Association analysis, fresh seed dormancy and screening of genotypes using SSR marker in groundnut (Arachis hypogaea L.)

THESIS

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By

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2019

CERTIFICATE – I

This is to certify that the thesis entitled **"Association analysis, fresh seed dormancy and screening of genotypes using SSR marker in groundnut *(Arachis hypogaea* L*).* "** submitted in partial fulfilment of the requirement of the degree of **MASTER OF SCIENCE in Agriculture (Genetics & Plant Breeding)** of the **Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior** is a record of the bonafide research work carried out by **Anil Nagar** under my guidance and supervision. The subject of the thesis has been approved by Students Advisory Committee and the Director of Instruction.

No part of the thesis has been submitted for any degree or diploma or has been published. All the assistance and help received during the course of investigations have been duly acknowledged by the scholar.

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Member	**(Dr. V.B. Singh)**	_____

CERTIFICATE – II

This is to certify that the thesis entitled "**Association analysis, fresh seed dormancy and screening of genotypes using SSR marker in groundnut *(Arachis hypogaea* L*).*** " submitted by **Anil Nagar** to the Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior in partial fulfilment of the requirements for the degree of Master of Science in Agriculture in the Department of **Genetics & Plant Breeding** , has been accepted after evaluation by the external examiner and approved by the Students Advisory Committee after an oral examination of the same.

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ABBREVIATION

AFLP	Amplifd Fragment Length Polymorphism
Cv	Cultivar
CATB	Cetyl Trimethyl Ammonium Bromide
dATP	Deoxy ribose adenosine tri-phosphate
dCTP	Deoxy cytidine tri-phosphate
dGTP	Deoxy guanosine tri-phosphate
dTTP	Deoxy thymidine triphosphte
dNTP	Deoxy ribonucleotide triphosphate
DNA	Deoxy ribose nucleic acid
DGR	Directorate of Groundnut Research
DDW	Double Distilled Water
EDTA	Ethylene Diamine Tetra acetic Acid
EST	Expressed Sequence Tag
FAO	Food and Agriculture Organization
G	Guanine
Ha	Hectare
IPS	Initial plant stand
ISSR	Inter Simple Sequence Repeat
Kb	Kilobases
Mg	Milli Gram
M	Mole
MAS	Marker Assisted Selection
Mm	Millimoles
MgCl2	magnesium chloride
Min	Minutes
Ml	Milliliters
Mt	million tones
Mg	Microgram
Ml	MicroLlitre
N	normal (solution)
NaCl	sodium chloride

NaOH	sodium hydroxide
Ng	Nanograms
P	Polymorphism
PCR	Polymerase Chain Reaction
%	Percent
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
Sec	Seconds
SSR	Simple Sequence Repeat
TBE	Tris-borate EDTA
Tm	Melting temperature
TAE	Tris base Acetic acid Glacial EDTA
USA	United States of America
V	Volts
Var	Variety
W	Watts
%	percentage
Σ	summation
√	square root
µl	micro litre
CD	Critical Difference
Cm	centimeter
CV	Coefficient of variation
Df	Degree of freedom
et al.,	and others
Fig.	Figure
gm	gram
GA	Genetic advance.
GCV	genotypic coefficient of variation.
Hbs	Heritability in broad sense
M	molar
mM	milli molar
No.	Number

PCV	phenotypic coefficient of variation
RCBD -	Randomised complete block design
SE	standard error
v/v	volume by volume
viz.,	namely
r_g	genotypic correlation coefficient
r_p	phenotypic correlation coefficient

Chapter-I

Introduction

Groundnut or peanut *(Arachis hypogeal* L*)* is one of the most important oilseed crop being cultivated in the semi-arid tropics. It belongs to the leguminous family. The cultivated species of groundnut is tetraploid (2n=4x=40) in nature and originated in South America. It is mainly self-pollinated crop. It exhibit low out crossing rates ranging from 0-8% through natural crossing. Groundnut is a rich source of edible oil (45-55%) and haulms and groundnut cake are important sources of animal feed.

India occupies the second position in production and first position in area in the world. In Globally, with annual all-season coverage of about 70 lakh hectares.. In kharif-2017, the all-India acreage was 41,52,500 hectares. Eight states viz., Gujarat, Andhra Pradesh, Rajasthan, Karnataka, Madhya Pradesh, Maharashtra, Tamil Nadu and Uttar Pradesh were identified to have acreages greater than one lakh hectares and these states jointly accounted for 95.5% of the national acreage . Globally, China rank first in production of groundnut followed by India, Nigeria and USA (FAO 2017). Top groundnut producing states in India are Gujrat, Rajasthan, Tamil Nadu, Andhra Pradesh, Karnataka ana Madhya pradesh. In Madhya Pradesh, total production of 312 thousand tones of peanuts.(Anonymous 2017).

The presence of genetic variability is also a pre-requisite for the success of plant breeding programmes. The importance of studying genetic variance was realized for the first time by Fisher (1918). He partitioned total genetic variance into additive, dominant and epistatic components. Additive genetic variance can be exploited for genetic advance through selection. The genetic inferences are obtained from phenotypic observations, which are the results of interaction of genotype with the environment. Therefore, the observed variability can be grouped under heritable and non heritable components and this can be estimated by parameters like heritability, genetic advance, genetic gain etc.

These parameters help the breeder in developing and formulating effective selection programmes.

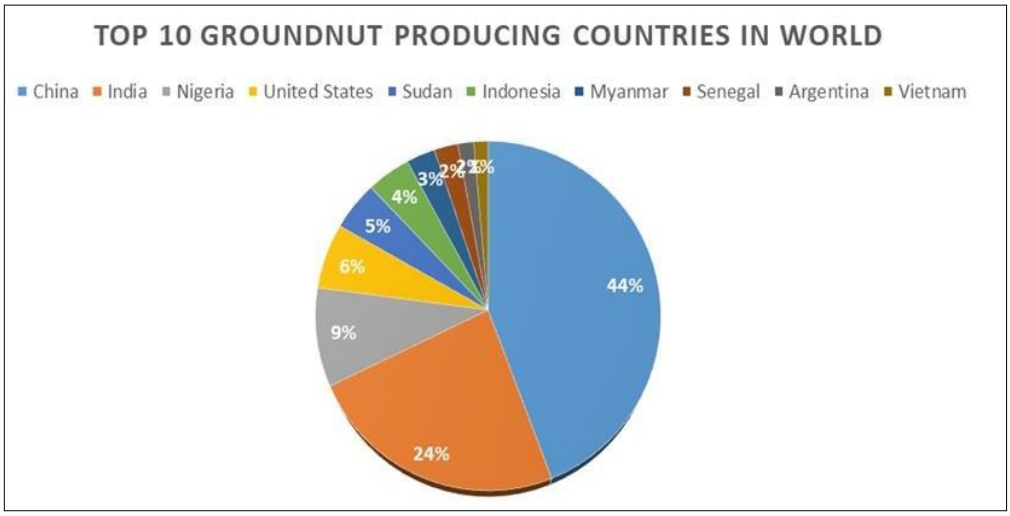


Fig 1.1: Top 10 groundnut producing countries in the world.

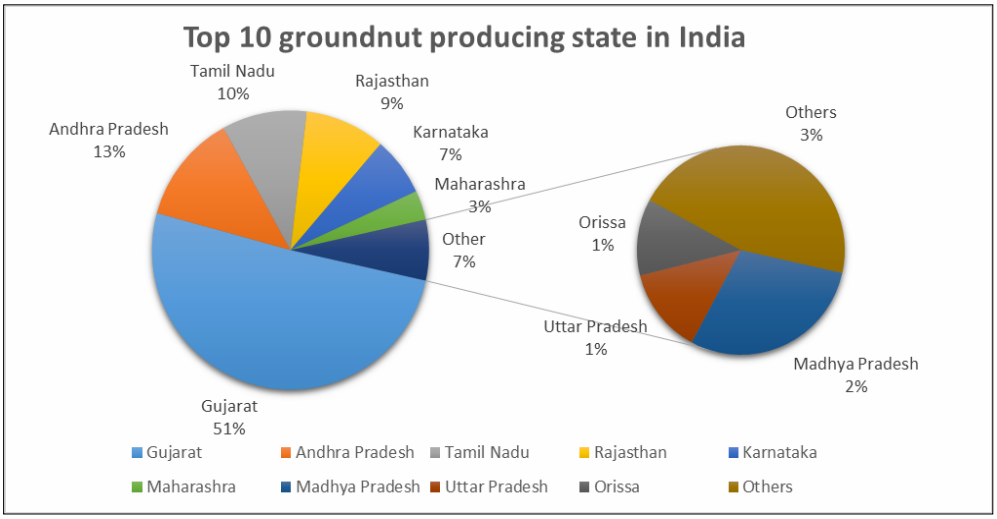


Fig 1.2: Top 10 groundnut producing state in the India

Further yield and oil content in groundnut are complex characters resulting from the interplay of various contributing characters, which have positive or negative association with these traits and among themselves. Therefore, direct selection for these traits, is not much effective. Hence, knowledge about association of characters which directly or indirectly contribute to yield and oil content is crucial. Correlation coefficients explain the degree of association

among the characters. The method of path coefficient analysis developed by Wright (1921) is helpful in partitioning of the correlation coefficients into direct and indirect effects and in the assessment of relative contributions of each component to these traits and aids in the formulation of effective selection criterion for their improvement.

Cultivated groundnut has two subspecies, *viz., Arachis hypogaea* spp. *fastigiata* and *Arachis hypogaea* spp. *hypogaea.* These two subspecies differ significantly in their seed dormancy after maturity. The cultivars of subspecies *hypogaea*, the *Virginia* type, are of long duration and possess seed dormancy for long periods after maturity. While those of *fastigiata*, the Spanish and Valenica types, are generally characterized by a short life cycle and non-dormant (Krapovickas, 1968). The longer periods of dormancy in the Virginia type do not allow immediate use of harvested seeds from one season for sowing in the next or for germination and other tests of seed quality. Therefore, a dormancy period of two to three weeks in erect bunch cultivars of groundnut is desirable. Dormancy in the early-maturing groundnut cultivars has been recognized as a desirable character (John *et al.,* 1948 and Ramachandran *et al.,* 1967). Appreciable dormancy observed in the freshly harvested seeds of several bunch groundnut cultivars, which disappears after curing is called fresh seed dormancy.

In groundnut breeding programs have also widely and extensively used phenotypic tools for selecting plants/progenies with desirable traits (Janila *et al*., 2013).Several efforts through conventional breeding have been made to enhance crop productivity. 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).

SSRs are therefore excellent choice of DNA markers for genetic mapping, genetic diversity analysis, gene mapping etc. in plants such as groundnut. Unlike RAPDs, SSR markers have proven to be reliable and reproducible, codominant and user friendly. 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 diversity, and phylogenetic analysis (Gupta *et al*., 1996).

Keeping views of all above facts, present study has been undertaken with the following objectives:

1. To study the genetic variability, heritability and genetic advance.
2. To study the character association and path coefficient analysis.
3. To study the fresh seed dormancy of the genotypes under study.
4. To screening of genotypes for foliar disease using gene based SSR marker.

Chapter-II

REVIEW OF LITERATURE

The relevant literatures related to various aspects of present study are reviewed under the following heads.

1. To study the genetic variability, heritability and genetic advance.
2. To study the character association and path coefficient analysis.
3. To study the fresh seed dormancy of the genotypes under study.
4. To screening of genotypes for foliar disease using gene based SSR marker.

Variability, Heritability and Genetic Advance

Sudhir Kumar *et al*. (2008) evaluated 64 genotypes of groundnut (39 accessions and 25 advance breeding lines) (*Arachis hypogaea* L.) for 14 quantitative characters and revealed wide range of variability. 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. Heritability and genetic advance were high for all the characters under study except for days to 50% flowering, days to maturity, sound mature kernel percent and oil content. Oil yield per plant recorded positive and significant association with matured pods per plants and kernel yield per plant. Path analysis showed high direct effects of kernel yield and oil content. He concluded that 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 pod and kernel traits using TMV 2 and 3 foliar disease resistant parents.. Among the crosses, TMV 2 x COG 0437 had higher mean performance for all the characters followed by TMV 2 x COG 438. High PCV and GCV were exhibited 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 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, (*Arachis hypogaea* L.). Fifty elite genotypes of bunch groundnut, (*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 m2 in biomass, 142–277 g m2 in pod weight, 91–216 g m2 in seed yield, 4–23 pods/plant, 1–3 seeds per pod, 245–594 g m2 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.

Rao *et al.* (2014) studied of Analysis of variance revealed the existence of significant differences among genotypes for all characters. High heritability coupled with high genetic advance as per cent of mean was observed for hundred kernel weight, Dry pod yield, kernel yield, plant height and number of pods per plant indicating the role of additive gene in expressing these traits. Dry pod yield was significant positively correlated with kernel yield, number of pods per plant, hundred kernel weight and dry haulm yield. Path coefficient analysis indicated that number of pods per plant and hundred kernel weight was important traits to be considered for realizing the improvement in yield.

Hampannavar *et al.* (2018) studied genetic parameters like variability, heritability and genetic advance as the percent of meanamong 144 groundnut genotypes. In all genotypes recorded the 13 different characters out of that plant height (cm), number of primary branches per plant, number of mature and immature pods per plant, kernel yield per plant, hundred kernel weight

(g), haulm yield per plant and dry pod yield per plant had high heritability and genetic advance as the percent of mean. The high heritability in addition to genetic advance as per cent of mean for most of the characters was observed indicating the presence of considerable genetic variation and additive gene effects.

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.

John *et al*. (2015) estimate of genotypic correlation coefficients in general higher than their corresponding phenotypic correlations indicating strong inherent association among the traits. Pod yield in groundnut is a complex and depends upon the interplay of number of components attributes. Primary yield components of groundnut *viz.,* pod size, sound mature kernels, shelling percentage, 100 kernel weight, kernel yield and number of mature pods per plant showed positive correlation with each other and with pod yield. A clear picture of contribution of each component is the final expression of character would emerge through the study of correlation and causation of path concept revealing different ways in which component attributes influence the complex traits. Path coefficient analysis helps in formulating the selection criteria based on these direct and indirect effects.

Hampannavar *et al.* (2018) studied 144 groundnut genotypes during *kharif*2015. The phenotypic and genotypic correlation coefficient kernel yield per plant, mature pods per plant, sound mature kernel and haulm yield per plant had significant positive correlation with dry pod yield per plant. The kernel yield had high direct effect on dry pod yield.

Raza *et al*. (2018) reported path analysis which was carried with 40 genotypes of groundnut for yield and its component traits. Path coefficient analysis indicated that kernel yield per plant exerted the highest positive direct effect on pod yield per plant. It was also revealed that plant height, days to maturity, seed calcium uptake and seed iron uptake contributed indirectly to pod yield per plant through kernel yield per plant. These characters also exhibited highly significant and desirable association with pod yield and among themselves. This information could be utilised in formulating a sound selection criterion in groundnut breeding programmes for genetic improvement of high yield potential genotypes with high nutrient uptake ability.

Fresh seed dormancy

Asibuo *et al.* (2008) conducted an experiment at Ghana (Africa) to determine the heritability of fresh seed dormancy in groundnut and to transfer this trait from dormant exotic lines (ICGV-86158 AND ICGV-87388) into two non- dormant groundnut varieties (Shitaochi and Aprewa), they reported that seed dormancy is controlled by monogenic inheritance with dormancy dominant over non dormant , as the results showed that more than 90% of the freshly harvested seeds of the non-dormant parents germinated before 14 days, whereas less than 10% of the seeds of the dormant parents germinated during the same period.

Gaikwad *et al.* (2010) studied six varieties RHRG-6021 and SB-XI were found to possesses fresh seed dormancy up to 10 DAPM, variety TPG-41 andRHRG-6083 up to 20 DAPM and variety TAG-24 up to physiological maturity, whereas variety JL-501 did not possesses fresh seed dormancy. These observations are based on periodical seed germination (%).There was no fresh seed dormancy in the variety JL-501 since it exhibited germination 54.67, 83.33, 95.33 and 95.33% at physiological maturity, 10, 20 and 30 DAPM, respectively. The significantly highest seedling vigour index-I was recorded in genotype SBXI (1879.05) harvested at M4 (30 DAPM) followed by genotype JL-501 at M4 (1751.99). The significantly lowest seedling vigour index-I was observedin combinations V1M1, V2M1, V5M1, V6M1, V5M2 and V6M2 (0.00). The significantly highest seedling vigour index-II was recorded in genotypeTPG-41 (416.47) harvested at M4 (30 DAPM) followed by genotype SBXI at M4 (406.04). The significantly lowest seedling vigour index-II was observed in treatment combinations *viz.,* V1M1, V2M1, V5M1, V6M1, V5M2 and V6M2 (0.00). Seedling vigour index-I and II were increased after physiological maturity in all six varieties of groundnut. The significantly lowest EC was observed in treatment combination V3M3 (0.23mmhos/cm). The significantly highest EC was recorded in genotype TPG-41 (0.53mmhos/cm) harvested at M2 (10 DAPM). These studies indicated that, after the physiological maturity fresh seed dormancy breaks down and germination percentage goes on increasing. Harvesting stages did not exhibit any

influence on EC values of groundnut seeds. The seeds which record high EC values have been found to be correlated with low germination percentage.

Issa *et al.* (2018) studied Inheritance of fresh seed dormancy in Spanish x Spanish crosses was studied with two sets of segregating populations, an F2 population derived from true F1 hybrids identified with peanut microsatellites markers and other populations (F2, BC1P1S and (BC1P2S) from randomly-selected F1 individuals. In the F2 population developed with true F1 hybrids, the chi square test was not significant for the deviation from the expected 3:1 (dormant: non-dormant) ratio. In addition, the bimodal frequency distribution curve with the F2 population gave more evidence that fresh seed dormancy is controlled by a single dominant gene. The average frequency (48%) of true F1 hybrids give evidence that deviations from expected ratios in the populations (F2 and BC1P1S) developed from non-tested F1 individuals, is most likely due to inadvertent selfs. This study emphasized the need to identify with molecular markers the cross progenies in self-pollinated crops as peanut before testing for any trait.

Kumar *et al.*(2018) studied 33 advanced breeding lines and two varieties during summer 2012 and 2013 Advanced breeding lines evaluated for fresh seed dormancy showed significant genetic variation for germination percent at weekly intervals, duration, intensity and degree of fresh seed dormancy in groundnut. It was concluded that three advanced breeding lines PBS-12171, PBS-12169 and PBS-18035hadmore than four week duration of fresh seed dormancy, highest intensity of fresh seed dormancy and degree of fresh seed dormancy during 2012 and 2013. Therefore, these genotypes were identified as new sources of fresh seed dormancy in groundnut.

Simple Sequence Repeats(SSRs)

What is SSR, Importance, principal, characters?

Cultivated peanuts are an allotetraploid (genome AABB) with a low genetic background. Among different marker systems analysed in the groundnut, like other plant species, SSR markers have been found more informative and useful for genetic analysis and breeding applications (Gupta

and Varshney 2000, Pandey et al. 2012) due to its nature of Co-dominance, Multi allelic and Uniformly dispersed throughout plant genome. In Groundnut SSR has proved to be useful for accession discrimination and assessment of genetic variation and hence work regarding primer development and screening genotypes are widely used in every crops therefore Guohao et al. (2003) developed microsatellite markers in cultivated peanut and reported that the GA/CT repeated the most frequently and dispersed. The primer pairs were designed for fifty-six different microsatellites, 19 of which showed a polymorphism among the genotypes studied. The average number of alleles per locus was 4.25 and up to 14 alleles were found at one locus. They suggested that microsatellite DNA markers produced a higher level of DNA polymorphism than other DNA markers along with this Ferguson et al. (2004) identified and characterized 110 sequence-tagged microsatellite (STMS) markers that revealed genetic variation in a diverse array of 24 peanut accessions. The simple sequence repeats (SSRs) were identified with a probe of two 27,648-clone genomic libraries.

Work done for molecular characterization using SSR markers in groundnut:

Subramanian *et al.*,(2000) analyzed 70 groundnut genotypes showing variability for many morphological, physiological and other characters, for polymorphism random amplified polymorphic DNA (RAPD) assay with 48 oligonucleotide primers in which only 7 (14.7%) primers yielded polymorphic amplification products among all.

He *et al.*,(2003) developed microsatellite markers in cultivated peanut and reported that the GA/CT repeated the most frequently and dispersed. The primer pairs were designed for fifty-six different microsatellites, 19 of which showed a polymorphism among the genotypes studied. The average number of alleles per locus was 4.25 and up to 14 alleles were found at one locus. They suggested that microsatellite DNA markers produced a higher level of DNA polymorphism than other DNA markers.

Ferguson *et al.*,(2004) identified and characterized 110 sequence-tagged microsatellite (STMS) markers that revealed genetic variation in a diverse array of 24 peanut accessions. The simple sequence repeats (SSRs) were identified with a probe of two 27,648-clone genomic libraries.

Hossain *et al.* (2006) studied 25 groundnut genotypes against leaf spot and rust diseases to select resistant sources. The genotypes were scored at 0-5 scale one week before harvest against both the diseases. The genotypes namely; 259/88 and 262/88 were found moderately resistant to both leaf spot and rust diseases. It was noted that genotype 269/89 was moderately resistant against leaf spot disease only and M-5 and 255/88 were moderately resistant to rust only. Leaf spots and rust moderately resistant genotypes had lower percentage defoliation.

Mace *et al.* (2006) conducted an experiment to identify diverse disease resistant germplasm lines for the development of mapping populations and their introduction into breeding programs. Twenty-three SSRs were screened across 22 groundnut genotypes with differing levels of resistance to rust and LLS. Overall, 135 alleles across 23 loci were observed in the 22 genotypes screened. Twelve of the 23SSRs (52%) showed a high level of polymorphism, with PIC values 0.5. Multi-dimensional scaling and cluster analyses revealed.

Cuc *et al.* (2008) reported 112 alleles obtained by 46 markers, a phenogram was constructed to understand the relationships among the 32 genotypes. Majority of the genotypes representing subspecies *hypogaea* were grouped together in one cluster, while the genotypes belonging to subspecies *fastigiata* were grouped mainly under two clusters.

Varshney *et al.* (2009) evaluated high level of natural variation in groundnut (*Arachis hypogaea* L.) germplasm lines by using SSR markers. A total of 59 unique alleles and 127 rare alleles were detected at almost all the loci assay

Khedikar *et al.* (2010) screened 1,089 simple sequence repeat (SSR) markers, out of which 67 (6.15%) were found polymorphic. Interestingly a major QTL associated with rust (QTLrust01), contributing 6.90–55.20%

variation, was identified by both CIM and single marker analysis (SMA). A candidate SSR marker (IPAHM 103) linked with this QTL was validated using a wide range of resistant/susceptible breeding lines as well as progeny lines of another mapping population (TG 26 × GPB).

Song *et al.* (2010) tested 610 ESTs that contained one or more SSRs from 12,000 peanut ESTs. The most abundant SSRs in peanut are trinucleotides (66.3%) SSRs and followed by dinucleotide (28.8%) SSRs. Results showed that polymorphism was very low in cultivars, while high level of polymorphism was revealed in wild type peanut.

Pandey *et al.* (2012) studied on highly informative genic and genomic SSR markers to facilitate molecular breeding 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 and concluded that out of 4485 markers 199 markers were working well and were highly polymorphic with >0.50 PIC values.

Sujay *et al.* (2012) identified tightly linked markers to these diseases, a total of 3,097 simple sequence repeats (SSRs) were screened on the parents of two recombinant inbred line (RIL) populations, namely TAG 24 9 GPBD 4 (RIL4) and TG 26 9 GPBD 4 (RIL-5), and segregation data were obtained for 209 marker loci for each of the mapping populations.

Anitha *et al.* (2014) worked on 64 groundnut genotypes to validate reported molecular markers associated with QTLs of pod and kernel traits for oil content; 100-pod weight (g), pod length (mm), kernel length (mm); 100-kernel weight (g) and days to maturity. Genotype data were generated using 64 genotypes and the seven markers associated with various phenotypic traits. They performed Single marker analysis to ascertain the relationship between the marker and traits. The results indicated that markers PM36, IPAHM103 and PGS19D09 for oil content; PM375 for 100-pod weight, pod length and kernel length; PM137 and PM384 for 100-kernel weight explained more than 10 percent of phenotypic variation.

Gajjar *et al.* (2014) investigated 22 SSR markers linked to rust and LLS diseases resistance with 95 diverse genotypes for marker validation, of which 16 SSRs could be validated. Among rust resistant varieties and germplasm lines, nearly perfect marker validation was recorded but for 30 wild *Arachis species*, marker validation was very poor. The results of principal coordinate analysis (PCoA) were comparable to the cluster analysis.

Kanyika *et al.* (2015) identified 376 informative markers, in which 139 (37%) have previously been mapped to the *Arachis* genome and can now be employed in Quantitative Trait Loci (QTL) mapping and the additional237 markers identified can be used to improve the efficiency of introgression of resistance to multiple important biotic constraints into farmer-preferred varieties of Sub-Southern Africa.

Zhou *et al.* (2016) conducted quantitative trait locus (QTL) analysis for LLS and three plant-type related traits including height of main stem (HMS), length of the longest branch (LLB) and total number of branches (TNB). The result of this study not only provided new favorable QTLs for fine-mapping, but also suggested that the relationship between LLS and plant-type-related traits of HMS, LLB and TNB should be considered while breeding for improved LLS resistance in peanut.

Divyadharsini *et al.* (2017) validated SSR markers in groundnut for disease resistance, considering the molecular analysis in CO7 × COG043709 were found to be polymorphic. The marker seq8D09 alone 19.0% of phenotypic variations respectively.

Pandey*et al*.,(2017) study has provided allele specific PCR-based markerfor late leaf spot and rust using QTL-seq approach and reported that these marker would be cost-effective and very easy to analysis.

Zongo *et al.* (2017) crossed an ELS susceptible genotype QH243C and an ELS resistant genotype NAMA and the F2 genotypic population and F3phenotypic progenies data were used for marker-trait association analysis. Parents are surveyed with 179 simple sequence repeat SSR markers out of which 103 SSR markers were found to be polymorphic between the parents.

Chapter – III

MATERIAL AND METHODS

The experimental materials used and the method applied during the present investigation entitled “Association analysis, fresh seed dormancy and screening of genotypes using SSR marker in groundnut *(Arachis hypogaea* L*).*” has been described below.

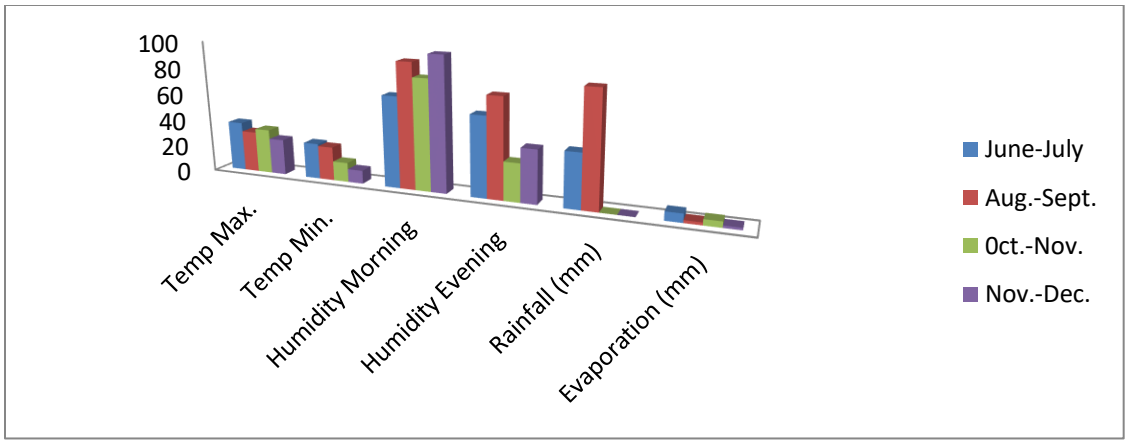
3.1 Experimental site

The field experiment has been conducted at Research Farm, Department of Genetics and Plant Breeding, RVSKVV Gwalior (M.P.) and molecular work was carried out at Plant Molecular Biology Laboratory, Department of Plant Molecular Biology and Biotechnology, College of Agriculture, Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior (M.P.), during 2018-2019.

3.1.1. Climatic condition during groundnut cropping monsoon season:

In India groundnut can ideally grow during monsoon and summer season. Kharif season crop accounts 80% of groundnut area. About 75% of the groundnut area lies in a low to moderate rainfall zone. Temperature between 25 – 30℃ suits best for the crop with an average rainfall ranges between 50-75 cm. Continuous rain, stagnant water and prolong drought causes major loss.

The experimental field of College of Agriculture, Gwalior is located at 26˚13 N latitude, 78˚14’ Elongitude and at an altitude of 211.5m above the sea level in gird belt. It has hot weather condition and during summers the temperature raises beyond 45℃.



3.2. EXPERIMENTAL MATERIAL

The plant materials consisted 32 uncharacterized germplasm lines and 4 varieties of Groundnut. Uncharacterized germplasm were received from ICRISAT, Hyderabad and DGR, Junagarh. Check varieties for the experiment includes GPBD4, Foliar disease resistance and a high yielding variety; KDG128, Foliar disease resistance and a high yielding variety;JGN3,Variety released from Madhya Pradesh sensitive to foliar diseases and Gangapuri, sensitive for foliar disease and good taste.

Material for fresh seed dormancy:

- Positive control parent : TG-26
- Negative control parent: JGN-3
- 36 genotypes

Details of Experiments:

Design	:	Randomized Complete Block Design
Number of Replication	:	3
Spacing	:	30 cm X 10 cm
Genotypes	:	32

1.Control ICRISAT-1	12.Control ICRISAT-13	23.ICRISAT-12
2.Control ICRISAT-2	13.Control ICRISAT-14	24.ICRISAT-13
3.Control ICRISAT-3	14.ICRISAT-1	25.ICRISAT-14
4.Control ICRISAT-4	15.ICRISAT-2	26.ICRISAT-15
5.Control ICRISAT-5	16.ICRISAT-3	27.GG7XGPBD4 (F_7)
6.Control ICRISAT-6	17.ICRISAT-4	28.GG2XICG1697 (F_7)
7.Control ICRISAT-7	18.ICRISAT-6	29.GG2XRHRG06083 (F_6)
8.Control ICRISAT-9	19.ICRISAT-7	30.GJG17XGPBD4 (BC_1F_6)
9.Control ICRISAT-10	20.ICRISAT-8	31.GG20xGBPD4 (F_8)
10.Control ICRISAT-11	21.ICRISAT-10	32.BAU13xCS196(F_8)
11.Control ICRISAT-12	22.ICRISAT-11	

Check variety :- JGN-3, Gangapuri, KDG128, GPBD4 .

Observations to be recorded:

S.NO.	Yield components		
1.	Days to 50 (%) flowering	7.	Kernel yield per plant (gm)
2.	Plant height (cm)	8.	Shelling (%)
3.	Number of primary branches per plant	9.	100 pod weight (gm)
4.	Number of secondary branches per plant	10	100 kernel weight (gm)
5.	Days to maturity	11	Sound mature kernel (%)
6.	pod yield per plant (gm)		

3.4 Observations recorded:

Observations were recorded on randomly chosen five competitive plants in each genotype in each replication for all the characters except days to 50 per

cent flowering. The mean values of five competitive plants the plants were selected excluding the border rows was expressed as mean of the respective character. The details of data recorded are as follows.

3.4.1 Days to 50 per cent flowering: Number of days taken from sowing to the attainment of flowering by 50 per cent of the plants in each plot and in each replication was recorded.

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

3.4.3 Primary branches per plant: Five plants were selected randomly after harvest in each plot and number of primary branches were recorded on them.

3.4.4Secondary branches per plant: Number of secondary branches were recorded from same five plants, which were selected for previous observation

3.4.5 Days to maturity: Number of days taken from date of sowing to physiological maturity of the plant was recorded as days to maturity.

3.4.6 Pod yield per plant (g): The dry weight of all filled and mature pods per plant was measured in grams after harvest, drying and cleaning.

3.4.7 Kernel yield per plant (g): The pods were shelled and kernel yield per plant was recorded in grams.

3.4.8100 pod weight (g): 100 pods weight was recorded by counting 100 pods randomly from pods of 5 plants in each replication and expressed in grams.

3.4.9 100 kernel weight (g): 100 kernel weight was recorded by counting 100 kernels randomly from bulked kernels of five plants in each replication and expressed in grams.

3.4.10 Shelling %: The ratio of kernel yield per plant to the pod yield was worked out and expressed in percentage.

$$\text{Shelling \%} = \frac{\text{Kernel weight}}{\text{Pod weight}} \times 100$$

3.4.11 Sound mature kernel percentage: Seed sample obtained from 100 gram shelled pods used to determine shelling percentage was taken, then well-developed kernels were sorted and sound mature kernel percentage was worked out as follows

$$\text{Sound mature kernel (\%)} = \frac{\text{Number of mature sound kernel}}{\text{Total number of kernels}} \times 100$$

Estimation of phenotypic and genotypic coefficients of variation:

The phenotypic and genotypic coefficients of variation in per cent were computed by the following formulae given by Burton (1952).

$$\text{Phenotypic Coefficient of Variation (PCV)} = \frac{\text{Phenotypic standard deviation}}{\text{Mean}} \times 100$$

$$\text{Genotypic Coefficient of Variation (GCV)} = \frac{\text{Genotypic standard deviation}}{\text{Mean}} \times 100$$

The PCV and GCV values are ranked as low, medium and high (Shivasubramanian and Menon, 1973) and are mentioned below:

0-10%- Low

10-20% - Moderate

>20%- High

Estimation of heritability and genetic advance:

Heritability in per cent in broad sense was estimated by the following formula given by Singh and Choudhary (1977):

$$\text{Heritability } (h^2) = \frac{\text{Genotypic variance}}{\text{Phenotypic variance}} \times 100$$

Heritability values are catagorised as low, moderate and high (Robinson *et al.,* 1949) and are given below,

0-30% - Low

30-60% - Moderate

60% and above - High

The estimates of expected genetic advance from selection, G(s), was obtained by the formula suggested by Robinson*et al.* (1949).

$G(s) = k \times h^2 \times \sigma p$

where,

k = Selection differential in standard deviation units which is 2.06 for 5% selection intensity,

h^2 = Heritability in broad sense, and

σ_p= Phenotypic standard deviation

Genetic advance was expressed as percentage of mean by using the formula suggested by Johnson *et al*. (1955).

Genetic advance as percentage of mean = $\frac{\text{Genetic advance}}{\text{Grand mean}} \times 100$

Genetic advance as percent of mean was classified as low, moderate and high (Johnson *et al*., 1955) and values are given below:

0-10% - Low

10-20% - Moderate

20% and above - High

Path coefficient analysis:

The proportion of direct and indirect contributions of various traits to the total correlation coefficients with seed yield per plant was estimated through path coefficient analysis as suggested by Wright (1921, 1934) and elaborated by Dewey and Lu (1959).

Path coefficient is a standardized partial regression, which measures the direct influence of one variable upon another and allows partition of correlation coefficient into components of direct and indirect effects.

To estimate various direct and indirect effects, the following set of simultaneous equations were formed and solved.

$r_{1y} = P_{1y} + r_{12}P_{2y} + r_{13}P_{3y} + \ldots + r_{1I}P_{Iy}$

$r_{2y} = r_{2y}P_{1y} + P_{2y} + r_{23}P_{3y} + \ldots + r_{2I}P_{Iy}$

$.r_{Iy} = r_{I1}P_{1y} + r_{I2}P_{2y} + r_{I3}P_{3y} + \ldots + P_{Iy}$

where,

r_{1y} to r_{Iy}= Coefficient of correlation between causal factor 1 to I and dependent character y,

r_{12} to $r_{I-1,I}$= Coefficient of correlation among causal factors themselves, and

P_{1y} to P_{Iy} = Direct effects of characters 1 to I on character y.

Residual effect, which measures the contribution of the characters not considered in the causal scheme, was obtained as:

Residual effect (P_{RY}) = $\sqrt{1 - R^2}$

where,

$$R^2 = \sum_{iy} P_{iy}^2 + 2\sum_{\substack{i \neq j \\ i > j}} P_{iy}\, P_{jy}\, r_{ij}$$

3.5 Estimation of fresh seed dormancy:

Appreciable dormancy observed in the freshly harvested seeds of several bunch groundnut cultivars which disappears after curing is called fresh seed dormancy. Fresh seed dormancy is characterized by its intensity and duration.

Fresh seed dormancy parameters were estimated using the method suggested by Kumar et al (1991).

1. Germination percentage : The percentage of germinated seed for entry at a given date was calculated by the following formula :

$$\text{Germination (\%)} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100$$

2.intensity of fresh seed dormancy : the intensity of dormancy was measured as percentage of non-germinated seed at 7 days after sowing.
Intensity of dormancy (%) =100-germination percentage.

3. Duration of fresh seed dormancy: duration of dormancy was measured by days taken to attend 50 per cent germination by a genotype.

3.6 Molecular analysis

3.6.1 Reagents for plant total genomic DNA isolation

0.5 M EDTA (pH 8.0): 186.1 g of sodium salt of EDTA was dissolved in 800 ml of MQ water, pH was adjusted to 8.0 with NaOH pellets. The final volume was adjusted to one litre with MQ water and sterilized by autoclaving.

4 M NaCl: 233.8 g of NaCl was dissolved in 800 ml of MQ water. The final volume was adjusted to one litre with MQ water and sterilized by autoclaving.

1M Tris-Cl (pH 8.0): 121.1 g of Tris-Cl salt was dissolved in 800 ml of sterile MQ water. pH was adjusted to 8.0with concentrated 1N HCl. The final volume was adjusted to one litre with MQ water and sterilized by autoclaving.

10% CTAB: 100 gm of CTAB powder was dissolved in sterile MQ water and the volume was adjusted to one litre.

Phenol : Chloroform : isoamyl alcohol

Buffer saturated phenol, chloroform and isoamyl alcohol were mixed in the ratio of 25: 24: 1. The equilibrated mixture was stored under a layer of 0.01 M Tris-HCl (pH 7.6) at 4^0C in dark glass bottle.

3.6.2 Total Genomic DNA isolation Using Modified CTAB method (Fig. 3)

- Pre-warmed (65°C) DNA extraction buffer was made and freshly 0.2% β-mercaptoethanol was added.
- Fresh young 2-3 pearl millet leaves cut and crushed in mortar pestle with DNA Extraction Buffer.
- Suspension was transferred in 2ml Eppendorf tube.
- Eppendorf tube subjected to water bath at 65°C for 45 minutes and mixed by gentle swirling by each 15 minute interval.
- The Tubes were allowed to cool to room temperature and the mixture emulsified with an equal volume (800µl) of phenol: chloroform: isoamyl alcohol (25: 24: 1) for 1-2 min by gentle inversion and centrifugation at 10,000 rpm for 10 min at room temperature.
- The separate aqueous phase was transferred from the contents to fresh clean 2 ml Eppendorf tube.
- 3.5 µl of RNase A (20 mg/ml) was added and the contents mixed and was incubated at 37°C for 30-45 min.
- $1/10^{th}$ volume of 3M sodium acetate (pH 5.2) and 2 volume of absolute ethanol was added to the aqueous solution, mixed by quick gentle inversion for five to six times and leaved for overnight at 4°C.
- Pellet the DNA by centrifugation of Eppendorf tube at 10000 rpm for 10 min and discard the supernatant.
- The DNA pellet was washed with 200 µl of 70% ethanol by centrifugation at 5000 rpm for 5 min, air dried properly till ethanol smell goes and dissolved finally in TE (100 mMTris and 50 mM EDTA, pH 8.0) buffer (100-200 µl) based on DNA pellet size and stored at 4°C for complete dissolution.

- Added 5 micro liters RNase @ 500 microliter DNA "50mg/ml stock" incubated at 30^0C for 30 minutes. Optional: Check the RNA presence by running 0.8% Agarose gel (RNA will appear at lower portion of gel).

3.6.3 Purification of DNA

Major contaminants in crude DNA preparation are RNA, protein and Inclusion of CTAB in DNA extraction buffer helps in elimination of polysaccharides from DNA. The RNA is removed by treating the sample with RNase, Protein and phenolic is removed by treating the sample with PCI (Phenol: Chloroforms: Isoamyl alchohal-25:24:1) (Fig.4.)

3.6.4 Quantification with Spectrophotometer

Spectrophotometer is a device which is very useful to check the quality and quantity of DNA. The working session of Spectrophotometer gets started with a blank reading first by loading NFW 1 µl to bring the device in calibrated form, then the main reading starts by loading 1 µl DNA sample. The reading is taken on the basis of ranges A 260/A280 and A260/A230. The best quality DNA comes under range of 1 to 2.5. Through Spectrophotometer the concentration of DNA in 1 µl can also be detected. In these samples of DNA as the concentration was too high therefore a precautionary measure of diluting it with required quantity of NFW was conducted to make it suitable for PCR amplification (Table 3.6) (Fig.7).

3.5.6 RNase treatment

5µL RNase A (10 mg/mL) was added to each sample and incubated at 37°C for 30 min to remove the RNA contamination from the samples (Fig.3).

.3.5.7 Agarose gel electrophoresis

The quality and quantity of DNA was checked by horizontal submarine gel electrophoresis on 0.8% agarose gel. DNA samples (2µl) were loaded with λ

uncut DNA ladder well in agarose gels and run at 80V for 60 min. The gel was stained with ethidiumbromide and observed under UV–transilluminator. The amount of fluorescence was proportional to the total mass of DNA (Fig.4). After quantification the DNA was diluted with distilled water. The final concentration of DNA obtained to be used for PCR was 25ng/µl.

Protocol for agarose gel electrophoresis

- Weight out the 0.8 g of agarose into plastic plate.
- 10% TBE (10x) buffer solution was made.(Table 3.5)
- Agarose and TBE buffer mixed in Erlenmeyer flask.
- Agarose / buffer mixture was melted. This is most commonly done by heating in a microwave.
- The flask was removed and swirled the content to mix well. Repeated until the agarose has completely dissolved.
- The agarose was allowed to cool at room temperature until the temperature reached at $65^{O}C$.
- 8% Ethidium Bromide was added.
- An appropriate comb placed into gel mold to create the wells.
- The molten agarose was poured into gel mold. The agarose allowed to set at room temperature and the comb removed.
- The gel was placed at gel electrophoresis unit.
- The DNA sample was loaded slowly and carefully into the gel with Lambda uncut DNA Ladder.
- Turn on the power.
- Run the gel until the DNA has migrated to an appropriate distance.
- When electrophoresis was completed turn off the power supply.
- Gel was removed from the gel electrophoresis unit.
- Gel was exposed to the UV transilluminator.
- DNA bands visualized as fluorescent bands.
- Picture/Snap of the gel was taken.
- The gel was disposed.

Table 3.6.1: Formation of TBE buffer(10x) for 1litre

S.No	Chemicals	Quantity(gm)
1	Tris – Cl	107.8
2	Boric acid	55.02
3	EDTA	9.36
Ph		**8**

Formation of primer working solution

Centrifuge the primer at 2000 rpm for 30 second.

Take 10µl Primer forward, 10µl Primer reverse and 80µl Nuclease free water into 1.5ml eppendorf tube.

3.6 PCR amplification

The PCR reaction was performed in Bio-Rad thermo cycler. The reaction components and reaction cycle is mentioned in table 3.6. The SSR primers (forward and reverse) used in this study is mentioned in table 3.7.

PCR process: Take 96 well plate and add 1µl of appropriate DNA sample in all wells. Prepare master mix (Table 3.8).

Table 3.6.2: PCR reaction components for SSR markers

S.No.	Reagent	Stock concentration	Quantity
1.	Nanopure H_2O		5.8 µL
2.	PCR Buffer	10X	1 µL
3.	$Mgcl_2$	25Mm	0.5 µL
4.	dNTPs (mix)	10mM	0.5 µL
5.	Primer	100pmol	1 µL
6.	TaqDNA polymerase	1 unit/	0.2 µL
7.	DNA template	30ng/ µG	1.0 µL
	Total		10 µL

Aliquot mastermix to each tube and close through optical sealing film. 96 well plate transfer into PCR machine and manually setup on PCR.

Table 3.6.3 PCR Program for different SSR Priming Steps

S.No.	Steps	Temperature (°C)	Duration
1.	Initial denaturation	95	3 min
2.	Denaturation	95	20 sec
3.	Annealing	50-55	20 sec
4.	Initial elongation	72	1.5 min
5.	Elongation	72	10 min
6.	Hold	15	Infinite
7.	Number of cycles		35

Formation of 3% agarose gel for PCR and loaded PCR product with 100kb DNA ladder and visualized the result in gel documentation (Fig.6).

Table 3.6.4.PCR reaction with dream taq components for SSR markers

S.No.	Reagent	Stock concentration	Quantity
1.	Primer	100	1 µL
2.	Nano pure H_2O		2.5 µL
3.	Ready to use master mix	2x	5 µL
4.	DNA template	30ng/ µG	1.5 µL
	Total		10 µL

Chapter-IV

RESULTS

The experimental results of the present investigation have been mentioned under following sub-headings.

4.1 Analysis of variance

4.2 Parameters of genetic variability

4.2.1 Mean and range

4.2.2 Genotypic and phenotypic coefficient of variation

4.2.3 Heritability

4.2.4 Genetic advance

4.3 Correlation coefficients

4.4 Path coefficients

4.5 Fresh Seed Dormancy

4.6 Screening of genotypes for foliar diseases using SSR markers

4.1 Analysis of variance:

The ANOVA (Table 4.1.1) *viz.* ,days to 50% flowering, plant height, number of primary branches per plant, number of secondary branches per plant, days to maturity, pod yield per plant(gm), kernel yield per plant (gm), shelling(%), 100 pod weight(gm), 100 kernel weight(gm) and sound mature kernel(%).

4.2Parameters of genetic variability:

The parameters of genetic variability *viz*., mean, range, phenotypic and genotypic coefficient of variation (%), heritability in broad sense (%), genetic advance and genetic advance as percentage of mean for each trait are presented in table 4.2.2.

4.1.1 Days to 50% flowering

Days to 50% flowering obtained the minimum value 25 days for CONTROL ICRISAT-2 and maximum value 35 days for ICRISAT-14 with average value as 30 days.

4.1.2 Plant height

The mean value of plant height was 29.06 cm that ranged from 22 cm (ICRISAT-12) to 36 cm (GG7 XGPBD4).

4.1.3 Number of primary branches per plant

Number of primary branches per plant obtained the minimum value 4 (CONTROL ICRISAT-11) and maximum value 9 (CONTROL ICRISAT-6) with average value as 6.69.

4.1.4 Number of secondary branches per plant

Number of secondary branches per plant ranged from 5 (ICRISAT-5) to 14 (BAU13XCS196). The average value was 9.71.

4.1.5 Days to maturity

Days to maturity ranged from 95 days (CONTROL ICRISAT-1) to 111 days (GG7XICG1697). The average value for the trait was 103.36 days.

4.1.6 Pod yield per plant

GG20XGPBD4 showed the least pod yield per plant (12 g), while ICRISAT-14 had the maximum 22.8 g with a mean of 14.80.

4.1.7 Kernel yield per plant

Kernel yield per plant ranged from 5.4 g (ICRISAT-10) to 12.6 g (ICRISAT-3) with a mean of 9.31.

4.1.8 Shelling %

Shelling % ranged from 56 (ICRISAT-11) to 82 (ICRISAT-6) with a mean value of 68.41.

4.1.9 100 pod weight

The mean 100 pod weight ranged from 64.1 g (CONTROL ICRISAT-10) to 71.7 g (GG7XGPBD4) with overall mean of 68.63 g.

4.1.10 100 kernel weight

The mean 100 kernel weight ranged from 36.1 g (CONTROL ICRISAT-1) to 48.3 g (ICRISAT-1) with a mean value of 42.85.

4.1.11 Sound mature kernel

The mean sound mature kernel ranged from 75 % (ICRISAT-13) to 85 % (ICRISAT-2) with a mean value of 79.8.

4.2.2 Genotypic and phenotypic coefficient of variation:

The genetic variability is the raw material of plant breeding on which selection acts to evolve superior genotypes. Thus, higher amount of variation present for a concerned character in the breeding materials, greater scope for its improvement through selection. The phenotypic coefficient of variation was significantly higher in magnitude than that of genotypic coefficient of variation for all the characters under study. Phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) are categorized as low (<10%), moderate (10-20%) and high (> 20%) as suggested by Sivasubramanian and Madhavamenon (1973). The GCV and PCV for yield and yield contributing characters have been presented in table 4.2.

Plant height had recorded the highest PCV (67.29%) followed by number of primary branches per plant (66.80%), days to 50% flowering (65.01%), number of secondary branches per plant (61.48%), kernel yield per plant (59.81%), days to maturity (57.74%), pod yield per plant (49.54%), 100 kernel weight (44.81%), sound mature kernel (34.71%), shelling percentage (26.29%) and 100 pod weight (23.81%).

number of primary branches per plant had recorded the highest GCV (57.82%) followed by days to 50% flowering (56.58%), days to maturity (53.02%), number of secondary branches per plant (43.71%), kernel yield per plant (42.83%), plant height (41.41%) and pod yield per plant (31.01%). Moderate

GCV recorded for 100 kernel weight (15.54%), sound mature kernel (13.90%), 100 pod weight (12.90%) and the low GCV recorded for shelling percentage (7.46%).

4.2.3 Heritability in Broad Sense (%):

Heritability in broad sense were estimated for all the 11 characters under study are presented in table 4.2. Heritability estimate were classified as high (> 70%), moderate (50- 70%) and low (< 50%). The high heritability estimated for days to maturity (84.31%), days to 50% flowering (75.75%), number of primary branches per plant (74.94%) and moderate heritability were recorded for kernel yield per plant (51.29%) and number of secondary branches per plant (50.54%). Low heritability were observed for pod yield per plant (39.19%), plant height (37.87%), 100 pod weight (29.33%), sound mature kernel (16.04%), 100 kernel weight (12.02%) and shelling % (8.05%).

4.2.4 Genetic advance as percentage of mean:

Genetic advance as percentage of mean were estimated for all the 11 characters under study are presented in table 4.2. The genetic advance was classified as suggested by Johnson et al. (1955) i.e., high (> 20%), moderate (10-20%) and low (< 10%). The high genetic advance as percentage of mean were observed for number of primary branches per plant (39.88%) followed by kernel yield per plant (20.71%), number of secondary branches per plant (20.54%) and moderate genetic advance as percentage of mean were recorded for days to 50% flowering (18.52%) followed by pod yield per plant (10.40%) and low genetic advance as percentage of mean was recorded for days to maturity (9.86%), plant height (9.74%), 100 pod weight (1.74%), 100 kernel weight (1.69%), sound mature kernel (1.28%), and shelling % (0.53%).

Fig.3. Field view of groundnut germplasms in experimental field.

Table 4.1.1: Mean sum of squares and their significance for plant traits in groundnut

S. No.	Characters	Mean sum of squares		
		Replication (2)	Genotypes (35)	Error (70)
1	Days to 50% flowering	1.36	22.29**	3.08
2	Plant height	231.11**	18.14**	8.18
3	No. of primary branches per plant	0.18	5.22*	0.75
4	No. of secondary branches per plant	2.79	5.53*	1.82
5	Days to maturity	8.78	63.51**	5.41
6.	Pod yield per plant	3.50	5.05*	2.21
7	Kernel yield per plant	50.58**	5.04*	1.62
8	Shelling (%)	10.56*	5.11*	4.35
9	100 Pod weight	1.93	5.03*	2.75
10	100 Kernel weight	7.21*	9.64**	7.57
11	Sound mature kernel (%)	12.78*	11.16**	8.07

* Significant at p=0.05, ** Significant at p=0.01Note: Figures in parentheses represent d.f.

Table 4.2.1: Estimates of range, mean and genetic parameters for different characters of groundnut

S. no.	Characters	Mean	Range	Coefficient of variation (%)		Broad sense Heritability(%)	Genetic advance	Genetic advance as % of mean
				PCV (%)	GCV (%)			
1	Days to 50% flowering	30.00	30	65.01	56.58	75.75	5.56	18.52
2	Plant height (cm)	29.06	29	67.29	41.41	37.87	2.83	9.74
3	No. of primary branches /plant	6.69	6.5	66.80	57.82	74.94	2.67	39.88
4	No. of secondary branches / plant	9.71	9.5	61.48	43.71	50.54	1.99	20.54
5	days to maturity	103.36	103	57.74	53.02	84.31	10.20	9.86
6	Pod yield per plant	14.80	17.4	49.54	31.01	39.19	1.54	10.40
7	Kernel yield per plant	9.31	9	59.81	42.83	51.29	1.93	20.71
8	Shelling %	68.41	71	26.29	7.46	8.05	0.36	0.53
9	100 pod weight	68.63	67.9	23.81	12.90	29.33	1.19	1.74
10	100 kernel weight	42.85	42.2	44.81	15.54	12.02	0.73	1.69
11	Sound mature kernel	79.80	80	34.71	13.90	16.04	1.02	1.28

4.3 Correlation coefficient:

Analysis of correlation coefficient revealed that the degree of association between yield and its components gives the mutual relationship between the yield components. It helps in direct selection of associated characters for the improvement of desirable traits. For the improvement of complex characters like kernel yield (g) per plant, pod yield (g) per plant for which direct selection could not effective, while selection for associated characters would be effective. In the present investigation, correlation coefficients were estimated among the characters under studied to find out association of kernel yield per plant with its components at genotypic as well as phenotypic levels.

4.3.1 Correlation between kernel yield per plant and other characters:

A perusal of Table 4.3.1 revealed that kernel yield per plant was positive and highly significant correlated with primary branches per plant (r_g=0.274**, r_p=0.159**), Kernel yield per plant showed significant negative association with 100 pod weight (r_g=-0.430*, r_g= -0.188**), days to maturity (r_g=-0.155**) and plant height (r_g=-0.333*).

4.3.2 Correlation among the characters:

4.3.2.1 Days to 50% flowering:

Days to 50% flowering had positive and highly significant association with pod yield per plant (r_g=0.249**), 100 kernel weight (r_g=0.879*, r_p=0.221**) and sound mature kernel (r_g=0.250**) and significant positive association with number of secondary branches (r_g=0.462*, r_p=0.325*), days to maturity (r_g=0.967*, r_p=0.720*), 100 pod weight (r_g=0.365*), while significant negative association with number of primary branches (r_g=-0.357*, r_p=-0.293**), plant height (r_g=-0.189**) and shelling % (r_g=-0.619*).

4.3.2.2 Plant height:

Plant height showed significant negative association with pod yield per plant ($r_{g=}$ -0.304**), 100 pod weight (r_g=-0.438*) and kernel yield per plant (r_g=-0.334).

4.3.2.3 Number of primary branches per plant:

Number of primary branches per plant exhibited high significant positive association with kernel yield per plant (r_g=0.275**, r_p=0.160**) and sound mature kernel % (r_g=0.228**), while significant negative association with number of secondary branches (r_g=-0.210**, r_p=-0.165**), shelling % (r_g=-0.785*, r_p=-0.237**), days to maturity (r_g=-0.186**) and 100 kernel weight (r_g=-0.198**).

4.3.2.4 Number of secondary branches per plant:

Number of secondary branches per plant exhibited significant positive association with days to maturity (r_g=0.350*, r_p=0.206**), shelling % (r_g=0.463*, r_p=0.215**), 100 kernel weight (r_g=0.154**) and pod yield per plant (r_g=0.348*), whereas, significant negative association with 100 pod weight (r_g=-0.672*, r_p=-.0.237**) and sound mature kernel (r_g=-1.614).

4.3.2.5 Days to maturity:

Days to maturity showed significant positive association with100 pod weight (r_g=0.388*, r_p=0.208**),100 kernel weight (r_g=0.346) and sound mature kernel % (r_g=0.433*), while significant negative association with shelling% (r_g=-0.496*, r_p=-0.192**) and kernel yield per plant (r_g=-0.155**).

4.3.2.6 Pod yield per plant:

Pod yield per plant observed significant positive association with 100 kernel weight (r_g=1.090*), 100 pod weight (r_p=0.212**) and sound mature kernel (r_g=0.371*), whereas, significant negative association with shelling % (r_g=-1.115*, r_p=-0.219**), and sound mature kernel (r_p=-0.210**).

4.3.2.7 Kernel yield per plant:

Kernel yield per plant were observed significant negative significant correlation with 100 pod weight (r_g=-0.430*, r_p=-0.188**) and sound mature kernel (r_g=-0.177**).

4.3.2.8 Shelling percentage:

Shelling percentage had significant positive association with sound mature kernel (r_g=0.310*), whereas, significant negative association with 100 kernel weight (r_g=-1.482*) and 100 pod weight (r_g=-1.728*, r_p= -0.219**).

4.3.2.9 100 pod weight:

100 pod weight showed significant positive association with 100 kernel weight (r_g=0.464*) and sound mature kernel (r_g=1.288*).

4.3.2.10 100 kernel weight:

100 kernel weight had significant negative association with sound mature kernel (r_g=-0.251*).

b) At environmental level:

Though, the correlation coefficients at the environmental level were estimated, but, since, these values are of no pertinent use to plant breeders their individual description has been obliterated.

Table 4.3.1: Genotypic and phenotypic correlation coefficients between kernel yield per plant and other characters in groundnut

S. No.	Characters	Genotypic Correlation Coefficient	Phenotypic Correlation Coefficient
1.	Days to 50% flowering	-0.0861	-0.0651
2.	Plant height	-0.3337*	-0.1428
3.	Number of primary branches per plant	0.2745**	0.1598**
4.	Number of secondary branches per plant	-0.0880	-0.0631
5.	Days to maturity	-0.1552**	-0.1236
6.	Pod yield per plant (gm)	-0.0634	-0.0524
7.	Shelling %	-0.0894	-0.1123
8.	100 pod weight	-0.4302*	-0.1882**
9.	100 kernel weight	0.0634	0.0224
10.	Sound mature kernel	-0.1292	-0.0687

Table 4.3.2: Estimates of correlation coefficients: genotypic (upper triangle of data) and phenotypic (lower triangle of data).

	Days to 50% flowering	Plant height	No. of primary branches / plant	No. of secondary branches /plant	days to maturity	Pod yield/plant	Kernel yield/plant	Shelling %	100 pod weight	100 kernel weight	Sound mature kernel
Days to 50% flowering	1.000	-0.189**	-0.357*	0.462*	0.967*	0.249**	-0.086	-0.619*	0.365*	0.879*	0.250**
Plant height	-0.112	1.000	0.018	0.040	-0.033	-0.304**	-0.334*	0.131	-0.438*	0.017	-0.085
No. of primary branches / plant	-0.293**	-0.088	1.000	-0.210**	-0.186**	0.046	0.275**	-0.785*	0.067	-0.198**	0.228**
No. of secondary branches /plant	0.325*	-0.017	-0.165**	1.000	0.350*	0.348*	-0.088	0.463*	-0.672*	0.009	-1.614*
days to maturity	0.720*	-0.051	-0.114	0.206**	1.000	0.055	-0.155**	-0.496*	0.388*	0.346*	0.433*
Pod yield/plant	0.128	-0.052	0.029	0.021	0.065	1.000	-0.063	-1.115*	0.143	1.090*	0.371*
Kernel yield/plant	-0.065	-0.143	0.160**	-0.063	-0.124	-0.052	1.000	-0.089	-0.430*	0.063	-0.177**
Shelling %	-0.091	0.021	-0.237**	0.215**	-0.192**	-0.219**	-0.112	1.000	-1.728*	-1.482*	0.310**
100 pod weight	0.145	-0.123	0.046	-0.237**	0.208**	0.212**	-0.188**	-0.219**	1.000	0.464*	1.288*
100 kernel weight	0.221**	-0.025	-0.062	0.154**	0.132	0.067	0.022	-0.100	0.028	1.000	-0.251**
Sound mature kernel	0.136	0.031	0.048	0.002	0.138	-0.210**	-0.069	0.035	-0.037	0.109	1.000

*Significant at 5% level of significance and ** Significant at 1% level of significance

Table 4.3.3: Estimates of environmental correlation coefficients for 11 characters in groundnut

Characters	Days to 50% flowering	Plant height	No. of primary branches / plant	No. of secondary branches /plant	days to maturity	Pod yield/plant	Kernel yield/plant	Shelling %	100 pod weight	100 kernel weight	Sound mature kernel
Days to 50% flowering		-0.029	-0.097	0.114	-0.273**	-0.021	-0.033	0.132	-0.065	-0.095	0.108
Plant height			-0.248**	-0.063	-0.105	0.107	0.008	-0.002	0.034	-0.039	0.072
No. of primary branches / plant				-0.100	0.169**	0.011	-0.030	-0.092	0.035	-0.005	-0.100
No. of secondary branches /plant					-0.081	-0.245**	-0.037	0.180**	0.037	0.230**	-0.173**
days to maturity						0.108	-0.078	-0.165**	0.047	0.059	-0.059
Pod yield/plant							-0.044	-0.028	0.250	-0.231**	-0.335
Kernel yield/plant								-0.141	-0.036	0.010	-0.049
Shelling %									0.057	0.051	0.000
100 pod weight										-0.074	-0.278**
100 kernel weight											0.167**

** Significant at 1% level of Significance

4.4. Path coefficients:

Correlation studies alone can't provide a clear cut picture of cause and effect of relationship between yield attributes and their extent of association. Path analysis given by Wright (1921) provides measure of direct and indirect effects of traits on yield, splitting the correlation coefficients into direct and indirect effects. Under studied, path coefficient analysis was carried out using phenotypic correlation, using kernel yield per plant as a dependent variable. Path coefficient analysis at both genotypic and phenotypic level presented in table 4.4.1. and table 4.4.2. respectively.

4.4.1. Genotypic Path analysis: direct and indirect effects of various traits on kernel yield per plant

4.4.1.1. Direct Effects:

Genotypic path coefficient analysis, the maximum positive direct effect of days to 50% flowering (1.5687) on kernel yield per plant followed by number of secondary branches per plant (1.1513), number of primary branches per plant (0.6761), 100 pod weight (0.5462) and 100 kernel weight (0.0005). Conversely, the highest negative direct effect on kernel yield per plant showed days to maturity (1.9961) followed by pod yield per plant (-1.1636), sound mature kernel (-0.5511), plant height (-0.2890) and shelling % (0.2561).

4.4.1.2. Indirect Effects:

Days to 50% flowering:

Days to 50% flowering exhibited positive indirect effect on kernel yield per plant via number of primary branches (0.6761) followed by number of secondary branches per plant (0.5321), 100 pod weight (0.1993), shelling % (0.1586), plant height (0.0545) and 100 kernel weight (0.0004). On the other hand, negative indirect effect on kernel yield per plant via days to maturity (-0.9307), pod yield per plant (-0.2896) and sound mature kernel (0.1379).

Plant height:

Plant height showed positive indirect effect on kernel yield per plant via pod yield per plant (0.3536) followed by days to maturity (0.0655), sound mature kernel (0.0468), number of secondary branches per plant (0.0462) and number of primary branches per plant (0.0121). On the other hand, negative indirect effect on kernel yield per plant via days to 50 % flowering (-0.2959) followed by 100 pod weight (-0.2394) and shelling % (-0.0335).

Number of primary branches per plant:

Number of primary branches per plant showed positive indirect effect on kernel yield per plant via days to maturity (0.3714) followed by shelling % (0.2010) and 100 pod weight (0.0364). Rest of the traits recorded either negative indirect effect on kernel yield per plant.

Number of secondary branches per plant:

Number of secondary branches per plant showed positive indirect effect on kernel yield per plant for days to 50% flowering (0.7251). On the other hand, negative indirect effect on kernel yield per plant via days to maturity (-0.6992) followed by pod yield per plant (-0.4054, 100 pod weight (-0.3672), sound mature kernel (-0.2200), number of primary branches per plant (-0.1423), shelling percentage (-0.1187) and plant height (-0.0116).

Days to maturity:

Days to maturity showed positive indirect effect on kernel yield per plant via days to 50 % flowering (1.5173) followed by number of secondary branches per plant (0.4033), sound mature kernel (0.2388), 100 pod weight (0.2116), shelling % (0.1271) and plant height (0.0095). Rest of the traits showed either negative indirect effect on kernel yield per plant.

Pod yield per plant:

Pod yield per plant showed positive indirect effect on kernel yield per plant via number of secondary branches per plant (0.4011) followed by days to 50 % flowering (0.3905), shelling % (0.2857), 100 pod weight (0.0780), plant height (0.0878), number of primary branches per plant

(0.0310) and 100 kernel weight (0.0005). Rest of the traits recorded either negative indirect effect on kernel yield per plant.

Shelling percentage:

Shelling percentage showed positive indirect effect on kernel yield per plant via pod yield per plant (1.2979) followed by days to maturity (0.9905). Rest of the traits recorded either negative indirect effect on kernel yield per plant.

100 Pod weight:

100 Pod weight showed positive indirect effect on kernel yield per plant via days to 50 % flowering (0.5723) followed by shelling % (0.4426), plant height (0.1267), number of primary branches per plant (0.0451) and 100 kernel weight (0.0002). Rest of the traits recorded either negative indirect effect on kernel yield per plant.

100 Kernel weight:

100 Kernel weight exhibited positive indirect effect on kernel yield per plant via days to 50% flowering (1.3794) followed by shelling % (0.3795), 100 pod weight (0.2533), sound mature kernel (0.1381) and number of secondary branches per plant (0.0105). Rest other traits recorded either negative indirect effect on kernel yield per plant.

Sound mature kernel:

Sound mature kernel showed positive indirect effect on kernel yield per plant via number of secondary branches per plant (0.4595) followed by 100 pod weight (0.4454), days to 50% flowering (0.3925), number of primary branches per plant (0.1828) and plant height (0.0245). Rest of the traits recorded either negative indirect effect on kernel yield per plant.

4.4.2.1. Phenotypic path analysis: **direct and indirect effect of various traits on seed yield per plant**

4.4.2.1. Direct Effects:

Phenotypic path coefficient analysis, the maximum positive direct effect of number of primary branches per plant (0.1420) on kernel yield per plant

followed by days to 50 % flowering (0.1391) and 100 kernel weight (0.0390). Conversely, the highest negative direct effect on kernel yield per plant were 100 pod weight (-0.2397) followed by days to maturity (-0.1619), plant height (-0.1510), shelling % (-0.1363), sound mature kernel (-0.0903), number of secondary branches per plant (-0.0861) and pod yield per plant (-0.0704).

4.4.2.2. Indirect Effects:

Days to 50% flowering:

Days to 50% flowering showed positive indirect effect on kernel yield per plant via plant height (0.0170) followed by shelling % (0.0124) and 100 kernel weight (0.0086). Rest of the traits recorded either negative indirect effect on kernel yield per plant.

Plant height:

Plant height showed positive indirect effect on kernel yield per plant via 100 pod weight (0.0295) followed by days to maturity (0.0083) and number of secondary branches per plant (0.0015). Rest of the traits recorded either negative indirect effect on kernel yield per plant.

Number of primary branches per plant:

Number of primary branches per plant showed positive indirect effect on kernel yield per plant via shelling % (0.0323) followed by days to maturity (0.0185), number of secondary branches per plant (0.0142) and plant height (0.0001). Remaining of the traits recorded either negative indirect effect on kernel yield per plant.

Number of secondary branches per plant:

Number of secondary branches per plant showed positive indirect effect on kernel yield per plant via days to 50% flowering (0.0453) followed by 100 kernel weight (0.0060) and plant height (0.0026). Rest of the traits recorded either negative indirect effect on kernel yield per plant.

Days to maturity:

Days to maturity showed positive indirect effect on kernel yield per plant via days to 50% flowering (0.1001) followed by shelling % (0.0262),

plant height (0.0078) and 100 kernel weight (0.0051). Remaining of the traits recorded either negative indirect effect on kernel yield per plant.

Pod yield per plant:

Pod yield per plant showed positive indirect effect on kernel yield per plant via shelling % (0.0298) followed by sound mature kernel (0.0190), days to 50% flowering (0.0177), plant height (0.0078), number of primary branches per plant (0.0041) and 100 kernel weight (0.0026). Rest of the traits recorded either negative indirect effect on kernel yield per plant.

Shelling percentage:

Shelling percentage exhibited positive indirect effect on kernel yield per plant via 100 pod weight (0.0525) followed by days to maturity (0.0311) and pod yield per plant (0.0154). Remaining of the traits recorded either negative indirect effect on kernel yield per plant.

100 Pod weight:

100 Pod weight showed positive indirect effect on kernel yield per plant via shelling % (0.0299) followed by days to 50% flowering (0.0201), plant height (0.0186), number of primary branches per plant (0.0066), sound mature kernel (0.0034) and 100 kernel weight (0.0011). Rest of the traits recorded either negative indirect effect on kernel yield per plant.

100 Kernel weight:

100 Kernel weight exhibited positive indirect effect on kernel yield per plant via days to 50% flowering (0.0308) followed by shelling % (0.0136) and plant height (0.0038). Rest other traits recorded either negative indirect effect on kernel yield per plant.

Sound mature kernel:

Sound mature kernel showed positive indirect effect on kernel yield per plant via days to 50% flowering (0.0189) followed by pod yield per plant (0.0148), number of primary branches per plant (0.0068) and 100 kernel weight (0.0042). Remaining of the traits recorded either negative indirect effect on kernel yield per plant.

Table 4.4.1: Genotypic path analysis for yield and characters attributing to it.

Characters	Days to 50% flowering	Plant height	No. of primary branches / plant	No. of secondary branches /plant	days to maturity	Pod yield/plant	Shelling %	100 pod weight	100 kernel weight	Sound mature kernel	Correlation with kernel yield
Days to 50% flowering	1.5687	0.0545	0.6761	0.5321	-1.9307	-0.2896	0.1586	0.1993	0.0004	-0.1379	-0.0861
Plant height	-0.2959	-0.2890	0.0121	0.0462	0.0655	0.3536	-0.0335	-0.2394	0.0000	0.0468	-0.3337
No. of primary branches per plant	-0.5604	-0.0052	0.6761	-0.2423	0.3714	-0.0534	0.2010	0.0364	-0.0001	-0.1490	0.2745
No. of secondary branches /plant	0.7251	-0.0116	-0.1423	1.1513	-0.6992	-0.4054	-0.1187	-0.3672	0.0000	-0.2200	-0.0880
days to maturity	1.5173	0.0095	-0.1258	0.4033	-1.9961	-0.0634	0.1271	0.2116	0.0002	-0.2388	-0.1552
Pod yield/plant	0.3904	0.0878	0.0310	0.4011	-0.1088	-1.1636	0.2857	0.0780	0.0005	-0.0656	-0.0634
Shelling %	-0.9711	-0.0378	-0.5306	0.0000	0.9905	1.2979	-0.2561	-0.9437	-0.0007	-0.1710	-0.0894
100 pod weight	0.5723	0.1267	0.0451	-0.7741	-0.7736	-0.1661	0.4426	0.5462	0.0002	-0.4495	-0.4302
100 kernel weight	1.3794	-0.0049	-0.1341	0.0105	-0.6912	-1.2678	0.3795	0.2533	0.0005	0.1381	0.0634
Sound mature kernel	0.3925	0.0245	0.1828	0.4595	-0.8648	-0.1384	-0.0795	0.4454	-0.0001	-0.5511	-0.1292

Residual = 0.930. Note: Values in red color across the diagonal denote direct effects

Table 4.4.2: Phenotypic path analysis for yield and characters attributing to it.

Characters	Days to 50% flowering	Plant height	No. of primary branches / plant	No. of secondary branches /plant	days to maturity	Pod yield/plant	Shelling %	100 pod weight	100 kernel weight	Sound mature kernel	Correlation with kernel yield
Days to 50% flowering	0.1391	0.0170	-0.0416	-0.0280	-0.1165	-0.0090	0.0124	-0.0347	0.0086	-0.0123	-0.0651
Plant height	-0.0156	-0.1510	-0.0125	0.0015	0.0083	0.0036	-0.0029	0.0295	-0.0010	-0.0028	-0.1428
No. of primary branches / plant	-0.0407	0.0001	0.1420	0.0142	0.0185	-0.0020	0.0323	0.0000	-0.0024	-0.0043	0.1598
No. of secondary branches /plant	0.0453	0.0026	-0.0234	-0.0861	-0.0334	-0.0014	-0.0293	0.0000	0.0060	-0.0002	-0.0631
days to maturity	0.1001	0.0078	-0.0162	-0.0178	-0.1619	-0.0046	0.0262	-0.0499	0.0051	-0.0124	-0.1236
Pod yield/plant	0.0177	0.0078	0.0041	-0.0018	-0.0105	-0.0704	0.0298	-0.0508	0.0026	0.0190	-0.0524
Shelling %	-0.0126	-0.0032	-0.0336	-0.0185	0.0311	0.0154	-0.1363	0.0525	-0.0039	-0.0032	-0.1123
100 pod weight	0.0201	0.0186	0.0066	0.0001	-0.0337	-0.0149	0.0299	-0.2397	0.0011	0.0034	-0.1882
100 kernel weight	0.0308	0.0038	-0.0088	-0.0133	-0.0214	-0.0047	0.0136	0.0000	0.0390	-0.0098	0.0224
Sound mature kernel	0.0189	0.0000	0.0068	-0.0002	-0.0223	0.0148	-0.0048	0.0000	0.0042	-0.0903	-0.3064

Residual = 0.9263. Note: Values in red color across the diagonal denote direct effects.

4.5. **Fresh Seed Dormancy:**

Analysis of variance for germination percentage at 7 days after sowing is presented in Appendix-II.

Duration of fresh seed dormancy:

Duration of dormancy, intensity of dormancy and mean value of germination percentage is presented in Table: 4.5.1. The result revealed that different duration of dormancy (ranged from 7 to >35 days). Genotypes TG-26 had highest >35 days duration of dormancy followed CONTROL ICRISAT-4, CONTROL ICRISAT-6, CONTROL ICRISAT-7, CONTROL ICRISAT-10, CONTROL ICRISAT-14, ICRISAT-1, ICRISAT-2, ICRISAT-3, ICRISAT-8, ICRISAT-10, ICRISAT-12, ICRISAT-11, GG7XGPBD4 and BAU13XCS196 had >28 days duration of dormancy. In contrast, non-dormant genotypes such as CONTROL ICRISAT-2, CONTROL ICRISAT-3 and ICRISAT-13 had lowest 7 days dormancy duration.

Intensity of fresh seed dormancy:

Intensity of fresh seed dormancy ranged from 3.7 to 100%. The highest (100%) intensity of dormancy were recorded in CONTROL ICRISAT-2, CONTROL ICRISAT-4, CONTROL ICRISAT-5, CONTROL ICRISAT-14, ICRISAT-2, ICRISAT-8, ICRISAT-12, GJG17 X GPBD4 and TG-26 genotypes. While the lowest were recorded in GPBD4 (3.7%), CONTROL ICRISAT-3 (4.5%) and CONTROL ICRISAT-1 (6.3%). This high variation could be due to genetic variation among the genotypes.

Table 4.5.1 Duration of dormancy, intensity of dormancy and mean value of germination percentage in 37 groundnut genotypes

Genotypes	Mean Value of germination percentage (%)	Duration of dormancy(days)	Intensity of dormancy(%)
CONTROL ICRISAT 1	93.7	28	6.3
CONTROL ICRISAT 2	0	7	100
CONTROL ICRISAT 3	95.5	7	4.5
CONTROL ICRISAT 4	0	>28	100
CONTROL ICRISAT 5	0	28	100
CONTROL ICRISAT 6	3.6	>28	96.4
CONTROL ICRISAT 7	2.6	>28	97.4
CONTROL ICRISAT 9	1.8	10	98.2
CONTROL ICRISAT 10	59.5	>28	40.5
CONTROL ICRISAT 11	8.6	28	91.4
CONTROL ICRISAT 12	17.2	21	82.8
CONTOLR ICRISAT 13	2.2	14	97.8
CONTROL ICRISAT 14	0	>28	100
ICRISAT 1	0.8	>28	99.2
ICRISAT 2	0	>28	100
ICRISAT 3	4.6	>28	95.4
ICRISAT 4	7.8	28	92.2
ICRISAT 6	18.97	21	81.03
ICRISAT 7	10	28	90
ICRISAT 8	0	>28	100

ICRISAT 10	0.6	>28	99.4
ICRISAT 11	29.6	>28	70.4
ICRISAT 12	0	>28	100
ICRISAT 13	92.4	7	7.6
ICRISAT 14	6.94	28	93.06
ICRISAT 15	17.9	21	82.1
GG7 X GPBD4 (F_7)	19.1	>28	80.9
GG2 X ICG1697 (F_7)	15.6	21	84.4
GJG17 X GPBD4 (BC_1F_6)	0	21	100
GG20 X GPBD4 (F_8)	0.67	10	99.33
BAU13 X CS196 (F_8)	1.8	>28	98.2
GG2 X RHRG06083 (F_6)	24	10	76
Gangapuri	2.44	14	97.56
JGN-3	27.3	10	72.7
GPBD4	96.3	14	3.7
KDG128	16.6	10	83.4
TG-26	0	>28	100

4.5 Screening of genotypes for foliar diseases using SSR markers:

A set of two allele specific SSR markers was taken to screen 32 genotypes of groundnut along with 4 check varieties against foliar diseases (Rust/LLS). Both the primers generated polymorphic bands among genotypes. Gel scoring was done with base pair analysis using ladder based on banding pattern. Data sheet was prepared to run in population structure and allele pattern A/A was used if band was in upper side and pattern B/B was used if the band was in lower side, in

heterozygous condition banding pattern was A/B and in case of no amplification it was represented as -/-.

The genetic diversity varied between 0.452 to 0.202 among the genotypes with an average of 0.33.Polymorphic Information Content (PIC) ranged from 0.39 to 0.19 with an average value of 0.29 (table 4.4.). The primer GMRQ786 showed highest genetic diversity while the other primer GMRQ517 showed the lowest frequency. The marker allele frequency (MAF) ranged from 0.69 to 0.89 with a mean value of 0.79.

4.6.1 Allele Specific SSR Marker presenting Major allele frequency (MAF), Genetic diversity and Polymorphic information content in Groundnut.

MARKER	MAF	GENETIC DIVERSITY	PIC
GMRQ786	0.69	0.452	0.39
GMRQ517	0.89	0.202	0.19
Mean	0.79	0.33	0.29

The genetic relationships among groundnut genotypes are presented in SSR based UPGMA tree (fig.1) all the genotypes were grouped into 5 clusters. The genotypes in Cluster 1: GG7XGPBD4, GJG17XGPBD4, ICRISAT 11, ICRISAT 13, ICRISAT 14, ICRISAT 15, ICRISAT 3, ICRISAT 7, JGN-3, BAU13XCS196, CONTROL ICRISAT 10, CONTROL ICRISAT 11, CONTROL ICRISAT 13, CONTROL ICRISAT 2, CONTROL ICRISAT 3, CONTROL ICRISAT 7, CONTROL ICRISAT 9, GANGAPURI, GG20XGPBD4, GG2XICG1697, GG2XRHRG06083; Cluster 2: CONTROL ICRISAT 5, CONTROL ICRISAT 6, ICRISAT 1, ICRISAT 10, ICRISAT 12, ICRISAT 2, ICRISAT 6, ICRISAT 8; Cluster 3: CONTROL ICRISAT 1, CONTROL ICRISAT 12; Cluster 4: KDG-128; Cluster 5: CONTROL ICRISAT 14, CONTROL ICRISAT 4, ICRISAT 4. Cluster 1 are showing distinct genotypes while Cluster 2 were grouped with moderately sensitive genotypes, while Clusters 3 and 4 were grouped with tolerant genotypes and Cluster 5 represented resistant genotypes

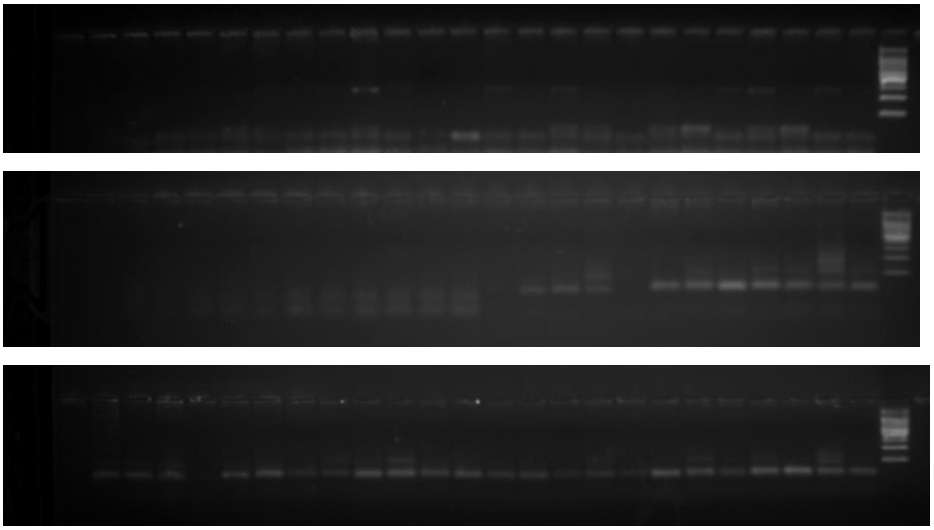


Fig.4. Allelic variation of groundnut germplasms using SSR analysis of GMRQ786 and GMRQ517 markers in 3% agarose gel.

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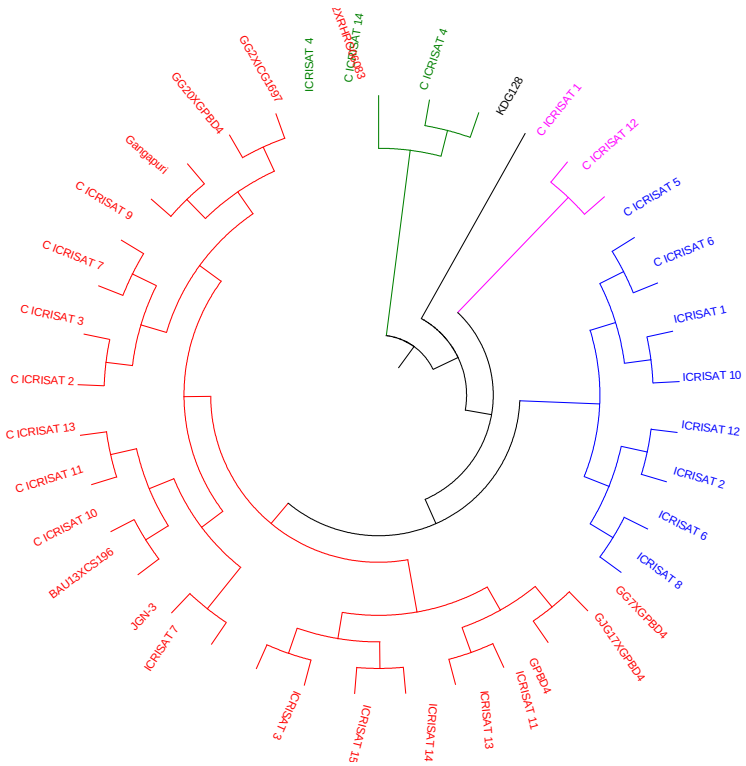

Fig 5.Dendogram of groundnut genotypes based on banding pattern analysis of gene based SSR markers.

Fig.6. Performance of molecular work at department of PMB&B Gwalior

Chapter- V

DISCUSSION

The long term objective of most plant breeding programmes is to increase yield potential of a crop with high quality and also by reducing the hindrance caused due to any disease or pest which is achieved by manipulating genes for its resistance and yield components. The selection of suitable parents for specific characters is a pre requisite for the successful hybridization programme in groundnut. The knowledge of genetic parameters like genotypic coefficient of variation, phenotypic coefficient of variation, heritability, genetic advance as well as correlation and path coefficient analysis and foliar diseases like rust or leaf spot are interfering with the yield and quality so, identifying the genotypes resistant for foliar diseases at molecular level are essential for making effective selections from the breeding material either for direct advancement or to formulate efficient crossing programme.

The present investigation was carried out to evaluate 36 genotypes of groundnut to obtain the information on mean performance, variability, heritability, genetic advance, character association, path coefficient analysis, fresh seed dormancy and genotypes resistant to foliar diseases. The findings of the present investigation have been interpreted and discussed in the chapter in the light of the similar research work carried out by other research workers.

Analysis of variance and Mean performance:

The results of analysis of variance based on randomized block design experiment are presented in Table 4.1.1 Among the characters under studied, all characters showed statistically significant mean sum of squares due to genotypes, viz., days to 50% flowering, plant height, number of primary branches per plant, number of secondary branches per plant, days to maturity, pod yield per plant, kernel yield per plant, shelling %, 100 pod weight, 100 kernel weight and sound mature kernel %. The mean performance of these genotypes for various characters is presented in

Appendix I. Range, mean, phenotypic and genotypic coefficients of variation, Heritability estimates and predicted genetic advance as per cent of mean for characters studied are presented in Table 4.1.2.

Mean performance revealed that all the genotypes showed significantly higher kernel yield per plant. Genotypes ICRISAT-14 showed significantly higher kernel yield with high pods yield per plant and maximum duration of 50% flowering, Genotype ICRISAT – 3 also showed higher kernel yield per plant. The increased kernel yield in genotypes GG7XGPBD4 was due to plant height and 100 pod weight. The genotypes CONTROL ICRISAT – 6, BAUXCS196 showed high kernel yield due to number of primary branches per plant and secondary branches per plant. Similarly, GG7XICG1697 also observed the high kernel yield potential as it took more duration to reach maturity. The genotypes ICRISAT - 6 recorded fairly high kernel yield potential which was due to fairly higher shelling %.ICRISAT - 1 registered superior performance for 100 kernel weight, while ICRISAT – 2 showed higher mean values for sound mature kernel and kernel yield per plant.

These result suggested that the genotypes ICRISAT – 14, ICRISAT – 3, GG7XGPBD4, CONTROL ICRISAT – 6, BAU13XCS196, GG7XICG1697, ICRISAT - 6, ICRISAT – 1, ICRISAT - 2 could be used as donors for kernel yield improvement in groundnut.

Phenotypic and genotypic coefficient of variation:

In present investigation among all the characters studied, number of primary branches per plant recorded the highest PCV and GCV followed by plant height, days to 50% flowering, days to maturity, number of secondary branches per plant, kernel yield per plant and pod yield per plant. Whereas, 100 kernels weight showed high PCV and moderate GCV followed by sound mature kernel and 100 pod weight, which suggested that these characters were least affect by environment. High proportion of GCV to PCV is desirable in process because it depicts that traits are much under the genetic control rather than the environment. The results are in confirmation Sudhir Kumar *et al*. (2008), Meta and Monpara (2010).

The minimum PCV and GCV values were seen in shelling % indicated that selection for these characters is less effective, these result were in agreement with Pradhan K. and Patra R.K. (2011).

Heritability (broad sense) and genetic advance:

Estimation of high heritability value coupled with high genetic advance were recorded for number of primary branches per plant. kernel yield per plant and number of secondary branches per plant showed high genetic advance and moderate heritability while days to 50% flowering showed high heritability and moderate genetic advance indicating that the inheritance of these traits were likely due to non additive gene effect and further limited scope of improvement for this traits. Days to maturity showed high heritability with low genetic advance indicating the influence of both additive and non additive gene action, similarly gene action is present. Similar result finding has been reported by John K. *et al.*(2012).

Low heritability with low genetic advance was observed for plant height, pod yield per plant, 100 pod weight, 100 kernel weight, shelling % and sound mature kernel indicating that these character was highly affect by environment and selection for these character is not effective.Similar result earlier reported by Mohan Vishnuwardhan K. *et al*. (2013)

Correlation analysis:

Correlation analysis revealed that kernel yield per plant was positive and highly significantly correlated at both genotypic and phenotypic level with number of primary branches per plant. While, kernel yield per plant was negatively correlated at both phenotypic as well as genotypic levels with 100 pod weight and significant negatively correlated at genotypic level with days to maturity and plant height. These findings in accordance with Korat *et al.* (2010), Babariya C. A. and Dobariya K. L. (2012), John *et al.* (2015).

Path analysis:

Direct and indirect effect of various traits on kernel yield per plant revealed that days to 50% flowering followed by number of secondary branches per plant, number of primary branches per plant, 100 pod weight

and 100 kernel weight exhibited highest positive direct effect. Days to maturity, pod yield per plant, sound mature kernel, plant height and shelling %, exhibited negative direct effect on kernel yield per plant but these characters recorded positive indirect effect on kernel yield per plant. Days to maturity via, days to 50% flowering, number of secondary branches per plant, sound mature kernel, 100 pod weight, shelling % and plant height. Pod yield per plant via, number of secondary branches per plant, days to 50% flowering, shelling %, 100 pod weight, plant height, number of primary branches per plant and 100 kernel weight. Sound mature kernel via, number of secondary branches per plant, 100 pod weight, days to 50% flowering, number of primary branches per plant and plant height. Plant height via, pod yield per plant, days to maturity, sound mature kernel, number of secondary branches per plant and number of primary branches per plant. Shelling % via, pod yield per plant and days to maturity.

The residual effect values was showed very low (0.930) thus, indicated majority of the factors influencing the kernel yield per plant was considered in the present study. Present finding are in confirmation with Sumathi *et al. (*2007),Rasheed *et al.* (2015)Hampannavar *et al*. (2018)Ganvit *et al*. (2018) and Raza *et al*. (2018).

Fresh seed dormancy:

Fresh seed dormancy in bunch type groundnut has a significant influence on kernel yield and quality. It is required to avoid economic loss in form of in–situ germination during unpredictable rainfall at maturity. Genotypes evaluated for fresh seed dormancy showed significant genetic variation for germination percentage at seven days after sowing. It was concluded that genotypes TG-26 had highest >35 days duration of dormancy followed CONTROL ICRISAT-4, CONTROL ICRISAT-6, CONTROL ICRISAT-7, CONTROL ICRISAT-10, CONTROL ICRISAT-14, ICRISAT-1, ICRISAT-2, ICRISAT-3, ICRISAT-8, ICRISAT-10, ICRISAT-12, ICRISAT-11, GG7XGPBD4 and BAU13XCS196 had >28 days duration of dormancy or moderate dormancy indicating that these genotypes had more than four week duration of dormancy. While, genotypes CONTROL ICRISAT-2, CONTROL ICRISAT-4, CONTROL ICRISAT-5, CONTROL ICRISAT-14, ICRISAT-2, ICRISAT-

8, ICRISAT-12, GJG17 X GPBD4 and TG-26 recorded highest intensity of fresh seed dormancy. Therefore, these genotypes were identified as new sources of fresh seed dormancy in groundnut. These results were in confirmation with the findings of Nagarjun and Radder. 1983, Asibuo *et al.* (2008), Gaikwad *et al.* (2010).

Screening of genotypes for foliar diseases using SSR markers

Molecular marker analysis on groundnut germplasm using a variety of molecular markers such as microsatellites or simple sequence repeats (SSRs), randomly amplified polymorphic DNAs (RAPDs) and amplified fragment length polymorphisms (AFLPs) in general has shown very low variation in cultivated gene pool because of the evolutionary genetic bottleneck in the form of polyploidy and self- pollination (Kochert *et al*., 1996, Subramanian *et al*., 2000, Varshney *et al.,* 2013). On the other hand, wild diploid *Arachis* species showed relatively higher variation, providing a rich source of genetic variation for genetic and genomic studies. Among different marker systems analysed in the groundnut, like other plant species, SSR markers have been found more informative and useful for genetic analysis and breeding applications (Gupta and Varshney 2000, Pandey *et al.,* 2012). The conventional breeding procedures employ hybridization, phenotype based selection followed by selection of promising breeding lines through yield evaluation traits. Recent advancement of genomic tools accelerated marker assisted breeding (MAB) to enhance efficiency of selection of target traits in groundnut (Janila *et al*. 2013, Kanyika *et al*. 2015).

Screening of groundnut genotypes for foliar fungal diseases was done using gene based SSR markers. The results indicated the presence of genetic diversity in a range between 0.452 to 0.202 among the genotypes bearing an average value of 0.33. The Polymorphic Information Content (PIC) values varied between 0.39 to 0.19 with an average value of 0.29, indicating the presence of variability in the genotypes at molecular level. The primer GRMQ786 showed highest genetic diversity. The marker allele frequency (MAF) ranged between 0.69 to 0.89 with a mean value of 0.79.

The genetic relationships among groundnut genotypes are presented in SSR based UPGMA tree (fig.1) all the genotypes were grouped into 5 clusters. Among which Clusters 3 (CONTROL ICRISAT 1, CONTROL ICRISAT 12) and Cluster 4(KDG-128) were grouped with tolerant genotypes and Cluster 5 (CONTROL ICRISAT 14, CONTROL ICRISAT 4, ICRISAT 4) represented resistant genotypes. These results were similar to the findings of These results were similar to the findings of He *et al.,*(2003), Ferguson *et al.,*(2004), Mace *et al.* (2006), Sujay *et al.* (2012), Divyadharsini *et al.* (2017)

It is concluded from experiment that genotypes was significant different for all the 11 characters. Estimation of high heritability values coupled with moderate genetic advances was recorded for number of days to 50% flowering.Genotypic and phenotypic correlation analysis revealed that only eight characters viz. plant height, number of primary branches per plant, number of days to 50% flowering, number of days to maturity, pod yield per plant, kernel yield per plant, 100 kernel weight,and sound mature kernel exhibited positive and significant association with kernel yield. Path analysis revealed that podyield per plant followed by kernel yield per plant,plant height,number of days to 50% flowering, 100 kernel weight, and shelling % exhibited highest positive direct effect on kernel yield per plant.

On the basis of fresh seed dormancy analysis genotypes , genotypes CONTROL ICRISAT-2, CONTROL ICRISAT-4, CONTROL ICRISAT-5, CONTROL ICRISAT-14, ICRISAT-2, ICRISAT-8, ICRISAT-12, GJG17 X GPBD4 and TG-26 were identified as new sources of fresh seed dormancy in groundnut. While screening the genotypes for foliar diseases the genotypes CONTROL ICRISAT 14, CONTROL ICRISAT 4, ICRISAT 4 were found to be resistant among all 36 genotypes.

Chapter-VI

SUMMARY,CONCLUSION AND SUGGESTIONS FOR FUTHER WORK

SUMMARY:

The present investigation **"**Association analysis, fresh seed dormancy and screening of genotypes using SSR marker in groundnut *(Arachis hypogaea* L*).*" were undertaken to understand phenotypic and genotypic coefficient of variation, heritability, genetic advance,correlation coefficient, path coefficient analysis, find out fresh seed dormancy among the genotypes and screening of genotypes for foliar disease using gene based SSR marker**.** Field experiment was conducted with 3 replication during kharif season 2018-19 at Research Farm, Department of Genetics and Plant Breeding, RVSKVV, College of Agriculture, Gwalior (MP) and molecular work was carried out at Plant Molecular Biology Laboratory, Department of Plant Molecular Biology and Biotechnology, College of Agriculture, Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior (M.P.). The observations recorded on 5 randomly selected plants of each genotype in each replication for days to 50% flowering, plant height (cm), number of primary branches per plant, number of secondary branches per plant, days to maturity, pod yield per plant(gm), kernel yield per plant (gm),shelling (%), 100 pod weight (gm), 100 kernel weight (gm) and sound mature kernel (%).

The analysis of variance revealed genotypes were significantly differed for all the characters, thereby, indicating variability for all the characters.

Number of primary branches per plant recorded the highest PCV and GCV followed by days to maturity, plant height, number of secondary branches per plant, kernel yield per plant and pod yield per plant. Whereas, 100 kernel weight, sound mature kernel and 100 pod weight showed high PCV and moderate GCV and the low PCV and GCV recorded for shelling %.

The high heritability estimated for days to maturity, number of primary branches per plant and days to 50% flowering. Moderate heritability recorded for kernel yield per plant and number of secondary branches per plant. Low heritability was recorded for plant height, pod yield per plant, kernel yield per plant, 100 pod weight, 100 kernel weight, shelling% and sound mature kernel.

The high genetic advance as percentage of mean (at 5% selection intensity) were recorded for number of primary branches per plant, kernel yield per plant and number of secondary branches per plant. The moderate genetic advance as percentage of mean were recorded for days to 50% flowering, days to maturity and pod yield per plant. While, low genetic advance were observed in plant height, 100 pod weight, 100 kernel weight, shelling % and sound mature kernel.

Genotypic and phenotypic correlation analysis revealed that kernel yield per plant was positive and high significantly correlated at both genotypic and phenotypic level with number of primary branches per plant. While, kernel yield per plant was negatively correlated at both phenotypic as well as genotypic levels with 100 pod weight and significant negatively correlated at genotypic level with days to maturity and plant height.

Genotypic path coefficient analysis the maximum positive direct effect of kernel yield per plant revealed that days to 50% flowering followed by number of secondary branches per plant, number of primary branches per plant, 100 pod weight and 100 kernel weight exhibited highest positive direct effect. Conversely, the highest negative direct effect on kernel yield per plant was registered by days to maturity, pod yield per plant, sound mature kernel, plant height and shelling percentage. The residual effect values was showed very low (0.930).

Genotypes tested showed different durations of dormancy and it’s ranged from 7 to >35 days. Genotypes TG-26 had highest >35 days duration of dormancy followed CONTROL ICRISAT-4, CONTROL ICRISAT-6, CONTROL ICRISAT-7, CONTROL ICRISAT-10, CONTROL ICRISAT-14, ICRISAT-1,

ICRISAT-2, ICRISAT-3, ICRISAT-8, ICRISAT-10, ICRISAT-12, ICRISAT-11, GG7XGPBD4 and BAU13XCS196 had >28 days duration of dormancy.High intensity of dormancy was recorded in CONTROL ICRISAT-2, CONTROL ICRISAT-4, CONTROL ICRISAT-5, CONTROL ICRISAT-14, ICRISAT-2, ICRISAT-8, ICRISAT-12, GJG17 X GPBD4 and TG-26. While, lowest have recorded in GPBD4 followed by CONTROL ICRISAT-3 and CONTROL ICRISAT-1.

The genotypes were screened for foliar diseases using SSR markers, thegenetic diversity in a range between 0.452 to 0.202 among the genotypes. The Polymorphic Information Content (PIC) values varied between 0.39 to 0.19. The primer GRMQ786 showed highest genetic diversity. The marker allele frequency (MAF) ranged between 0.69 to 0.89.The genetic relationship showed based on UPGMA tree indicated that the genotypes CONTROL ICRISAT 14, CONTROL ICRISAT 4, ICRISAT 4 belonging to cluster 5 were found to be resistant.

CONCLUSION

The following relevant conclusions could be drawn from the present study:

- The estimation of high heritability value coupled with high genetic advance were recorded for number of primary branches per plant indicating that the inheritance of these characters was most likely due to additive gene effects and direct selection for these traits would be more effective for desired genetic improvement.
- Kernel yield per plant was positively and significantly correlated at both genotypic and phenotypic level with number of primary branches per plant indicating that direct selection for these trait may lead increase in genetic potential of kernel yield.
- Path analysis revealed that the traits *viz.* days to 50% flowering, number of secondary branches per plant, number of primary branches per plant, 100 pod weight and 100 kernel weight exhibited the highest positive direct

effect on kernel yield per plant and some traits have indirect positive effect on kernel yield per plant and each traits be given preference in selecting the superior types.

- On the basis of fresh seed dormancy analysis genotypes CONTROL ICRISAT-2, CONTROL ICRISAT-4, CONTROL ICRISAT-5, CONTROL ICRISAT-14, ICRISAT-2, ICRISAT-8, ICRISAT-12, GJG17 X GPBD4 and TG-26 were identified as new sources of fresh seed dormancy in groundnut.
- On the basis of screening the genotypes for foliar disease using SSR markers, the genotypes CONTROL ICRISAT 14, CONTROL ICRISAT 4, ICRISAT 4 were found to be resistant.

Suggestion for future work:

- The genetic variability reported for different characters in relation in kernel yield should be exploited.
- Characters showing high heritability with high genetic advance should be utilized in selection.
- Selection should be more effective for the character, days to 50% flowering. Since, this trait was under the influence of additive gene action and highly significant and positive correlation with kernel yield.
- Direct selection should be done for characters such as days to 50% flowering, number of secondary branches per plant, number of primary branches per plant, 100 pod weight and 100 kernel weight.
- Further studies to determine the heritability of fresh seed dormancy in groundnut should be done.
- The five groundnut genotypes identified with foliar disease resistance in cluster 5 namely CONTROL ICRISAT 14, CONTROL ICRISAT 4, ICRISAT 4. These genotypes could be used for further hybridization programme and other crop improvement programs in groundnut.

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Appendix-I : Mean performance of 11 characters in 36 groundnut genotypes

Genotypes	Days to 50% flowering	Plant height (cm)	No. of primary branches per plant	No. of secondary branches per plant	Days to maturity	Pod yield per plant (gm)	Kernel yield per plant (gm)	Shelling %	100 pod weight (gm)	100 Kernel Weight (gm)	Sound mature kernel (%)
CONTROL ICRISAT 1	28.00	31.07	8.00	8.33	98.33	16.03	11.33	67.67	68.70	39.70	80.40
CONTROL ICRISAT 2	26.00	27.33	6.67	8.00	98.67	15.07	9.67	69.33	67.00	41.70	79.70
CONTROL ICRISAT 3	26.00	30.67	6.67	7.00	99.00	14.90	9.00	67.67	69.60	39.50	79.00
CONTROL ICRISAT 4	27.00	24.67	8.00	8.33	98.33	15.03	10.00	68.33	69.10	42.40	77.00
CONTROL ICRISAT 5	26.00	30.17	7.33	7.67	99.67	13.77	9.67	69.33	68.40	41.80	78.00
CONTROL ICRISAT 6	27.00	28.00	9.33	9.00	97.33	14.03	9.00	67.67	69.40	42.90	81.10
CONTROL ICRISAT 7	27.00	28.00	7.33	8.67	98.33	14.20	10.00	68.33	67.40	41.40	76.00
CONTROL ICRISAT 9	26.00	33.50	7.00	9.00	98.67	14.17	7.33	69.33	68.30	42.90	79.40
CONTROL ICRISAT 10	26.00	34.00	7.00	10.33	96.67	13.30	9.67	69.67	67.90	42.70	81.70
CONTROL ICRISAT 11	26.00	31.07	4.00	9.67	96.67	14.93	9.00	70.33	68.60	41.60	79.00
CONTROL ICRISAT 12	27.00	27.33	6.00	10.67	97.00	13.87	10.00	69.00	68.90	42.70	78.00
CONTOLR ICRISAT 13	26.00	30.67	9.00	11.67	97.00	15.13	9.67	69.33	66.80	43.20	81.00
CONTROL ICRISAT 14	27.00	24.67	7.00	9.00	97.33	15.03	9.00	67.67	67.40	45.40	80.70
ICRISAT 1	32.00	30.17	7.33	9.00	106.67	16.53	10.00	68.33	67.90	45.50	78.70

ICRISAT 2	33.00	28.00	7.00	9.67	105.33	14.43	9.67	69.33	68.40	41.20	78.73
ICRISAT 3	32.00	31.07	6.33	11.00	107.00	13.90	9.00	69.00	66.50	43.70	80.80
ICRISAT 4	33.00	27.33	5.33	10.33	108.00	14.73	10.00	72.00	68.40	45.10	82.80
ICRISAT 6	32.00	30.67	5.00	11.33	108.33	14.73	7.33	67.67	69.00	41.50	80.20
ICRISAT 7	31.00	24.67	9.00	11.67	106.33	14.73	9.67	69.33	68.70	41.00	81.20
ICRISAT 8	32.00	30.17	8.00	11.67	106.33	15.10	9.00	67.67	67.40	41.90	80.50
ICRISAT 10	32.00	28.00	5.00	11.00	105.67	13.37	10.00	68.33	67.40	42.40	80.40
ICRISAT 11	32.00	31.07	6.00	12.33	105.33	13.90	8.33	69.33	66.87	42.70	77.70
ICRISAT 12	31.00	27.33	5.67	10.00	107.33	14.00	10.67	68.33	66.90	40.30	84.20
ICRISAT 13	31.00	30.67	6.00	9.33	104.33	13.67	10.00	68.67	68.10	40.90	77.37
ICRISAT 14	32.00	24.67	5.00	11.00	108.00	17.40	7.33	69.33	70.00	40.50	77.00
ICRISAT 15	32.00	30.17	7.67	8.00	106.33	13.20	10.67	67.67	69.00	43.20	79.30
GG7 X GPBD4	31.00	28.00	7.33	9.00	109.33	12.43	7.00	69.33	71.50	42.30	83.90
GG2 X ICG1697	31.00	31.67	7.67	10.67	109.67	16.93	10.67	66.00	68.00	45.90	78.70
GJG17 X GPBD4	32.00	30.87	6.00	9.33	108.33	13.93	8.00	68.33	69.70	43.20	82.10
GG20 X GPBD4	32.00	28.67	5.33	8.67	108.33	12.43	11.33	67.67	69.50	44.80	78.00
BAU13 X CS196	31.00	31.07	7.67	8.00	109.00	16.60	6.33	65.33	71.43	44.10	79.70
GG2 X RHRG06083	32.00	27.33	8.33	9.67	109.00	16.47	10.67	65.67	71.13	43.20	81.00
Gangapuri	33.00	30.67	4.67	11.67	104.00	15.93	7.67	69.33	67.80	45.00	77.40
JGN-3	33.00	24.67	6.00	9.33	102.33	15.20	10.00	66.33	70.30	46.10	79.70
GPBD4	33.00	30.17	5.00	8.67	101.33	16.00	7.33	68.33	69.50	44.60	82.00
KDG128	32.00	28.00	6.00	11.00	101.67	17.63	11.00	67.67	69.87	45.50	80.40
mean	30.00	29.06	6.69	9.71	103.36	14.80	9.31	68.41	68.63	42.85	79.80
SE(m)	1.03	2.73	0.25	0.61	1.80	0.74	0.54	1.45	0.92	2.52	2.69
CD 5%	3.13	8.32	0.76	1.85	5.50	2.25	1.65	4.42	2.80	7.71	8.22
CV %	5.85	9.84	12.93	13.87	2.25	10.04	13.68	3.05	2.42	6.42	3.56

Appendix- II : ANOVA for germination percentage at seven days after sowing

Source of variation	Degree of freedom	Sum of squares	Mean sum of squares	F value
Replication	1	12.43	6.22	9.19
Germination percentage	37	93669.62	2601.93	3848.45
Error	37	48.68	0.68	
Total	74	93730.73		

Appendix-III : COMMON REAGENTS AND BUFFERS USED

REAGENTS FOR PLANT TOTAL GENOMIC DNA ISOLATION

1.1. PREPARATION OF COMMONLY USED STOCK SOLUTION

Solution	Method of prepration
0.5 M EDTA (pH 8.0	186.1 g of sodium salt of EDTA was dissolved in 800 ml of MQ water, pH was adjusted to 8.0 with NaOH pellets. The final volume was adjusted to one litre with MQ water and sterilized by autoclave
4 M NaCl	233.8 g of NaCl was dissolved in 800 ml of MQ water. The final volume was adjusted to one litre with MQ water and sterilized by autoclaving.
1M Tris-Cl	121.1 g of Tris-Cl salt was dissolved in 800 ml of sterile MQ water. pH was adjusted to 8.0with concentrated 1N HCl. The final volume was adjusted to one litre with MQ water and sterilized by autoclaving.
10% CTAB	100 gm of CTAB powder was dissolved in sterile MQ water and the volume was adjusted to one litre.

Phenol : Chlorofrm : isoamyl alcohol	Buffer saturated phenol, chloroform and isoamyl alcohol were mixed in the ratio of 25 : 24 : 1. The equilibrated mixture was stored under a layer of 0.01 M Tris-HCl (pH 7.6) at 40C in dark glass bottle.
Chloroform : isoamyl alcohol	Buffer saturated chloroform and isoamyl alcohol were mixed in the ratio of 24 : 1.
3M Sodium acetate (pH 4.8)	408.1 g of NaOAc.3H2O was dissolved in 800 ml of MQ water. The pH was adjusted to 4.8 with glacial acetic acid. The final volume was adjusted to one litre with MQ water and sterilized by autoclaving.
Ethidium bromide (10 mg ml-1)	1.0 g of ethidium bromide was added to 100 ml of distilled water and stirred on a magnetic stirrer for several hours to ensure that the dye has dissolved. The solution was transferred to a dark bottle and stored at room temperature.

DNA Extraction buffer

Chemical	**Concentratioon**	**Volume ml**
1 M Tris-CL (pH 8.0)	100mM	5.0
0.5 M EDTA (pH 8.0)	20mM	2.0
4 M NaCl	1.4M	17.5
10% CTAB	2% w/v	10.0
B-mercaptoethanol	0.2%w/v	0.2
Milli-Q water	-	15.3
Total	-	50

TE Bufer	10 mM Tris-HCl (pH 8.0) 1 mM EDTA (pH 8.0)

DNA ELECTROPHORESIS RE-AGENTS

50X TAE	Tris base Glacial acetic acid 0.5M EDTA PH 8.0 Distilled water to 1 Liter	**242.0g** **57.1 ml** **100ml**
Loading dye	1% Bromophenol blue Glycerol 10% SDS 0.5M EDTA 10X TAE Distilled water	**200**
10x TBE Buffer pH 8.3	89Mm Tris HCL 89 mM Boric acid 2.5 mM EDTA Adjust to pH 8.0 with acetic acid store buffer as TBE 10x stock solution.	**107.81g** **55.02 g** **9.36 g**
Tracking dye	Bromophenol blue Xylene cyanolDissolve the powdered dye in sucrose	**0.25 %** **0.25%**

	solution and filter by Whatman No. 1, divide in aliquots and store below 00C.	

VITA

The author **‘ANIL NAGAR’** S/o Shri K.N.NAGAR was born on 15 january 1993 at village and post-Birkhadi, Teh- Gohad, Dist- Bhind (M.P.). She passed high-school certificate (10^{th}) during 2008 from, D.A.V. public school kirandul, Dist-Dantewada (C.G.) in first division with 83.33% marks and Higher-Secondary school certificate (10+2) in 2010 from D.A.V. public school kirandul, Dist-Dantewada (C.G.) in first division with 90.60% marks. He passed undergraduate degree ‘B.Sc. (Agriculture)’ from Rvskvv Gwalior through in 2017 achieving 75.70% marks.

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