensional scaling by Principal Component Analysis (PCA) of 14 groundnut genotypes using Jaccard’s similarity coefficient of RAPD profile data.
Fig.5 Three Dimensional scaling by Principal Component Analysis (PCA) of 14 groundnut genotypes using Jaccard’s similarity co-efficient of RAPD profile data.
4.8.2 Principal component analysis

Principle component analysis was also done to visualize genetic relationships among the varieties (Figure 5 & 6). The results were very much similar to UPGMA results. Two Dimensional and Three Dimensional scaling showed four clusters (I to IV). Cluster-I had six accession i.e. “DhS-102, Dh-206, TCGS-159, Dh-108, CSMG-2014 and KGN-34”. Cluster-II had three accessions i.e. “Dh-216-1, ALG-234 and DRT-53”. Cluster-III had four accessions i.e. “Dh-8, Dh-209, Dh-204 and AG-2006-15”. Cluster-IV had only one accession i.e. “ICGV-95401”. Only difference is that, three Dimensional scaling shows vertical diversity between the accessions which is in cluster-I. Based on study the large range of similarity values for related accessions using RAPD provides greater confidence for the assessment of genetic diversity and relationships. This characterization of 14 genotypes will be very significantly helpful to the background selections during back cross of breeding programs. Dwivedi et al. (2001) studied on genetic diversity among selected groundnut germplasm by RAPD analysis. He used both multidimensional scaling and unweighted pair-group method with arithmetic averages (UPGMA) dendrograms and concluded the existence of five distinct clusters. However, this classification could not be related to known biological information about the accessions falling into different clusters.
SUMMARY AND CONCLUSION

The present investigation “Studies on genetic diversity of groundnut (*Arachis hypogaea* L.) using morphological and molecular markers, was undertaken with the following objectives:

(i) Study the nature and magnitude of variability of morpho-metric traits among advance breeding lines of groundnut belonging to seven major groundnut growing states of India.

(ii) Study the nature and magnitude of character association among morphological traits.

(iii) Assess patterns of phenotypic diversity among advance breeding lines of groundnut belonging to several major groundnut growing states of India.

(iv) Identify agronomically beneficial breeding lines for broadening the genetic base of groundnut cultigens.

(v) Molecular characterization of intra-accession variation of selected groundnut genotypes and assessment of genetic diversity on the basis of polymorphic banding pattern.

The 27 breeding lines with 2 released varieties evaluated in R.B.D for yield and its component traits grown in *rabi* seasons showed wide variation in all the 12 traits including yield. The genotypic source of variations for all the twelve characters were highly significant except haulm yield per plant at 1% level of
significance. The replication source of variation was highly significant for only number of branches per plant at 1% level of significance.

Mean performance of 29 genotypes for all the twelve morphometric traits is observed for *rabi* season. Highest number of pods per plant and number of kernels per plant were observed in UG 3 but The test genotypes exhibited sound matured kernel per cent minimum of 82.98 % in UG 3. The highest harvest index (36.65 %) was observed in Dh 108. Dh 108 was also highest in both kernel yield per plant (10.49 g) and pod yield per plant (14.74 g) per plant.

The P.C.V. and G.C.V. estimates were high for plant height, number of branches per plant, number of pods per plant, number of kernels per plant, hundred kernel weight, harvest index percentage, kernel yield per plant and pod yield per plant, there by suggesting ample scope for improvement through selection among these advance breeding lines. Low values of G.C.V. was observed in days to 50% flowering, sound mature kernel percentage, shelling percentage and haulm yield per plant indicated the need to create variability either by hybridization or mutation followed by selection.

High heritability estimates were observed for 100 kernel weight, shelling percentage, pod yield per plant, harvest index and number of pods per plant indicating less influence of environment on these characters. Along with high heritability, high genetic advance as percentage of mean has been noticed for pod yield per plant, hundred kernel weight, number of pods per plant and harvest index. Therefore it was clear that these four traits were less influenced by the environmental changes due to the presence of additive gene action in their
expressions. Hence improvement of these traits would be more effective through the selection. This showed additive type of gene action and indicated phenotypic selection to be effective for pod yield per plant, hundred kernel weight, number of pods per plant and harvest index. Heritability and genetic advance as percent of mean were moderate for days to 50% flowering, number of kernels per plant, number of branches per plant, kernel yield per plant and plant height. Low genetic advance along with low heritability estimate for sound mature kernel percent, and haulm yield per plant was observed in present study. This indicated the additive and non additive gene actions that have a role in their inheritance and phenotypic selection would be effective to some extent.

Pod yield per plant and kernel yield per plant exhibited significant positive correlation and both these traits also exhibited significant positive correlation with plant height, number of pods per plant, 100 kernel weight, number of kernels per plant and harvest index at both phenotypic and genotypic level. This indicated the strong association of harvest index, number of kernels per plant and number of pods per plant with pod yield and kernel yield per plant in *rabi* season. Both phenotypic and genotypic correlation coefficient for number of kernels per plant were highly significant and positive with plant height, number of pods per plant, kernel yield per plant, harvest index and pod yield per plant. Number of pods per plant were significant and positively correlated with plant height, number of branches per plant, number of kernels per plant, kernel yield per plant and pod yield per plant both at phenotypic and genotypic levels. Plant height was significant and positively correlated with number of pods per
plant, number of kernels per plant, kernel yield per plant and pod yield per plant both at phenotypic and genotypic level. Hundred kernel weight was significant and positively correlated with harvest index percentage both at phenotypic and genotypic level. Harvest index percentage was significant and positively correlated with hundred kernel weight, number of kernels per plant, kernel yield per plant and pod yield per plant both at phenotypic and genotypic level. In contrast to this, days to 50% flowering did not show any significant correlation with pod yield and other yield contributing characters. From this studies, it can be concluded that number of pods per plant and number of kernels per plant are two most important morphological traits for selecting better genotypes with high kernel yield and pod yield per plant. The highest phenotypic correlation as well as genotypic correlation was observed between kernel yield per plant and pod yield per plant. But the sound mature kernel percent is negatively correlated with number of kernels per plant, harvest index and both pod and kernel yield per plant.

The path coefficient analysis showed low residual effect (0.089) during rabi indicating that most of the major yield components were included in the study. Harvest index percentage had the highest direct positive effects on pod yield per plant followed by haulm yield per plant. Also kernel yield per plant, hundred kernel weight, number of pods per plant and plant height were observed to be the major indirect contributor towards pod yield through harvest index percentage. Present study thus indicated that prime emphasis should be given to
harvest index percentage followed by number of kernels per plant and kernel yield per plant.

The smallest $D^2$ estimate (9.044) was observed between ALG 234 and AG 2006-15, so these genotypes were much similar in many traits. The largest $D^2$ estimate (640.258) was obtained between ALG 234 and GPBD 5, which indicated the maximum diversity. On the basis of average $D^2$, shelling percentage contributed maximum divergence followed by harvest index, kernel yield per plant, hundred kernel weight and all other characters. Sound mature kernel percent, Plant height, pod yield per plant, days to 50% flowering, number of branches per plant, haulm yield per plant, number of pods per plant and number of kernels per plant contributed least to $D^2$ estimates.

On the basis of critical $D^2$ values (83.614), 29 genotypes were classified into 8 clusters. In the present study, advance breeding lines of major groundnut growing states of India were grouped into eight different clusters. This indicated the large diversity existing in the advance breeding lines of groundnut giving the opportunity for further improvement in groundnut. Thus recent released varieties contain sufficient diversity though groundnut itself has narrow genetic base.

Cluster III was the largest, accommodating as many as 10 genotypes and cluster VII and VIII was smallest with one genotype each. Cluster IV, V and VI possessing two genotypes each. Cluster I and II possessing six and five genotypes, respectively. Two genotypes (UG 3 and DhS 102) remained in isolated clusters. The clustering pattern of genotypes showed that the genotypes
of different origins were clubbed into one cluster whereas the genotypes belonging to same state or origin were grouped into different clusters indicating that the geographic distribution was not the sole criterion of genetic diversity. Lowest intra-cluster distance was observed between cluster IV (24.933). Highest intra-cluster distance was observed within cluster VI followed by cluster V. Intercluster distance is the main criterion for selection of genotypes. Maximum inter-cluster distance was observed between cluster VI and cluster VIII followed by cluster VI and cluster VII. In this contest, the genotypes from cluster VI and VIII or cluster VI and VII could be selected as parents for hybridization. Cluster VIII exhibited high value of days to 50% flowering, number of branches per plant and haulm yield per plant. Cluster VII exhibited high value of plant height, number of pods per plant, number of kernels per plant, harvest index percentage and pod yield per plant. Cluster IV exhibited high value of hundred kernel weight. Cluster V exhibited high value of sound mature kernel percent. Cluster I exhibited high value of shelling percent. Cluster VI and IV exhibited high value of kernel yield per plant. Thus the advanced breeding line ALG 234 from cluster I and GPBD 5 from cluster IV, exhibiting highest genetic diversity of 640.258 may be selected as parents in the future hybridization programme to give maximum high yielding segregates with desirable features.

The canonical analysis revealed that values in both vectors (Vector I and II) for plant height, 100 kernel weight, kernel yield per plant and harvest index were positive. It was observed that the important characters responsible for genetic divergence were shelling percentage and kernel yield per plant in the first
axis and harvest index and kernel yield per plant in the second axis. Such results indicated that these characters contributed maximum towards total divergence of the genotypes and it was also suggested that attention should be given for these five characters for yield improvement of groundnut.

In molecular analysis, a total of fourteen accessions of Groundnut (*Arachis hypogaea* L.) collected from different parts of Odisha and were used for genetic diversity analysis in the present study. A total of 27 bands were detected ranging in size from 200 bp to 2250 bp among the 14 genotypes. The number of bands produced with primer, OPW-1 is 10, OPE-19 is 9 and OPW-8 is 8 with a mean of 9 bands per primer. Of a total 27 RAPD bands, all 27 (100%) were found to be polymorphic. The maximum number of polymorphic bands (10) was obtained using OPW-1 primer whereas the minimum number of polymorphic bands (8) was obtained in Primer OPW-8.

Genetic relationship between groundnut genotypes were determined on the basis of Jaccard’s pair wise similarity coefficients. The range of pair wise similarity varies from 0.00 to 0.833. Highest similarity coefficient was observed between ‘Dh-204’ and ‘Dh-209’ (0.833) followed by ‘Dh-108’ and ‘TCGS-159’(0.692), while minimum similarity was observed in between ‘ICGV-95401’ and ‘DhS-102’(0.067), whereas no similarity was observed in between ‘Dh-216-1’ and ‘DhS-102’(0.00). Accessions ‘Dh-204’ and ‘Dh-209’ showed highest similarity coefficient, indicating that they are belonging to similar genetic background.
Following data analysis based on Jaccard’s similarity coefficient, a
dendrogram was plotted for the data set. It depicted that, at genetic distance 0.00,
two distinct clusters were formed having single root. Two major clusters (I-II)
were identified from the dendrogram, which were found distinct from each other
having only 0% similarity. The highest number of genotypes (13) was included
in the Cluster I whereas cluster II was represented by only one genotypes.
Cluster I was further subdivided into two sub-clusters (IA and IB). All the
genotypes re-presented in Cluster I were similar with respect to most of the
morphological characters. The sub-cluster IA was represented by 8 genotypes.
Cluster IA further classified into two minor sub clusters AI and AII. AI was
represented by one genotypes and AII was represented by 7 genotypes. Similarly
cluster IB is divided into two minor subclusters BI and BII. Sub cluster BI
represented by 4 genotypes. Sub cluster BII represented by only one genotype.
The genotypes namely ‘Dh-204’ and ‘Dh-209’ exhibited the maximum similarity
(83.3%). From the clustering pattern it showed that distinct genome specific
group exists among the studied 14 accessions in groundnut. These genetic
distances are maintained as per geographical distribution of the species which
can be exploited for further breeding programme between out groups and core
groups.

Principle component analysis was also done to visualize genetic
relationships among the varieties. The results were very much similar to
UPGMA results. Two Dimensional and Three Dimensional scaling showed four
clusters (I to IV). Cluster-I had six accession i.e. “DhS-102, Dh-206, TCGS-159,
Dh-108, CSMG-2014 and KGN-34”. Cluster-II had three accessions i.e. “Dh-216-1, ALG-234 and DRT-53”. Cluster-III had four accessions i.e. “Dh-8, Dh-209, Dh-204 and AG-2006-15”. Cluster-IV had only one accession i.e. “ICGV-95401”. Only difference is that, three Dimensional scaling shows vertical diversity between the accessions which is in cluster-I. Based on study the large range of similarity values for related accessions using RAPD provides greater confidence for the assessment of genetic diversity and relationships. This characterization of 14 genotypes will be very significantly helpful to the background selections during back cross of breeding programs.


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APPENDIX-I

REAGENTS AND SOLUTIONS:

A. 1.0 M Tris-HCl (pH 8.0)

Dissolve 12.11gm Tris HCl in sterile de-ionized water, adjust pH to 8.0 with conc. HCl, and make up volume to 100 ml with de-ionized water and autoclave at 15 psi for 20 min.

B. 0.5 M EDTA (pH 8.0)

EDTA (dissolved salt; Mw = 372.3) = 18.61gm

Dissolve, 18.61gm EDTA in sterile de-ionized water, adjust pH to 8.0 with 5N NaOH, make up volume to 100 ml with de-ionized water and autoclaved at 15 psi for 20 min.

C. 5 M NaCl

NaCl = 29.2 gm
Dissolve 29.2gm NaCl in sterile de-ionized water, make up volume to 100 ml with de-ionized water and autoclave at 15 psi for 20 min

D. Extraction buffer

1M Tris-HCl (pH 8.0) = 10 ml
0.5 M EDTA (pH 8.0) = 2 ml
3 M NaCl = 46.6 ml
2% CTAB (w/v) = 2 g
0.2% β-Mercaptoethanol = 0.2ml

Dissolve the above and make up to 100 ml with de-ionized water and autoclave at 15 psi for 20 min.

E. 10% working C-TAB

10% CTAB = 10 g
5 M NaCl = 14 ml

Dissolve the above in water, make up to 100 ml and autoclave at 15 psi for 20 min.

F. 3M NaOAC (pH 4.8)

Sodium Acetate = 24.61 gm

Dissolve 24.61 gm NaOAC in sterile de-ionized water, adjust pH to with glacial acetic acid, make up volume to 100 ml with de-ionized water and autoclave at 15 psi for 20 min.

G. Chloroform : Iso-amyl alcohol mixture (24:1) (100 ml)

Chloroform = 96 ml
Iso-amyl alcohol = 4 ml
H. 70% ethanol (100ml)

Absolute alcohol = 70 ml
Double distilled water = 30 ml

I. RNase stock

1 M Tris- HCl (pH 8.0) = 100 µl
5 M NaCl = 300 µl
RNase = 10 mg

Adjust volume to 1 ml with de-ionized water, boil for 15 min and allow to cool slowly and stored at -20ºC

J. TE (10:1)

1 M Tris-HCl (pH 8.0) = 1.0 ml
0.5 M EDTA (pH 8.0) = 0.2 ml

Dissolve the above and make up volume to 100 ml with de-ionized water and autoclave at 15 psi for 20 min.

K. 10 X TBE (pH 8.0)

Tris base = 108.0 g
Boric acid = 55.0 g
EDTA (0.5M) = 20ml

Dissolve the above and make up volume to 1000 ml with double distilled water.

L. DNA quantification
1. DNA standard assay solution

<table>
<thead>
<tr>
<th>Component</th>
<th>Low range DNA assay (10-50 ng/ml find DNA conc.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOECHST 33258 Stock soln.</td>
<td>10µl</td>
</tr>
<tr>
<td>10 × TNE</td>
<td>10ml</td>
</tr>
<tr>
<td>Distilled water</td>
<td>90ml</td>
</tr>
</tbody>
</table>

The above solutions were mixed and volume made up to 100ml.

The solution was prepared afresh as and when necessary and stored in amber coloured bottle.

2. Hoechst Dye (H-33258) 10 × solution

Add 10ml distilled water to 10mg of Hoechst 33258 and stored at 4°C for up to 6 months in an amber coloured bottle.

3. 10 × TNE buffer stock solution (100:10mM EDTA:2M NaCl, pH-7.4)

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
<th>Amount taken</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris</td>
<td>100mM</td>
<td>12.11g</td>
</tr>
<tr>
<td>EDTA</td>
<td>10mM</td>
<td>3.72g</td>
</tr>
<tr>
<td>NaCl</td>
<td>2mM</td>
<td>116.89g</td>
</tr>
</tbody>
</table>

4. DNA standard-Low range Standard (1mg/ml)

<table>
<thead>
<tr>
<th>Components</th>
<th>Volume (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calf Thymus DNA (1 mg/ml)</td>
<td>100</td>
</tr>
<tr>
<td>Chemical</td>
<td>Manufacture</td>
</tr>
<tr>
<td>---------------------------</td>
<td>-------------</td>
</tr>
<tr>
<td>TNE buffer (10 × )</td>
<td>100</td>
</tr>
<tr>
<td>Sterile distilled water</td>
<td>800</td>
</tr>
</tbody>
</table>

### APPENDIX-II

#### 1. LIST OF CHEMICALS AND THEIR MANUFACTURES:

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Manufacture</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1. DNA extraction</strong></td>
<td></td>
</tr>
<tr>
<td>(a) CTAB buffer</td>
<td></td>
</tr>
<tr>
<td>100mM Tris (pH 8.0)</td>
<td>Qualigens</td>
</tr>
<tr>
<td>1.4 M NaCl</td>
<td>Qualigens</td>
</tr>
<tr>
<td>2% CTAB</td>
<td>Qualigens</td>
</tr>
<tr>
<td>0.2% β-Mercaptoethanol</td>
<td>Amresco</td>
</tr>
</tbody>
</table>
2. DNA Purification

(a) Phenol:Chloroform:Isoamyl alcohol

(b) RNase solution

Rnase
Tris HCl (pH 7.5)
NaCl

(c) Sodium Acetate

3. DNA Quantification

(a) 10× TNE Buffer

100 mM Tris
2M NaCl
10 mM EDTA

(b) Hoechst 33258

(c) Calf Thymus DNA

(d) Assay Buffer (in dark bottle)
10 × TNE buffer (10 µl)
Double distilled water (90 ml)

4. **RAPD analysis**
(a) Taq DNA polymerase (1U/reaction)  
Bangalore Genei
(b) Buffer (10 × ) (2 µl/reaction)  
Bangalore Genei
(c) MgCl$_2$ (25 mM) (1.2 µl/reaction)  
Bangalore Genei
(d) dNTP mixture (2 µl/reaction)  
Bangalore Genei
(e) Primer (2 µl/reaction)  
Bangalore Genei

5. **Agarose gel electrophoresis**
(a) Agarose  
Bangalore Genei
(b) 1× TBE buffer  
Bangalore Genei
(c) Ethidium bromide solution  
Sigma
(d) Loading dye (10×)  
Bangalore Genei
(e) DNA Ladder  
Bangalore Genei
2. **LIST OF INSTRUMENTS USED IN**: 

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Manufacture</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA extraction, purification, quantification.</td>
<td></td>
</tr>
<tr>
<td>(a) Electronic balance</td>
<td>AICOSET</td>
</tr>
</tbody>
</table>

A. *DNA extraction, purification, quantification.*

(a) Electronic balance AICOSET
(b) Micropipette (2µl,20µl,100µl,1000µl)  NICHIPET
(c) Water bath  JULABA
(d) Layophilizer  SAVANT
(e) Deep Freezer (-20°C)  LAB-LINE
(f) Refrigerator  GODREJ
(g) Fume hood  ATLANTIS
(h) Table top centrifuge  SIGMA 4K15
(i) Vortex  SPHNIX
(j) Eppendorf tubes(0.5ml,2ml)  TARSON
(k) Tips (200µl,1000µl)  TARSON
(l) Quant Fluorimeter  BIORAD
(m) Quartz Cuvette  SIGMA
(n) pH meter  SYSTRONIC
(o) Water purification system  MILLIPORE
(p) Hybridization oven  AMERSHAM
(q) Vertical autoclave  YORCOZX

B. RAPD analysis

(a) Thin walled PCR tubes  AXYGEN
(b) PCR  PQLAB
(c) Laminar air flow  
(d) Micro centrifuge  
(e) Pipettman  
(f) Pipette tips  

**C. Gel Electrophoresis**  
(a) Gel electrophoretic unit  
(b) UV transilluminator  
(c) Power pack (300)  
(d) Microwave oven  
(e) Laboratory shaker  
(f) Gel Doc. System