GENETIC AND QTL ANALYSIS FOR KERNEL IRON AND ZINC CONCENTRATIONS IN GROUNDNUT
(*Arachis hypogaea* L.)

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M.Sc. (Ag.)

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BY

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2015

DECLARATION

I, **KURAPATI SADAIAH,** hereby declare that the thesis entitled "**GENETIC AND QTL ANALYSIS FOR KERNEL IRON AND ZINC CONCENTRATIONS IN GROUNDNUT [*Arachis hypogaea* L.]"** submitted to **Professor Jayashankar Telangana State Agricultural University** for the degree of **Doctor of Philosophy in Agriculture** is a result of the original research work done by me. I also declare that the thesis or part thereof has not been published earlier elsewhere in any manner.

Place: Hyderabad **(K. SADAIAH)**

Date: **I. D. No. RAD/12-37**

CERTIFICATE

Mr. KURAPATI SADAIAH has satisfactorily prosecuted the course of research and that the thesis entitled "**GENETIC AND QTL ANALYSIS FOR KERNEL IRON AND ZINC CONCENTRATIONS IN GROUNDNUT [*Arachis hypogaea* L.]"** submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that neither the thesis nor its part thereof has been previously submitted by him for a degree of any University.

Date: **(K. RADHIKA)**

Chairperson

CERTIFICATE

This is to certify that the thesis entitled "**GENETIC AND QTL ANALYSIS FOR KERNEL IRON AND ZINC CONCENTRATIONS IN GROUNDNUT** [*Arachis hypogaea* L.]" submitted in partial fulfillment of the requirements for the degree of **DOCTOR OF PHILOSOPHY IN AGRICULTURE** of the Professor Jayashankar Telangana State Agricultural University, Hyderabad is a record of the bonafide research work carried out by **KURAPATI SADAIAH** under my guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee.

No part of the thesis has been submitted for any other degree or diploma or has been published. The published part has been fully acknowledged. All the assistance and help received during the course of investigation have been duly acknowledged by the author of the thesis.

Thesis approved by the Student's Advisory Committee

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(K. SADAIAH)

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LIST OF SYMBOLS AND ABBREVIATIONS

%	:	Per cent
°C	:	Degree Centigrade
ANOVA	:	Analysis of Variance
B_1	:	Progeny of backcross of F_1 with parent 1
B_2	:	Progeny of backcross of F_1 with parent 2
bp	:	base pair
Ca	:	Calcium
cm	:	centimeter
cM	:	centiMorgan
C.D.	:	Critical Difference
CGIAR	:	Consultative Group on International Agricultural Research
CoV	:	Covariance
CTAB		Cityl Tri methyl Ammonium Bromide
Cu	:	Copper
C.V.	:	Coefficient of Variation
d	:	Additive
d.f.	:	Degrees of freedom
DNA	:	Deoxyribonucleic Acid
dNTP	:	deoxyribonucleotide tri-phosphate
EMS	:	Error Mean Sum of square
EDTA	:	Ethylene diaminetetraacetic acid
et al.	:	and other workers
Fig.	:	Figure
F_1	:	First filial generation
F_2	:	Second filial generation
Fe	:	Iron
g	:	Gram
GCV	:	Genotypic Coefficient of Variation
GA	:	Genetic Advance
GAM	:	Genetic Advance as a percentage of Mean
h	:	dominance
ha^{-1}	:	Per hectare

$h^2_{(b)}$	:	Heritability in broad sense
$h^2_{(n)}$	:	Heritability in narrow sense
i	:	Additive × additive
ICGV	:	ICRISAT Groundnut Variety
ICP-OES	:	Inductively Coupled Plasma Optical Emission Spectrometry
ICRISAT	:	International Crops Research Institute for Semi-Arid Tropics
Inc.	:	Incorporated
j	:	Additive × dominance
K	:	Potassium
kg ha^{-1}	:	Kilograms per hectare
l	:	Dominance × dominance
LSD	:	Least Significant Difference
m	:	Mean
Mg	:	Magnesium
m. ha	:	Million hectares
mt	:	Million tonnes
mg	:	Milli gram
mg kg^{-1}	:	milli gram per kilogram
µl	:	Micro litre
µM	:	Micro molar
mM	:	Millimolar
Mn	:	Manganese
N	:	Nitrogen
ng	:	Nanogram
No.	:	Number
P	:	Phosphorous
p		Probability
P_1	:	Parent 1
P_2	:	Parent 2
PCV	:	Phenotypic Coefficient of Variation
ppm	:	Parts per million
P_2O_5	:	Phosphorous pentoxide
r	:	Correlation coefficient
R^2		Phenotypic variance

R.E.	:	Restriction endonuclease
rpm	:	Revolution per minute
RNA	:	Ribonucleic acid
Rnase	:	Ribonuclease
S	:	Sulphur
S.E.	:	Standard error
S.Em	:	Standard error mean
SSR	:	Simple Sequence Repeats
TE	:	Tris-EDTA
USA	:	United States of America
UV	:	Ultra Violet
V	:	Volt
viz.	:	Namely
WHO	:	World Health Organisation
Zn	:	Zinc

Abstract

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ABSTRACT

The present investigation was carried out to identify the molecular markers associated with the kernel iron and zinc concentrations and to study the gene action involved in the inheritance of the traits under concern using generation mean analysis by conducting two separate experiments at ICRISAT, Patancheru. Besides that, studies were also made to estimate the nature and magnitude of genetic effects and to understand the association of kernel iron and zinc concentrations with grain yield and other agronomic traits.

In the first experiment, an attempt was made to identify the molecular markers associated with the kernel iron and zinc concentrations using $F_{2:3}$ mapping population of a cross between a high kernel iron and zinc containing parent, ICGV 06099 and a low kernel iron and zinc containing parent, ICGV 93468. Parental polymorphism survey was conducted with 200 SSR markers, out of which thirty three markers were found polymorphic between the parents. Out of 33 polymorphic SSR markers, three markers *viz.*, SEQ1B09, IPAHM245 and SEQ9G05 showed significant association with the kernel iron concentration with a phenotypic variation of 0.23, 2.19 and 6.34 %, respectively, towards the trait and three markers *viz.*, GM2638, IPAHM245 and SEQ9G05 showed significant association with phenotypic variation of 1.75, 2.25 and 6.01 %, respectively towards kernel zinc concentration. Validation of these markers in another $F_{2:3}$ population derived from the cross ICGV 06040 × ICGV 87141 also showed the strong association of these markers with the trait of interest.

Studies on genetic parameters in $F_{2:3}$ population of the cross ICGV 06099 × ICGV 93468 revealed that PCV was moderately higher than GCV for all the traits including kernel iron and zinc concentrations. Heritability (broad sense) was also found to be higher for kernel iron (64.24 %) and zinc (62.21 %) concentrations. However, low genetic advance as per cent of mean was recorded for the traits understudy. Correlation studies revealed significant positive association between kernel iron and zinc

concentrations. However, these micronutrient concentrations did not show any significant association with pod yield.

In the second experiment, six generations (P_1, P_2, F_1, F_2, B_1 and B_2) each of two crosses (ICGV 06040 × ICGV 87141 and ICGV 06099 × ICGV 93468) were evaluated in compact family block design during post-rainy season, 2013-14 at ICRISAT, Patancheru. Observations were recorded on important agronomic traits along with kernel iron and zinc concentrations which were estimated using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES).

Analysis of variance showed significant differences among the generations of both the crosses for days to emergence, days to maturity, hundred kernel weight, shelling percentage (in the cross ICGV 06040 × ICGV 87141 only), pod yield per plant, kernel iron and zinc concentrations. The phenotypic coefficient of variation was moderately higher than genotypic coefficient of variation for all the traits under study including kernel iron and zinc concentrations which suggested moderate influence of environment on these traits. High heritability (broad sense) coupled with moderate genetic advance as per cent of mean was observed for kernel iron and zinc concentrations in the cross ICGV 06040 × ICGV 87141 indicating that these traits were governed by additive gene action and that selection will be effective, whereas moderate heritability (broad sense) was observed for the same traits in the cross ICGV 06099 × ICGV 93468. Significant negative heterobeltiosis and residual heterosis over better parent for kernel iron and zinc concentration was observed in the cross ICGV 06040 × ICGV 87141 suggesting outperformance of better parent over F_1 and F_2 whereas significant negative heterosis for kernel zinc concentration was observed in the cross ICGV 06099 × ICGV 93468. Correlation studies showed highly significant positive correlation between kernel iron and zinc concentrations in both the crosses, indicating the possibility of simultaneous improvement of both the traits. Kernel iron and zinc concentrations did not show any significant association with pod yield per plant suggesting that no penalty will be there on yield while selecting for kernel iron and zinc concentrations. Positive significant association between 100-kernel weight and kernel zinc concentration was observed indicating the chance of improvement of zinc concentration in bold seeded genotypes.

Generation mean analysis revealed that at least one of the scaling tests to a maximum of three scaling tests *viz.,* A, B and C were significant for the above mentioned traits which indicated the presence of non-allelic interactions. For kernel iron and zinc concentrations additive gene action and additive × additive interaction were positively significant in the cross ICGV 06040 × ICGV 87141 whereas only additive gene action was significant in positive direction in the cross ICGV 06099 × ICGV 93468. However, the magnitude of additive gene effect was higher than the interaction component for the traits under concern. The signs of dominance (*h*) and dominance × dominance (*l*) were opposite for kernel iron and zinc concentrations along with the other traits indicating the presence of duplicate type of epistasis. Selection among parental lines and pedigree method of breeding may be profitable to exploit additive component of gene action for bringing about improvement for kernel iron and zinc concentrations in groundnut.

Introduction

Chapter I

INTRODUCTION

Groundnut also called peanut is one of the principal oil as well as economic crops of the world. It is utilised for human consumption as a vegetable oil and food crop, as a green manure and as fodder for livestock. India is the fourth largest oil producing country in the world, next only to USA, China and Brazil. India occupies the place of pride as the world's second largest producer of groundnut with a total production of 9.47 million metric tons (FAOSTAT, 2014). Groundnut, soybean and mustard together contribute about 85 per cent of the country's oil production and about 80 per cent of total groundnut production in India is crushed for oil extraction, thus improvement in kernel nutrient concentration and quality is of interest to plant breeders.

Micronutrient deficiencies have increased over recent decades due to a generalized decrease in the quality of poor people's diets both in developed and developing countries and even in areas where food is not a limiting factor (Welch and Graham, 1999 and Graham *et al*., 2001). Micronutrient malnutrition affects more than one-half of the world's population, especially women and pre-school children (UNSCN, 2004). Furthermore, micronutrient deficiencies are more widespread than deficiencies caused by inadequate consumption of energy or protein. Breeding crop plants for higher micronutrient concentration, an approach termed as bio-fortification has become an active goal of plant breeding programs in the developing world at both the international and national agricultural research centers (Welch, 2002 and Bouis, 2003). It aims on the development of micronutrient-dense staple crops using the best traditional breeding practices and modern biotechnology.

Micronutrient deficiencies are predicted to affect human population, with Iron Deficiency Anaemia (IDA) being an especially common health concern affecting at least two billion people. IDA is caused by low consumption of iron especially in reproductive age women and developing adolescents (Welch, 1999). Zinc deficiency is suspected to be equally as common but has not been as well documented as IDA (Welch and Graham, 2002). While IDA causes losses in work productivity and developmental problems, zinc deficiency causes lowered disease immunity and stunting. Improving iron and zinc densities of staple crops by breeding offers a cost-effective and sustainable solution to reduce micronutrient malnutrition in resource poor communities.

Poor consumers in developing countries acquire roughly one-half of their total iron intakes and a higher percentage of zinc intakes from staple foods. Bio-fortification, wherever possible, is a cost effective and sustainable solution for tackling the micronutrient deficiencies as the intake of micronutrients is on a continuing basis with no additional costs to the consumer in the developing countries (Kumar *et al*., 2011). It has the potential to help to alleviate the suffering, death, disability and failures to achieve human potential, which results from micronutrient deficiency related diseases. In comparison to other strategies, it provides a truly feasible means of reaching out to remote and rural areas to deliver naturally fortified foods to population groups with limited access to diverse diets, supplements and commercially fortified foods (Bouis *et al.,* 2011).

Results from germplasm screening suggest that the iron and zinc concentration of staple foods can be doubled through conventional breeding. This result, in turn, implies that iron and zinc intakes in poor people's diets can be increased by 50 per cent. This should result in an appreciable improvement in nutrition and health even for those whose intakes remain below recommended daily intakes.

Groundnut is valued as a rich source of energy contributed by oil (48-50 %) and protein (25-28 %) in the kernels. In addition, groundnut kernels also contain antioxidants, vitamins and are rich in mono-unsaturated fatty acids (Janila *et al.,* 2013). They contain vitamin E, and many important B-complex group of vitamins like thiamin, pantothenic acid, vitamin B-6 and niacin. Of the 20 minerals necessary for normal body growth and maintenance, seven, including iron and zinc are present in peanut. Groundnut is a dietary source of biologically active polyphenols, flavonoids and isoflavones but lacks completely in Vitamin-A (Misra, 2006). Developing countries, where micronutrient deficiencies are widespread, contribute world's maximum peanut area and production (FAOSTAT, 2011). Thus, peanut can contribute significantly towards reduction of protein-energy and micronutrient malnutrition (Janila *et al.,* 2014). If there is sufficient genetic variation for the density of micronutrients in edible parts of the crop, bio-fortification can be achieved through plant breeding (Mayer *et al.,* 2008). In groundnut genetic variability was reported for iron and zinc concentration (Upadhyaya *et al*., 2012 and Janila *et al*., 2014) and thus bio-fortification is possible.

Groundnut products can be promoted as nutritional foods by mixing with some essential minerals to fight energy, protein, and micronutrient malnutrition among poor. Project peanut butter is an organisation devoted to fight against malnutrition producing peanut butter with all essential nutrients and energy to serve malnourished children in

Africa. Groundnut based Plumpy'nut, a ready to use therapeutic food, has helped to save the lives of thousands of malnourished children in Niger (UNICEF, 2007).

In order to realize the potential impact of the micronutrient-dense cultivars, the micronutrient-rich cultivars must be delivered in high-yielding backgrounds with farmer's preferred traits (Kumar *et al*., 2010a). However, limited information is available on the components of genetic variance controlling iron and zinc concentrations which show quantitative inheritance. The term epistasis was coined by Bateson (1909) to describe a situation where an action of one gene masks the effect of other at different loci like the phenomenon of complete dominance in which one allele at same locus mask the effect of other. The estimation of epistasis assumes more significance in view of the fact that in its presence, variance component estimates are likely to be biased, hence, inferences drawn from such estimates are most likely to be misleading. Generation mean analysis is a powerful statistical procedure for detection of epistasis using several basic generations from a cross between two inbred lines.

Generation mean analysis is often used to estimate components of mean (additive and dominance effects and interaction) of individual traits. Mather (1949) introduced tests for epistasis, through scaling test. Hayman (1958) described the procedure for partitioning of generation mean into six parameters *viz.,* mean (*m*), additive (*d*), dominance (*h*), additive x additive (*i*), additive x dominance (*j*) and dominance x dominance (*l*) gene effects. Gamble (1962) proposed a model for partitioning the estimation of additive, dominance and epistasis effects from six generations *viz.*, P_1, P_2, F_1, F_2, BC_1 and BC_2 of a cross. This model is considered to be a perfect fit and is not materially different from that proposed by Hayman and Mather (1955). In groundnut, generation mean analysis was carried out to understand the gene action for yield and its contributing characters (Shobha *et al.,* 2010 and Venuprasad *et al.,* 2011) and to study the inheritance pattern for leaf rust and late leaf spot (Janila *et al.,* 2013). However, gene action studies for kernel iron and zinc concentrations in groundnut have not been documented till now.

The inheritance of quantitative traits is a moving target. The expression of these traits is affected not only by large number of genes governing them but also by environmental effects. However, selection for such traits is practiced only in advanced breeding lines, as biochemical estimation for these traits in segregating populations is high resource requiring, cumbersome and time consuming. Thus it seems very complex and challenging to the breeder to undertake quality improvement in large scale breeding programmes through conventional breeding approaches.

Molecular markers offer great scope for improving the efficiency of conventional plant breeding. With the advent of molecular markers, by using segregating populations for the trait of interest for breeders, it has now become routine to map genes or Quantitative Traits Loci (QTLs) and identify valuable alleles for the corresponding traits. The process of constructing linkage maps and conducting QTL analysis to identify genomic regions associated with traits is known as QTL mapping (McCouch and Doerge, 1995). Once the trait is mapped, the markers associated with them can be efficiently employed in breeding programmes through Marker-Assisted Selection (MAS). Markers not only eliminate the need of chemical analysis and phenotypic evaluation in the early generation breeding program, but also minimize the time required to develop new genotypes with desirable traits in the kernelling stage itself, instead of waiting until harvest.

Recently some efforts have been made to locate and tag the traits associated with oil concentration and other yield contributing traits in groundnut based on bulk segregant analysis by using SSR markers (Gomez *et al*., 2009). However, not much effort has been made to locate the QTLs responsible for kernel iron and zinc concentrations in groundnut.

A thorough knowledge of the genetics of characters will help the plant breeder to choose the best breeding scheme in attaining desired objectives. In case of groundnut, both continuous and discontinuous variations have been observed for agronomically important characters (Pattanashetti *et al.,* 2008). Phenotypic Coefficient of Variation (PCV) and Genotypic Coefficient of Variation (GCV) provide an idea about the range of variability present in the material used for the experiment. Heritability and genetic advance helps a breeder to know whether the selection will be effective or not in the improvement of a particular character.

In order to achieve the goal of increased production and quality the knowledge of direction and magnitude of association between various traits is essential for plant breeders. The correlation co-efficient provides a reliable measure of association among the characters and helps to differentiate vital associations useful in breeding from those of the non-vital ones (Falconer, 1981).

Therefore, keeping all the above points in view, the present investigation was undertaken with the following objectives.

- Polymorphism survey between the parents with contrasting kernel iron and zinc concentrations using molecular markers

- Development of $F_{2:3}$ mapping populations using parents with diversified levels of kernel iron and zinc concentration
- Identification of molecular markers linked to the putative genomic regions (QTLs) controlling kernel iron and zinc concentration using $F_{2:3}$ mapping population.
- Validation of putative QTLs in an alternate mapping population
- Studying the gene action governing kernel iron and zinc concentrations.

Review of Literature

Chapter II

REVIEW OF LITERATURE

Groundnut (*Arachis hypogaea* L.), an annual leguminous oilseed crop, is valued as a rich source of high quality edible oil and protein. It is cultivated primarily in the semi-arid tropical regions of Asia and Africa, which together account for over 96 % of world's groundnut area and 92 % of total global groundnut production (Janila *et al.*, 2013). Though groundnut is rich in oil and proteins, it contains traces of essential minerals and lacks vitamin-A (Misra, 2006), thus improvement in kernel mineral concentration and vitamin-A is essential to fight against malnutrition.

Iron and zinc are essential micronutrients in human diet. To develop varieties with high concentration of these elements, it is a prerequisite to identify germplasm with high concentration of both these elements and understand their genetic mechanism (Qin *et al.,* 2012). The choice of selection and breeding procedures for genetic improvement of any crop is largely dependent on the knowledge of type and relative amount of genetic components and the presence of non-allelic interactions for different traits in the plant material under investigation. Assessment of genetic effects involved in the expression of quantitative traits in groundnut can be accomplished by generation mean analysis, which is a simple but useful technique for estimating genetic effects for a polygenic trait. Its greatest merit lies in its ability to estimate epistatic genetic effects such as additive × additive, dominance × dominance and additive × dominance. The information so obtained would have a direct bearing on the breeding programme for further tangible advancement of the crop. Furthermore, micronutrient accumulation in groundnut kernels and its genetic mechanism have not been explored so far.

Since their discovery molecular markers have been frequently used to identify genomic regions and alleles associated with the trait of interest with precision using QTL (Quantitative Trait Loci) mapping (McCouch and Doerge, 1995). It provides a powerful genetic approach in identifying novel genes affecting a certain trait (Vert *et al.* 2002). Several researchers have focussed on micronutrient variation in crops like rice, wheat, common bean *etc.* But information on QTL identification for micronutrient concentration is very limited (Gregorio *et al.,* 2000; Guzma´n-Maldonado *et al.,* 2003 and Gelin *et al.,* 2007) and no literature is available on QTL analysis for iron and zinc concentration in groundnut kernel.

A brief review of literature available on the above aspects is presented in this section, under the following sub-headings:

2.1 Quantitative Trait Loci (QTL) Analysis

2.2 Gene effects

2.3 Variability, heritability and genetic advance

2.4 Heterosis and inbreeding depression

2.5 Correlation studies

2.1 QUANTITATIVE TRAIT LOCI (QTL) ANALYSIS

The cultivated species, *A. hypogaea,* with a large and tetraploid genome, is probably derived from a unique cross between the wild diploid species *A. duranensis* (A-genome) and *A. ipaënsis* (B-genome) resulting in a hybrid followed by spontaneous chromosome duplication (Kochert *et al.,* 1996 and Seijo *et al.,* 2004). Although cultivated peanut is a tetraploid, genetically it behaves as diploid (Stalker *et al.,* 1991). It has been concluded that the A and B genomes contributed nearly equal amounts of DNA to the domesticated peanut (Singh *et al.,* 1996).

In case of cultivated peanut, low levels of genetic variation due to single hybridization event and tetraploid nature of the genome of cultivated peanut have been responsible for the slow progress in the area of developing genomic resources such as molecular markers and genetic maps. However, as a result of concerted efforts in the area of *Arachis* genomics and several molecular studies have been initiated towards QTL mapping and molecular breeding for resistance or tolerance to biotic and abiotic stresses for peanut improvement (Varshney *et al.,* 2010)

The regions within genomes that contain genes associated with a particular quantitative trait are known as Quantitative Trait Loci (QTLs). The process of constructing linkage maps and conducting QTL analysis to identify genomic regions associated with traits–is known as QTL mapping or genome' mapping (McCouch and Doerge, 1995 and Mohan *et al.,* 1997).

Finding genes which control the accumulation of iron and zinc in kernels of major crops is the precondition for bio-fortified breeding program (Jin *et al.,* 2013). Studies have shown that the iron and zinc metabolism, involving processes of mobilization, uptaking, translocation and accumulation, is a complex process regulated by many genes (Bashir *et al.,* 2012 and Kobayashi and Nishizawa, 2012). QTL mapping is a powerful approach to study and manipulate complex traits that are important in agriculture, including mineral concentration (Kaiyang *et al.,* 2008). It provides

information on the chromosomal location of the target loci without any prior knowledge of the genes related to the trait and also may be applied in breeding program using Marker-Assisted Selection (MAS) (Collard *et al.*, 2005 and Ghandilyan *et al.*, 2006). However, reports of QTLs on kernel micronutrient concentration are limited.

Since QTL analysis for kernel iron and zinc concentration in groundnut was not documented before and limited information is available in other related crops, the literature below covers a range of crops on which this aspect was studied. The following review will give an impression about the QTL analysis done on kernel iron and zinc concentrations in several important crops.

The discussed literature suggests that much of the work on QTL analysis for kernel iron and zinc concentrations was carried out in important cereal crops like wheat, rice, maize and pearl millet also in legumes like common bean, clover and soybean which resulted in the identification of key QTLs for the above mentioned traits. The population selected for their study was dominated by Recombinant Inbred Lines (RILs) developed through Single Seed Descent (Goulden, 1941) method for 5-6 generations followed by Double Haploid (DH) lines.

Though there are different types of QTL analysis methods, Composite Interval Mapping (CIM) (Zeng, 1994) was used predominantly because of its high precision to detect the QTL. QTL cartographer v2.5 (Wang *et al.*, 2007) software was most commonly used to detect the QTL and its contribution towards the phenotypic variance for the character studied.

Wheat, being an important cereal crop, attempts were made to locate QTLs responsible for grain iron and zinc concentration. Most of the studies (Table 2.1) revealed that QTLs for kernel iron were located on chromosomes 2A (Tiwari *et al.*, 2009, Hakimeh *et al.*, 2013 and Jayasudha *et al.*, 2014), 3D (Genc *et al.*, 2009 and Hakimeh *et al.*, 2013) and 7A (Tiwari *et al.*, 2009) whereas, QTLs for kernel zinc concentration were located on chromosomes 4A (Shi *et al.*, 2008 and Hakimeh *et al.*, 2013) and 7A (Shi *et al.*, 2008 and Tiwari *et al.*, 2009) contributing to the maximum phenotypic variation for the traits in question.

Similarly, in rice, QTLs for grain iron (Table 2.1) were identified on chromosome 2 (Stangoulis *et al.*, 2007 and Oliveira *et al.*, 2009) and 9 (Oliveira *et al.*, 2009 and Kaiyang *et al.*, 2008) whereas, for grain zinc concentration QTLs were identified on chromosomes 5 (Oliveira *et al.*, 2009, Kaiyang *et al.*, 2008 and Gande *et al.*, 2014) and 12 (Stangoulis *et al.*, 2007 and Oliveira *et al.*, 2009) contributing to the maximum phenotypic variation for the traits understudy. However, the earlier reports of

Anuradha *et al.* (2012) revealed that QTLs for kernel iron and zinc were co-located on chromosomes 7 and 12.

Studies on QTL analysis in maize (Table 2.1) revealed the presence of QTLs for kernel iron concentration on chromosomes 5 (Lung'aho *et al.*, 2011 and Jin *et al.,* 2013) and 2 (Lung'aho *et al.* 2011 and Simic *et al.,* 2012) whereas, for kernel zinc concentration QTLs were identified on chromosomes 2 and 5 (Jin *et al.,* 2013) and 4 (Simic *et al.,* 2012) with higher phenotypic variation for the traits understudy.

Though, literature on QTL mapping for kernel iron and zinc in legumes is scanty few attempts were made to identify the QTLs in common bean, clover and soybean. Results revealed that in common bean (Table 2.1), QTLs for both grain iron and zinc were co-located on B6 linkage group (Cichy *et al.,* 2009 and Blair *et al.,* 2009) and on B5 (Cichy *et al.,* 2009) with higher phenotypic expression.. In Clover, chromosome 7 carries the QTLs for both kernel iron and zinc with moderate phenotypic variance (Klein and Grusak 2009). In Soybean, chromosome 20 carries QTLs for kernel iron concentration and chromosomes 7 and 18 carry QTLs for kernel zinc concentration with moderate amount of phenotypic variance (King *et al.,* 2014).

Table 2.1. Review on Quantitative trait Loci (QTL) for kernel iron and zinc densities in different crops

S No.	Population size	Mapping population	Trait	No. of QTL detected	Software and Method	Chromosome	Closely linked markers (LOD score)	Position (cM)	R^2 or PV%	Reference
WHEAT (*Triticum aestivum* L.)										
1	119	Doubled Haploid (DH) population	Zn	4	QTL Cartographer v2.0; Composite Interval Mapping (CIM)	4A, 4D, 5A and 7A	P3446-205—CWM145 (2.14), Xgwm192—WMC331 (4.22), Xgwm291—Xgwm410 (3.63) and WMC488—P2071-180 (2.08), respectively.	--	6.8, 11.9, 10.9 and 5.3, respectively.	Shi *et al.* (2008)
2	90	Doubled Haploid (DH) population	Fe	1	WGIAM v 1.4; Whole Genome Average Interval Mapping	3D	gdm8-gdm136	--	1.10	Genc *et al.* (2009)
			Zn	4		3D, 4B, 6B and 7A	Gdm136-gwm3, wms149-gwm113, barc146a-p41/m48-76 and gwm282-gwm63, respectively.	--	43.50, 7.9, 14.5 and 6.9, respectively.	
3	93	Recombinant Inbred Lines (RIL) population	Fe	2	QTL Cartographer v2.5; Composite Interval Mapping (CIM)	2A and 7A	Xwmc382-Xbarc124 (3.3) and Xgwm473-Xbarc29 (3.2), respectively.	23.6 and 153.8, respectively.	12.6 and 11.7, respectively.	Tiwari *et al.* (2009)
			Zn	1		7A	Xcfd31-Xcfa2049 (4.2)	72.6	18.8	
4	118	RILs	Fe	6	QTL Cartographer v2.5; Composite Interval Mapping (CIM)	2A, 3D, 4D, 7B, and 7D	Xgwm312-Xgwm817 (3.91), Xgwm817-Xgwm630 (3.71), Xgwm1047-Xgwm383 (2.76), Xgwm4670-Xgwm194 (2.54), Xgwm767-Xgwm3036 (2.52) and Xbarc184-Xgwm1055 (2.78), respectively.	51.5, 53.5, 107, 72.5, 121 and 23.5, respectively.	29.1	Hakimeh *et al.* (2013)
			Zn	2		1A and 4A	Xgwm3094-Xgwm164 (2.97) and Xgwm4026-Xgwm1081 (2.67), respectively.	39 and 48.5, respectively.	45.51	
5	185	RILs	Fe	5	QTL IciMapping v.3.2; Composite Interval Mapping (CIM)	1A, 2A and 3B	1046200|F|0 1228280|F|0 (0.52), 2289695|F|0 1218555|F|0 (0.93), 1708014|F|0 1000008|F|0 (2.08), 1081485|F|0 1216621|F|0 (0.52) and 1,015.23–1,022.28 3022954|F|0 (8.61), respectively.	56, 227, 346, 162 and 1022, respectively.	5.56, 7.48, 16.55, 5.6 and 25.95, respectively.	Jayasudha *et al.* (2014)
			Zn	5		2A, 2B, 3D, 6A and 6B	1126272|F|0 2255234|F|0 (2.1), 989092|F|0 1101425|F|0 (0.6), 1094214|F|0 1057342|F|0 (1.15), 998265|F|0 3026160|F|0 (1.23) and 1001916|F|0 1129916|F|0 (0.89), respectively.	146, 966, 57, 327 and 1433, respectively.	5.20, 16.46, 4.75, 6.99 and 9.7, respectively	

S No.	Size of population	Mapping population	Trait	No. of QTLs detected	Software and Method	Chromosome	Closely linked markers (LOD score)	Position (cM)	R^2 or PV%	Reference
RICE (*Oryza sativa* L.)										
6	129	Double Haploid lines (DH)	Fe	3	QTL Cartographer v2.5; Composite Interval Mapping (CIM)	2, 8 and 12	--		17, 18 and 14, respectively.	Stangoulis *et al.* (2007)
			Zn	2		1 and 12	--		15 and 13, respectively.	
7	85	Introgression Lines (ILs)	Fe	1	MAP MANAGER QTX software. Model QTXb17; Single Point Analysis	2 and 9	RM6641 and RM296, respectively.	--	5	Oliveira *et al.* (2009)
			Zn	3		5,8 and 12	RM1089, RM152 and RM3331, respectively.	--	5, 19 and 9, respectively.	
8	120	DH	Fe	14	QTL cart. 2.5; Composite Interval Mapping (CIM)	6 and 1	6022-6022 (4.05) and 1024-1026 (3.24), respectively.	--	10-21.1	Qin *et al.* (2012)
9	241	RILs	Fe	2	QTLMapper1.0	1 and 9	RG236-C112 (7.66) and C472-R2638 (4.25), respectively.	--	25.81 and 11.11, respectively.	Kaiyang *et al.* (2008)
			Zn	3		5, 7 and 11	R3166-RG360 (4.27), RM234-R1789 (1.8) and C794-RG118 (5.65), respectively.	--	12.34, 5.3 and 18.61, respectively.	
	168	RILs	Fe	7	QTL Cartographer v2.5; Composite Interval Mapping (CIM)	1, 5, 7 and 12	RM243-RM488 (21.9), RM488-RM490 (21.9), RM574- RM122 (25.3), RM234- RM248 (27.9), RM248-RM8007 (27.2), RM17- RM260 (33.8) and RM260- RM7102 (33.4)	--	69, 69.2, 69.2, 69, 69, 71 and 71.	Anuradha *et al.* (2012)
			Zn	6		3, 7 and 12	RM7-517 (3.04), RM234-RM248 (2.6), RM248- RM8007 (2.6), RM501-OsZip2 (3), RM17-RM260 (3.1) and RM260- RM7102 (2.9)	--	31, 35, 35, 29, 35 and 34	
10	160	RILs	Zn	4	SPSS 16.0 (SPSS Inc.); Single Marker Analysis (SMA)	3, 4,5 and 7	OsNAC, OsZIP8a, OsZIP8c and OsZIP4, respectively.	--	4.5, 19.0, 5.1 and 10.2, respectively.	Gande *et al.* (2014)
MAIZE (*Zea mays* L.)										
11	218	$F_{2:3}$ population	Fe	5	QTL Cartographer v2.5; Composite Interval Mapping	5	umc1429–umc1060 (3.49)	--	16.9	Jin *et al.* (2013)
			Zn	5		2, 5 and 10	bnlg1633–bnlg1138 (3.01), umc1536–bnlg1633 (3.17), umc1429–umc1060 (5.58) and umc1506–umc2350 (4.23).	--	5.9-17.6	

S No.	Size of population	Mapping population	Trait	No. of QTLs detected	Software and Method	Chromosome	Closely linked markers (LOD score)	Position (cM)	R2 or PV%	Reference
12	172	F_4	Fe	4	PLABQTL; Composite Interval Mapping	2,6 and 8	--	--	6.8-7.5	Simic *et al.* (2012)
			Zn			4	--	--	7.8	
13	224	RILs	Fe	3	QTL Cartographer v2.5; CIM.	2, 5 and 9	--	--	9.3-12	Lung'aho *et al.* (2011)
BEAN (*Phaseolus vulgaris* L.)										
14	73	RILs	Zn	4		3, 9	--	--	7.1-1.3	Gelin *et al.* (2007)
15	110	RILs	Fe	5	QTL Cartographer v2.5; Composite Interval Mapping (CIM)	B4, B6, B7, B6 and B6	BMc127 (2.74), R0405B (4.71), BMc248 (2.99), BM158 (5.10) and BM158 (5.42), respectively.	--	10.82, 21.27, 9.57, 19.8 and 19.26	Blair *et al.* (2009)
			Zn	8		B6, B8, B6, B8, B2,B3, B6 and B6	V1001B (5.24), H1201A (4.44), BM158 (4.07), H1201A (2.85), PV15 (3.92), BMd1 (2.92), BM158 (5.34) and BM158 4.92, respectively.	--	38.42, 17.83, 14.29, 10.05, 11.94, 10.52, 17.36 and 29.91	
16	**77**	RILs	Fe	6	QTL Cartographer v2.5; Composite Interval Mapping (CIM)	B1, B5, B6, B9, B11 and B8	fi n (12.65) GGAT02 (3.91) BM170 (6.9) GCTC02 (5.1) GGAG01 (5.23) and M12.1600A (3.39), respectively.	--	8-36	Cichy *et al.* (2009)
			Zn	4		B1,B6,B11,B5	fin (7.05), AGAT05 (8.42), CTTA02 (2.89) and CGTC01 (2.76), respectively.	--	9-39	
CLOVER (*Medicago truncatula*)										
17	93	RILs	Fe	--	QTL Cartographer v2.5; Composite Interval Mapping (CIM)	7			21.2	Klein and Grusak (2009)
			Zn	--		4, 7 and 8	--	--	24.2, 17.9 and 8.9, respectively.	
SOYBEAN (*Glycine max*)										
18	92	$F_{2:4}$	Fe	1	MapQTL6; Multiple-QTL Mapping (MQM)	20	pa 515-1-Satt239 (4.7)	4.0	21.5	King *et al.* (2014)
			Zn	2		7 and 18	pk 417H-pk 70T (3.0) pa 890V-pK 493H (2.9)	65.9 and 124.6, respectively.	23.4 and 18.5, respectively.	

2.2 GENE EFFECTS

To develop a plant genotype with desirable combination of traits comprehensive information regarding genetic mechanism controlling various traits is considered a pre-requisite to launch a breeding programme (Rehman *et al.*, 2009).

Improving iron and zinc densities of staple crops by breeding offers a cost-effective and sustainable solution to reduce micronutrient malnutrition in resource poor communities. An understanding of the genetics of these micronutrients can help to accelerate the breeding process (Velu *et al.*, 2011a).

The estimation of epistasis assumes more significance in view of the fact that in its presence, variance component estimates are likely to be biased hence inferences drawn from such estimates are more likely to be misleading. The magnitude of the bias depends upon the relative magnitude of epistatic effects compared to the deviations of additive (*d*) and dominance (*h*) type of prevailing epistasis and direction of dominance. The existence of large array of interactions in a polygenic system causes over-estimation of heritability (narrow sense) thereby causing an additional bias in predicted gains. Generation mean analysis (GMA) is a simple but useful technique for estimating gene effects for a polygenic trait, its greatest merit lying in the ability to estimate epistatic gene effects such as additive × additive (aa), dominance × dominance (dd) and additive × dominance (ad) effects (Singh and Singh, 1992).

This technique has been used to carry out gene action studies on iron and zinc concentration and yield contributing traits in groundnut.

Sangha *et al.* (1990) using GMA, observed that dominance × dominance epistasis was important for pod yield in groundnut in the cross M13 X Acc.1978.

Ali *et al.* (1999) conducted generation mean analysis experiment by involving two crosses *viz.*, No.334 x ICGSE 4 and NC 9 x ICGSE4 to study the gene action governing 100 kernel weight and reported that additive gene action had a predominant role in governing the character mentioned.

Gene action studies carried out by Venkateswarlu *et al.* (2007a) in groundnut involving eight parents and 28 single crosses without reciprocals in diallel mating design revealed the importance of both additive and non-additive gene action in governing the kernel yield per plant, shelling percentage, sound mature kernel weight and oil concentration.

Jivani *et al.* (2009) conducted an experiment in diallel mating design involving eight parents and 28 single crosses (without reciprocals) to study the gene action

governing 100 kernel weight and kernel yield per plant and concluded that both additive and non-additive gene action had equal role to govern the above mentioned traits.

Rehman *et al.* (2009) evaluated 55 genotypes of mung bean in Randomized Complete Block Design (RBD) to study the gene action for kernel yield per plant and concluded that additive gene action played an important role in governing the kernel yield.

Generation mean analysis experiment was conducted in groundnut by Shobha *et al.* (2010) involving three crosses *viz.*, TMV 2 × ICGV 97150, TMV 2 × COG 0437 and TMV 2 × COG 0438 to study the gene action governing 100 kernel weight, kernel yield per plant and shelling percentage and reported that along with additive and dominance gene action, additive × additive and dominance × dominance components of epistasis played an important role in governing the above mentioned traits.

Vekariya *et al.* (2011) examined the gene action in groundnut for several traits by evaluating 50 genotypes in Randomized Complete Block Design (RBD) and mentioned the predominance of additive gene action in controlling the kernel yield per plant.

Venuprasad *et al.* (2011) carried out research in groundnut to study the gene action governing kernel traits *viz.,* kernel size, kernel weight and kernel length by conducting generation mean analysis involving six crosses and concluded that additive gene action alone governed the kernel size whereas, additive and additive × additive and dominance and dominance × dominance gene actions played an important role in governing kernel weight and kernel length, respectively.

Alam *et al.* (2013) conducted a 10 ×10 half diallel experiment on groundnut to ascertain the gene action and genetic parameters controlling days to 50 % flowering and 100 kernel weight. The estimates of gene effects indicated that significance of both additive and dominance gene action in governing days to 50 % flowering and 100 kernel weight.

Rai *et al.* (2014) evaluated 15 genotypes of groundnut in Randomized Complete Block Design (RBD) to study the gene action of several agronomical traits and revealed the importance of additive gene action governing the shelling percentage.

Since the literature on gene action studies for kernel iron and zinc concentrations, yield and its contributing traits in groundnut is scanty, a brief description about the gene action controlling the above mentioned traits in other important crops is furnished below:

Literature on days to flowering (Table 2.2) suggests the predominant role of additive gene action in dolichos bean (Parmer *et al.,* 2013) and pigeon pea (Santosh *et al.,* 2014) in governing the trait concerned and additive and dominant gene action in chickpea (Deb and Khaleque, 2009). In mung bean, additive and dominance × dominance interaction (Khattak *et al.,* 2004) and additive, dominance and additive × additive (Singh *et al.,* 2006) type of epistatic interactions were found to be influencing the trait understudy.

Santosh *et al.* (2014) observed the predominant role of additive gene action in governing branching and growth habit in pigeon pea. Whereas, in chickpea additive and dominant gene actions (Deb and Khaleque, 2009 and Biranvand *et al.,* 2013) had equal importance in governing the trait understudy. Singh *et al.* (2001) observed influence of non-additive gene action in governing number of primary branches in garden pea (Table 2.2).

Role of additive gene action in governing days to pod maturity was reported by in dolichos bean (Parmer *et al.,* 2013) and in pigeon pea (Santosh *et al.,* 2014). Role of non-additive along with additive gene action was reported in mung bean (Khattak *et al.,* 2004 and Noorka *et al.,* 2014) and lentil (Akbari *et al.,* 2013). Importance of epistatic gene actions in governing days to pod maturity was also reported in mung bean by Singh *et al.* (2006).

Based on the available literature (Table 2.2) it was observed that 100-kernel weight, which also gives knowledge on kernel size, was majorly governed by additive gene action in maize (Azizi *et al.,* 2006), wheat (Fatehi *et al.,* 2008), common bean (Mulugeta *et al.,* 2013) and in pigeon pea (Santosh *et al.,* 2014). Along with additive gene action Singh *et al.* (2006) observed the role of all three types of epistatic gene interactions (additive × additive (*i*), additive × dominance (*j*) and dominance × dominance (*l*)) in governing 100-kernel weight in mung bean. Sundari *et al.* (2012) found the involvement of additive and additive × additive gene action in controlling the 1000-seed weight in sesame.

Influence of many genes and involvement of several gene actions was expected in controlling a complex economic trait, seed yield. In garden pea, Kalia and Sood (2009) observed the predominant role of additive gene action in governing the pod yield per plant. Along with additive gene action dominance gene action also plays an important role in governing kernel yield per plant in crops like maize (Azizi *et al.,* 2006). In wheat, Fatehi *et al.* (2008) observed the importance of dominance ×

dominance gene interaction along with dominance gene action in governing the grain yield per plant.

In sesame, role of additive and dominance (Sharmila *et al.,* 2007), additive and additive × additive interaction (Sundari *et al.,* 2012) and additive ×additive and dominance × dominance epistatic interactions (Jawahar *et al.,* 2013) were observed in governing the seed yield. In chickpea, Biranvand *et al.* (2013) observed the effect of additive and dominance gene actions in governing the seed yield per plant. Influence of epistatic gene actions along with additive gene action in governing kernel yield was reported by Singh *et al.* (2006) in mung bean and Akbari *et al.* (2013) in lentil.

Hazem *et al.* (2013) in faba bean reported the predominance of additive and dominance gene actions in governing shelling and sound mature kernel percentages.

Oil content in groundnut is an important trait based on which the economic importance of the variety will be measured. Attempts were made by scientists to understand the gene action governing this trait and found the influence of both additive and non-additive gene action (Venkateswarlu *et al.,* 2007a) on the trait understudy in groundnut. Jawahar *et al.* (2013) reported the influence of epistatic gene action in controlling oil concentration in sesame.

Study on gene actions governing protein content (Table 2.2) revealed the importance of additive gene action in chickpea (Santos *et al.,* 2012) and dolichos bean (Parmer *et al.,* 2013). Role of dominant gene action along with additive gene action for protein concentration was reported in faba bean by Hazem *et al.* (2013).

Comprehensive studies to understand the gene action governing kernel iron and zinc concentrations were made by several workers (Table 2.2) which revealed the importance of additive gene action in governing the concerned traits in different crops *viz.,* maize (Arnold *et al.,* 1977; Long *et al.,* 2004 and Chakraborti *et al.,* 2011), pearl millet (Brkic *et al.,* 2003; Velu *et al.,* 2011a and Rai *et al.,* 2012) and rice (Zhang *et al.,* 2004). Additive and non-additive gene actions were observed in sorghum (Kumar *et al.,* 2013) and both additive and dominant gene actions were observed in common bean (Silva *et al.,* 2013).

Table 2.2. Review on gene action governing various agronomic traits along with kernel iron and zinc concentrations in different crops

S. No	Crop	Trial information	Analysis method and Population size	Trait	Predominant gene action	Reference
DAYS TO FLOWERING						
1	Mung bean	ML-5 × NM 54	Generation mean analysis (Six parameter model)	Days to first flowering	Additive and dominance × dominance	Khattak *et al.* (2004)
		6601 × NM 92			Additive and non-additive	
2	Mung bean	ML 1271 × MUL81	Generation mean analysis (Six parameter model) P_1, P_2 and F_1-5 Plants; B_1, B_2 and F_2- 10 Plants	Days to 50 per cent Flowering	Additive, dominance, additive × additive	Singh *et al.* (2006)
		VC 6370-30-65 × MUL 81				
		ML 1271 x LM 51				
		VC 6370-30-65 × LM 51				
3	Chickpea	RBH-228 × ICC-4918, RBH-228 x Nobin, Nobin x ICC-4918	Generation Mean Analysis (Five parameter model)	Days to first flower	Additive and dominance	Deb and Khaleque (2009)
4	Dolichos bean	Randomized Complete Block Design (RBD)	30 genotypes	Days to 50 per cent flowering	Additive	Parmer *et al.* (2013)
5	Pigeonpea	Randomized Complete Block Design (RBD)	38 genotypes	Days to 50 per cent flowering	Additive	Santosh *et al.* (2014)
GROWTH AND BRANCHING HABIT						
6	Garden pea	Randomized Complete Block Design (RBD)	Seven parents and 21 single crosses evaluated in diallel mating design	Number of primary branches	Non-additive	Singh *et al.* (2001)
7	Chickpea	RBH-228 × ICC-4918, RBH-228 × Nobin, Nobin × ICC-4918	Generation mean analysis (Five parameter model)	Number of primary and secondary branches	Additive and dominance	Deb and Khaleque (2009)
8	Chickpea	Randomized Complete Block Design (RBD)	Six parents and 15 single crosses evaluated in diallel mating design	Secondary branches per plant	Additive and dominance	Biranvand *et al.* (2013)
9	Pigeonpea	Randomized Complete Block Design (RBD)	38 genotypes	Number of primary and secondary branches	Additive	Santosh *et al.* (2014)

S. No	Crop	Trial information	Population size and Analysis method	Trait	Predominant gene action	Reference
DAYS TO MAURITY						
11	Mung bean	ML-5 × NM 54	Generation mean analysis (Six parameter model)	First pod maturity	Additive	Khattak *et al.* (2004)
		6601 × NM 92			Additive and non-additive	
12	Mung bean	ML 1271 × MUL81	Generation mean analysis (Six parameter model) P_1,P_2 and F_1-5 Plants; B_1, B_2 and F_2- 10 Plants	Days to maturity	Additive, dominance, dominance × dominance	Singh *et al.* (2006)
		VC 6370-30-65 × MUL 81			Additive, dominance, additive × additive and dominance × dominance	
		ML 1271 × LM 51			Additive, dominance and Additive × additive	
		VC 6370-30-65 × LM 51			Additive, Additive × additive and dominance × dominance	
13	Mung bean	MN-51, MN - 92, MN - 96, MN - 98 and 00TM-12	Five parents and 10 single crosses evaluated in diallel mating design	Days to first pod maturity	Additive and non-additive	Noorka *et al.* (2014)
14	Lentil	Randomized Complete Block Design (RBD)	Six parents and 15 single crosses evaluated in diallel mating design	Days to maturity	Additive and non-additive	Akbari *et al.* (2013)
15	Dolichos bean	Randomized Complete Block Design (RBD)	30 genotypes	Days to maturity	Additive	Parmer *et al.* (2013)
16	Pigeonpea	Randomized Complete Block Design (RBD)	38 genotypes	Days to maturity	Additive	Santosh *et al.* (2014)
100- KERNEL WEIGHT (g)						
17	Maize	B73 × Mo17	Generation mean analysis (Six parameter model)	100- kernel weight	Additive	Azizi *et al.* (2006)
		B73 × K74/1				

S. No	Crop	Trial information	Population size and Analysis method	Trait	Predominant gene action	Reference
18	Wheat	Falat × Line 30 (breeding line)	Generation mean analysis (Six parameter model)	1000-kernel weight	Additive	Fatehi *et al.* (2008)
19	Mung bean	ML 1271 × MUL81	Generation mean analysis (Six parameter model) P_1, P_2 and F_1- 5 Plants B_1, B_2- 10 Plants F_2- 10 Plants per row	100- kernel weight	Additive, additive × additive and dominance × dominance	Singh *et al.* (2006)
		VC 6370-30-65 × MUL 81			Additive, dominance, additive × additive, additive x dominance and dominance × dominance	
		ML 1271 × LM 51			Additive × additive, additive x dominance and dominance × dominance	
		VC 6370-30-65 × LM 51			Additive, dominance, additive x dominance and dominance × dominance	
		TMV 2 × COG 0438			Additive	
20	Sesame	Vm, X-79-1, EC 351187, EC 359007	Generation mean analysis (Five parameter model) Five parents, 10 F_1s, 10 F_2s, and 10 F_3 plants each.	1000 kernel weight	Additive and additive × additive	Sundari *et al.* (2012)
21	Common bean	Diallel mating design	Eight parents and 28 single crosses	1000 kernel weight	Additive	Mulugeta *et al.* (2013)
22	Pigeonpea	Randomized Complete Block Design (RBD)	38 genotypes	100 kernel weight	Additive	Santosh *et al.* (2014)
POD YIELD PER PLANT (g)						
23	Maize	B73 × Mo17	Generation mean analysis (Six parameter model)	Kernel yield	Additive	Azizi *et al.* (2006)
		B73 × K74/1			Dominance	
24	Wheat	Falat × Line 30 (breeding line)	Generation mean analysis (Six parameter model)	Kernel yield per plant	Dominance and dominance × dominance	Fatehi *et al.* (2008)

S. No	Crop	Trial information	Population size and Analysis method	Trait	Predominant gene action	Reference
25	Mung bean	ML 1271 × MUL81	Generation mean analysis (Six parameter model) P_1, P_2 and F_1 - 5 plants, B_1, B_2- 10 plants, F_2- 10 plants per row	seed yield per plant	Additive, additive × additive and dominance × dominance	Singh *et al.* (2006)
		VC 6370-30-65 × MUL 81			Additive, dominance additive × additive, additive x dominance and dominance × dominance	
		ML 1271 × LM 51			Additive, Dominance Additive × additive, Additive x Dominance and Dominance × dominance	
		VC 6370-30-65 × LM 51			Additive, dominance additive x dominance and dominance × dominance	
26	Sesame	VS 9510 × Co1	Generation mean analysis (Six parameter model)	Seed yield per plant	Additive and dominance	Sharmila *et al.* (2007)
		NIC 7907 × TMV 3				
		Cianno 13/10 × VRI 1				
		Si 1115/1 × TMV 3				
27	Sesame	Vm, X-79-1, EC351187, EC359007 and EZ351881	Generation mean analysis (Five parameter model) 5 parents, 10 F_1s, 10 F_2s, 10 F_3 plants	Seed yield per plant	Additive and additive × additive	Sundari *et al.* (2012)
28	Sesame	KMR-108 × JCS-507, KKS-98049 × IS 562 B, S 0018 × SI-3171, KKS-98049 × TKG-22 and CST 2001-5 × KMS 5-396	Generation mean analysis (Six parameter model) P_1, P_2 and F_1-30 plants F_2- 400 plants BC_1 and BC_2- 200 plants	Seed yield per plant	Additive × additive and dominance × dominance	Jawahar *et al.* (2013)
29	Garden pea	Diallel mating design	Eight parents and 28 single crosses	Pod yield per plant	Additive	Kalia and Sood (2009)
30	Lentil	Randomized Complete Block Design (RBD)	Six parents and 15 crosses evaluated in diallel mating design	Seed yield	Additive and Non-additive	Akbari *et al.* (2013)
31	Chickpea	Randomized Complete Block Design (RBD)	Six parents and 15 single crosses evaluated in diallel mating design	Seed yield per plant	Additive and dominance	Biranvand *et al.* (2013)

S. No	Crop	Trial information	Population size and Analysis method	Trait	Predominant gene action	Reference
SHELLING (%) and SOUND MATURE KERNEL (%)						
32	Faba bean	Evaluation for genetic parameters in RBD	Five parents *viz.,* Misr 2, Giza 429, Misr 1,Giza 843 and Giza 40 and their 10 single crosses	Shelling outturn	Additive and dominance	Hazem *et al.* (2013)
OIL CONTENT (%)						
33	Groundnut	Evaluation for genetic parameters in RBD	Eight parents and 28 single crosses evaluated in diallel mating design	Oil content (%)	Additive and non-additive	Venkateswarlu *et al.* (2007a)
34	Sesame	KMR-108 × JCS-507, KKS-98049 × IS 562 B, S 0018 × SI-3171, KKS-98049 × TKG-22 and CST 2001-5 × KMS 5-396	Generation mean analysis (Six parameter model) P_1, P_2 and F_1-30 plants F_2- 400 plants B_1 and B_2- 200 plants	Oil content (%)	Additive × additive and dominance × dominance	Jawahar *et al.* (2013)
PROTEIN CONCENTRATION (%)						
35	Cowpea	IT97K-1042-3 × BRS Tapaihum and IT97K-1042-3 × Canapu	Generation mean analysis (six parameter model)	Kernel Protein	Additive	Santos *et al.* (2012)
36	Faba bean	Evaluation for genetic parameters in RBD	Five parents *viz.,* Misr 2, Giza 429, Misr 1,Giza 843 and Giza 40 and their 10 single crosses	Protein concentration	Additive and dominance	Hazem *et al.* (2013)
37	Dolichos bean	Evaluation for genetic parameters in RBD	30 genotypes	Kernel Protein	Additive	Parmer *et al.* (2013)

S. No	Crop	Trial information	Population size and Analysis method	Trait	Predominant gene action	Reference
KERNEL IRON AND ZINC CONCENTRATION (mg kg^{-1})						
38	Rice	Diallel mating design	7 parents and 42 F_1s	Grain Fe and Zn	Additive	Zhang *et al.* (2004)
39	Maize	Diallel mating design	6 inbreds and 14 F_1s	Kernel Fe and Zn	Additive	Arnold *et al.* (1977)
40	Maize	Diallel mating design	14 inbreds and 91 F_1s	Kernel Fe and Zn	Additive	Long *et al.* (2004)
41	Maize	CM145 × V334 CM128 × V340	Generation mean analysis (Six parameter model) 10 ears each from the P_1 and P_2, 20 from F_1, 60 from F_2, and 40 each from BC (P_1) and BC (P_2).	Kernel Fe and Zn	Additive	Chakraborti *et al.* (2011)
42	Pearl millet	Diallel mating design	8 inbreds and 28 F_1s	Grain Fe and Zn	Additive	Brkic *et al.* (2003)
43	Pearl Millet	Diallel mating design	10 parents and 90 F_1s	Grain Fe and Zn	Additive	Velu *et al.* (2011a)
44	Pearl Millet	Advanced breeding lines (F_7) and early generation progenies (S_1-S_3)	F_7 (lines) and S_1-S_3 (232 progenies)	Grain Fe and Zn	Additive	Rai *et al.* (2012)
45	Sorghum	Diallel mating design	Five inbreds lead 20 F_1 s six inbreds lead 30 F_1 s and four parents lead 12 F_1s	Grain Fe	Additive and non-additive	Kumar *et al.* (2013)
				Grain Zn	Additive	
46	Common bean	Partial Diallel design	--	Seed Fe	Additive and dominance	Silva *et al.* (2013)
				Seed Zn	Additive	

2.3 VARIABILITY, HERITABILITY AND GENETIC ADVANCE

The genetic variability has to be looked into for planning suitable measures for crop improvement. This necessitates a thorough knowledge of variability owing to genetic factors, actual genetic variation heritable in the progeny and the genetic advance that can be achieved through selection (John and Reddy, 2014). The information on the nature and magnitude of variability of different quantitative and qualitative traits in any crop species plays a vital role while formulating efficient breeding programmes. Superior genotypes can be isolated by selection if considerable genetic variation exists within the population. Besides genetic variability, heritability and genetic advance also play a vital role in the improvement of any trait.

Genetic variability is an essential prerequisite for crop improvement programme for obtaining high yielding varieties, through the estimation of different genetic parameters like components of variances, genotypic and phenotypic coefficients of variability, heritability and genetic advance (Younis *et al*., 2008). In genetic studies, characters with high genotypic coefficient of variation indicate the potential for an effective selection (Sadiq *et al.,* 1986). Determining the components of variability in yield and its components enable us to know the extent of environmental influence on yield, taking into consideration of the fact that yield and its component traits are quantitative characters that are affected by environments (Ahmed *et al*., 2007). Heritability provides information about the extent of which a particular genetic character can be transmitted to the successive generations (Mangi *et al*., 2010). High heritability indicates less environmental influence in the observed variation (Mohanty, 2003 and Eid, 2009). Genetic advance, which estimates the degree of gain in a trait obtained under a given selection pressure, is an important parameter that guides the breeder in choosing a selection programme (Hamdi *et al*., 2003). High heritability and high genetic advance for a given trait indicates that it is governed by additive gene action and, therefore, provides the most effective condition for selection (Tazeen *et al*., 2009).

The available literature on variability, heritability and genetic advance studies for yield and its contributing traits and kernel iron and zinc concentrations was summarised in the table 2.3.

Upon reviewing the literature, it was observed (Jonah *et al.*, 2012; Rai *et al.*, 2014 and Satyanarayan *et al.*, 2014) that in groundnut days to field emergence had low

to moderate broad sense heritability and low to high genetic advance but reported lower GCV % compared to PCV % indicating higher level of environmental influence on the trait understudy.

Days to flowering recorded higher broad sense heritability coupled with low genetic advance and almost same levels of GCV and PCV suggesting less influence of environment on flowering (Vishnuvardhan *et al.,* 2013, John and Reddy, 2014 and Satish, 2014). However, John and Reddy (2014) has reported higher GCV (455.87 %) for the trait understudy. Alam *et al.* (2013) recorded low narrow sense heritability for this character.

Zaman *et al.* (2011) recorded moderate broad sense heritability coupled with low genetic advance for days to maturity along with low and almost equal GCV and PCV for the trait understudy. But moderate to high broad sense heritability was reported by Vishnuvardhan *et al.* (2013), Rai *et al.* (2014) and Satyanarayan *et al.* (2014).

Growth and branching habit along with number of primary branches plays an important role in attaining higher yield. Satish (2014) obtained higher broad sense heritability coupled with low genetic advance and moderate GCV and PCV for this trait. Almost similar type of results were obtained by Nath and Alam, (2002), Vishnuvardhan *et al.* (2013) and Satyanarayan *et al.* (2014).

Parameswarappa *et al.* (2005) and Jonah *et al.* (2012) recorded higher broad sense heritability and low genetic advance coupled with low and equal levels of GCV and PCV for shelling percentage.

Vishnuvardhan *et al.* (2013) and Satyanarayan *et al.* (2014) carried out experiments in groundnut to study various genetic parameters and reported moderate to high broad sense heritability coupled with low genetic advance, low GCV and low to moderate PCV for sound mature kernel percentage.

Nath and Alam (2002) reported higher broad sense heritability, moderate genetic advance, GCV and PCV for 100 kernel weight in groundnut. Similar results were reported by Parameswarappa *et al.* (2005). However, Jonah *et al.* (2012) recorded high GCV and PCV for this trait. Alam *et al.* (2013) has reported moderate narrow sense heritability for this trait. Janila *et al.* (2014) also obtained higher broad sense heritability whereas Satish (2014) recorded high broad sense heritability along with moderate genetic advance, GCV and PCV for 100 kernel weight.

Parameswarappa *et al.* (2005) observed higher broad sense heritability, low genetic advance and moderate GCV and PCV for pod yield per plant in groundnut. These results were in accordance with the findings of Jonah *et al.* (2012), Satish (2014)

and Satyanarayan *et al.* (2014). Whereas, Jonah *et al.* (2012) and Gadakh *et al.* (2013) reported higher narrow sense heritability for the trait understudy.

Parameswarappa *et al.* (2005) and Noubissie *et al.* (2012) were reported higher broad sense heritability coupled with low to medium levels of genetic advance and moderate and higher levels of GCV and PCV for oil and protein content, respectively, Shukla and Rai (2014) partially supported the above results by reporting higher levels of broad sense heritability but with higher and moderate levels of genetic advance for oil and protein content, respectively and higher GCV and PCV for both the traits understudy.

Janila *et al.* (2014) reported higher levels of broad sense heritability for kernel iron and zinc concentrations.

2.4 HETEROSIS AND INBREEDING DEPRESSION STUDIES

The term heterosis, as is now widely used, refers to the phenomenon in which the F_1 hybrids obtained by crossing the two genetically dissimilar gametes or individuals, shows increased or decreased vigour over the parents. Shull (1908) referred to this phenomenon as the stimulus of heterozygosis. Whereas inbreeding depression is the reduced biological fitness in a given population as a result of inbreeding.

The magnitude of heterosis provides information on the extent of genetic diversity of parents which helps in the selection of superior parents for hybrid breeding. However, in a self-pollinated crop like groundnut, where commercial production of hybrids is not feasible owing to the inherent problem associated with it (Verma and Ranwah, 2012), it is desirable to identify the crosses which exhibit cross vigour preferably when one of the parents is of acceptable commercial quality (Isleib and Wynne, 1983). In addition, the heterotic crosses can also produce desirable transgressive segregants in their advanced generations if parents are having dispersed dominance.

In groundnut, heterosis cannot be exploited for increased value through commercial hybrids due to cleistogamous nature of flower and poor kernel recovery during hybridization (Gor *et al.,* 2012). Hence, the heterosis assumes importance in breeding as heterotic crosses have the potentiality to throw out superior segregants in subsequent generations. The estimates of heterosis and inbreeding depression provide information about the nature of gene action involved in the expression of yield and related traits.

The information is also essential to formulate efficient breeding programmes for the improvement of the crop. Though there are a number of reports on heterosis, information was limited in case of inbreeding depression especially for the traits like kernel mineral concentration, pod yield and yield components in groundnut. Study of heterosis together with inbreeding depression has a direct bearing on the breeding methodology to be followed in varietal improvement (John *et al.*, 2014). Therefore, in the present investigation study on heterosis and inbreeding depression was also made to support the understanding of gene action involved in the control of kernel iron and zinc concentrations along with the identification of better cross combination between two crosses *viz.*, ICGV 06040 × ICGV 87141 and ICGV 06099 × ICGV 93468 for the trait of interest. A brief review of heterosis and inbreeding depression studies in groundnut and other related crops is presented below:

Dwivedi *et al.* (1989) conducted studies in groundnut to identify the heterotic F_1s in a 8 × 8 full diallel experiment and observed significant negative heterosis for pod weight and kernel weight but a positive significant heterosis was observed for pod weight per plant. However, a negative significant heterosis was observed for shelling percentage in almost all the cross combinations indicating the inefficacy of hybrid breeding in groundnut.

Vyas *et al.* (2001) studied heterosis in groundnut using six parents and their fifteen crosses along with check TAG-24 and results revealed that four out of fifteen crosses showed positive and high heterosis over mid parent and better parent for dry pod yield per plant and kernel yield per plant. In addition, GG-2 × GG-4 cross combination also showed good heterosis for early flowering.

Venkateswarlu *et al.* (2007b) carried out experiments involving a set of 28 crosses and their corresponding eight parents to get information on the extent of heterosis over better parent and mid parent for yield attributes in groundnut. Results revealed the existence of positive significant heterosis for pod yield per plant in most of the crosses whereas negative significant heterosis was observed for shelling percentage and sound mature kernel percentage.

Table 2.3. Review on variability, heritability and genetic advance of various traits along with kernel iron and zinc concentrations in groundnut

S.No.	Crop	Trial information	GCV (%) and PCV (%)	Genetic advance	Heritability (%)	Reference
DAYS TO EMERGENCE						
1	Bambara groundnut	12 accessions were evaluated	Moderate GCV (13.86) and high PCV (21.85)	Moderate (13.9)	Moderate broad sense heritability (40.8)	Jonah *et al.* (2012)
2	Groundnut	12 genotypes were evaluated in RBD	Low GCV (8.09) and moderate PCV (16.77)	low (5.22)	Low broad sense heritability (26.28)	Rai *et al.* (2014)
3	Groundnut	Evaluation of 14 lines in RBD	moderate GCV (15.12) and high PCV (27.95)	High (40.96)	Low broad sense heritability (29.28)	Satyanarayan *et al.* (2014)
DAYS TO FLOWERING						
4	Groundnut	10 ×10 half- diallel experiment	--	--	Low narrow sense heritability (38.0)	Alam *et al.* (2013)
5	Groundnut	8 × 8 half- diallel experiment evaluated in RBD	Low GCV (5.17) and low PCV (5.62)	Low (2.28)	High broad sense heritability (84.53)	Vishnuvardhan *et al.* (2013)
6	Groundnut	28 F_2's sown in RBD		Low (3.57)	High broad sense heritability (85.36)	John and Reddy (2014)
7	Groundnut	37 advanced breeding lines	High GCV (455.87)	Low (4.86)	High broad sense heritability (91.8)	John *et al.* (2014)
8	Groundnut	16 genotypes were evaluated in RBD	Low GCV (3.40) and low PCV (3.81)	Low (2.23)	high broad sense heritability (79.0)	Satish (2014)
DAYS TO MATURITY						
9	Groundnut	34 genotypes were evaluated in RBD	Low GCV (3.25) and low PCV (4.22)	Low (1.99)	Moderate broad sense heritability (31.85)	Zaman *et al.* (2011)

S.No.	Crop	Trial information	GCV (%) and PCV (%)	Genetic advance	Heritability (%)	Reference
10	Groundnut	8 × 8 half- diallel experiment evaluated in RBD	Low GCV (2.09) and Low PCV (2.20)	Low (3.89)	High broad sense heritability (89.69)	Vishnuvardhan *et al.* (2013)
11	Groundnut	12 genotypes were evaluated in RBD	Low GCV (0.87) and Low PCV (1.27)	Low (1.46)	Moderate broad sense heritability (47.01)	Rai *et al.* (2014)
12	Groundnut	Evaluation of 14 lines in RBD	Low GCV (1.35) and Low PCV (1.48)	Low (2.92)	High broad sense heritability (82.28)	Satyanarayan *et al.* (2014)
GROWTH AND BRANCHING HABBIT						
13	Groundnut	15 accessions were evaluated in RBD	Moderate GCV (18.59) and high PCV (20.09)	Low (2.67)	High broad sense heritability (85.58)	Nath and Alam (2002)
14	Groundnut	8 × 8 half- diallel experiment evaluated in RBD	Moderate GCV (14.61) and high PCV (21.67)	Low (1.20)	Moderate broad sense heritability (45.44)	Vishnuvardhan *et al.* (2013)
15	Groundnut	16 genotypes were evaluated in RBD	Moderate GCV (11.76), moderate PCV (12.45)	Low (1.43)	High broad sense heritability (89.0)	Satish (2014)
16	Groundnut	Evaluation of 14 lines in RBD	Low GCV (7.61) and Moderate PCV (10.36)	Low (1.55)	Moderate broad sense heritability (54.05)	Satyanarayan *et al.* (2014)
SHELLING PERCENTAGE (%)						
17	Groundnut	Evaluation of 48 lines in RBD for shelling percentage	Low GCV (6.5) and Low PCV (6.83)	Low (6.97)	High broad sense heritability (79.0)	Parameswarappa *et al.* (2005)
18	Bambara groundnut	12 accessions were evaluated for shelling percentage	Low GCV (5.88) and Low PCV (6.38)	Low (4.2)	High broad sense heritability (69.8)	Jonah *et al.* (2012)

S.No.	Crop	Trial information	GCV (%) and PCV (%)	Genetic advance	Heritability (%)	Reference
SOUND MATURE KERNEL PERCENTAGE (%)						
19	Groundnut	8 × 8 half- diallel experiment evaluated in RBD for sound mature kernel percentage	Low GCV (6.41) and moderate PCV (10.46)	Low (6.99)	Moderate broad sense heritability (37.53)	Vishnuvardhan *et al.* (2013)
20	Groundnut	Evaluation of 14 lines in RBD for sound mature kernel percentage	Low GCV (4.69) and low PCV (5.59)	Low (7.39)	High broad sense heritability (70.44)	Satyanarayan *et al.* (2014)
100-KERNEL WEIGHT (g)						
21	Groundnut	15 accessions were evaluated in RBD	Moderate GCV (11.67) and moderate PCV (13.04)	Moderate (19.82)	High broad sense heritability (97.89)	Nath and Alam (2002)
22	Groundnut	Evaluation of 48 lines in RBD	Moderate GCV (11.00) and moderate PCV (11.13)	Moderate (10.4)	High broad sense heritability (81.0)	Parameswarappa *et al.* (2005)
23	Bambara groundnut	12 accessions were evaluated	High GCV (24.01) and High PCV (25.73)	Moderate (13.4)	High broad sense heritability (94.8)	Jonah *et al.* (2012)
24	Groundnut	10 × 10 half- diallel experiment	--	--	moderate narrow sense heritability (35.0)	Alam *et al.* (2013)
25	Groundnut	Evaluation of 46 genotypes in 8 × 8 Alpha lattice design	--	--	High broad sense heritability (91.0)	Janila *et al.* (2014)
26	Groundnut	16 genotypes were evaluated in RBD	Moderate GCV (13.57), moderate PCV (13.64)	Moderate (13.24)	high broad sense heritability (98.0)	Satish (2014)
POD YIELD PER PLANT (g)						
27	Groundnut	Evaluation of 48 lines in RBD	Moderate GCV (16.61) and moderate PCV (17.80)	Low (7.9)	High broad sense heritability (87.0)	Parameswarappa *et al.* (2005)

S.No.	Crop	Trial information	GCV (%) and PCV (%)	Genetic advance	Heritability (%)	Reference
28	Groundnut	10 × 10 half- diallel experiment	--	--	Moderate narrow sense heritability (41.0)	Alam *et al.* (2013)
29	Bambara groundnut	12 accessions were evaluated	Moderate GCV (19.42) and high PCV (29.38)	High (22.5)	High broad sense heritability (70.1)	Jonah *et al.* (2012)
30	Groundnut	16 genotypes were evaluated in RBD	High GCV (22.04) and high PCV (22.42)	High (731.42)	high broad sense heritability (96.0)	Satish (2014)
31	Groundnut	Evaluation of 14 lines in RBD	High GCV (22.43) and high PCV (26.05)	Low (5.15)	High broad sense heritability (74.14)	Satyanarayan *et al.* (2014)
OIL CONCENTRATION (%)						
32	Groundnut	Evaluation of 12 lines in RBD	--	Low (3.7)	Moderate broad sense heritability (52.0)	Noubissie *et al.* (2012)
33	Groundnut	Evaluation of 48 lines in RBD	Low GCV (4.17) and low PCV (4.61)	Low (3.29)	High broad sense heritability (82.0)	Parameswarappa *et al.* (2005)
34	Groundnut	Evaluation of 30 genotypes in RBD	Low GCV (9.43) and low PCV (9.87)	Low (9.0)	High broad sense heritability (91.8)	Shukla and Rai (2014)
PROTEIN CONCENTRATION (%)						
35	Groundnut	Evaluation of 12 lines in RBD	--	Low (4.7)	High broad sense heritability (64.0)	Noubissie *et al.* (2012)
36	Groundnut	Evaluation of 48 lines in RBD	Moderate GCV (13.21) and moderate PCV (13.49)	Low (4.72)	High broad sense heritability (96.0)	Parameswarappa *et al.* (2005)
37	Groundnut	Evaluation of 30 genotypes in RBD	Low GCV (6.41) and low PCV (8.31)	Low (3.39)	Moderate broad sense heritability (59.57)	Shukla and Rai (2014)

S.No.	Crop	Trial information	GCV (%) and PCV (%)	Genetic advance	Heritability (%)	Reference
KERNEL IRON CONCENTRATION (mg kg^{-1})						
38	Groundnut	Evaluation of 46 genotypes in 8 × 8 Alpha lattice design	--	--	High broad sense heritability (81.0)	Janila *et al.* (2014)
KERNEL ZINC CONCENTRATION (mg kg^{-1})						
39	Groundnut	Evaluation of 46 genotypes in 8 × 8 Alpha lattice design	--	--	High broad sense heritability (92.0)	Janila *et al.* (2014)

Velu *et al.* (2011a) conducted studies to identify the heterotic hybrid combinations in pearl millet for kernel iron and zinc concentrations and reported the positive significant heterosis of F_1 over mid parent for grain iron and zinc concentrations. These results were in agreement with the earlier findings of Aruselvi *et al.* (2006).

Gor *et al.* (2012) evaluated thirty-six crosses generated in a diallel fashion excluding reciprocals using nine genotypes to study the extent of heterosis and inbreeding depression for pod yield and yield attributing characters in groundnut and observed considerable heterobeltiosis for the number of mature pods per plant, harvest index and biological yield, while the traits like pod and kernel yield per plant showed low heterobeltiosis, and it was absent or in negative direction for the traits like days to 50% flowering, days to maturity, 100-kernel weight, shelling outturn and sound mature kernels in all the crosses. High inbreeding depression was recorded for days to maturity and 100-kernel weight, while fully matured kernels and biological yield did not show any inbreeding depression.

Verma and Ranwah (2012) carried out research in groundnut with fifty-three genotypes including twelve lines, three testers, thirty-six crosses and two checks in two environments and observed positive significant heterosis in only two crosses out of thirty-six for pod yield per plant.

Waghmode *et al.* (2013) attempted a diallel cross analysis using seven parents to study the extent of heterosis in F_1 hybrids over mid and better parent in groundnut and found only one hybrid to show negative significant heterosis over mid and better parent for days to maturity whereas almost ten out of twenty one hybrid combinations exhibited positive significant heterosis over mid and better parents for pod yield per plant.

John *et al.* (2014) estimated heterosis and inbreeding depression for 28 crosses in groundnut and concluded that as many as twenty-two crosses showed positive significant heterosis along with higher inbreeding depression for pod yield per plant and a negative significant heterosis was observed for days to maturity coupled with low inbreeding depression.

Prabhu *et al.* (2014) used eighteen F_1 hybrids obtained by crossing six lines and three testers in a line × tester mating design to generate information on the extent of heterosis over better parent, mid-parent and standard parent in groundnut and observed positive significant mid and better parent heterosis in majority of the cross combinations for pod yield per plant and 100-kernel weight whereas negative significant heterosis was

observed for shelling percentage and sound mature kernel percentage in most of the crosses understudy.

2.5 CORRELATION STUDIES

The degree of association between two variables is measured by the correlation coefficient which indicates the relationship between these two variables. It is the regression coefficient which measures the change in one variable for a unit change in other variable. Most of the characters of breeder's interest are complex and are the result of interaction of a number of components (Korat *et al.*, 2010). Understanding the relationships among yield and yield components is of paramount importance for making the best use of these relationships in selection.

In peanut breeding programs, the selection of productive lines based on the phenotypic traits of pods is difficult. Therefore, the selection of yield-related traits either directly or indirectly is highly useful for breeders, particularly when working with divergent or segregating populations. Correlation analysis is useful in this regard since information on the nature and magnitude of interrelationships among traits is not only helpful to define the selection potential of an isolated trait but also detects the effects of one particular trait due to the selection for another (Cruz and Regazzi, 1997).

In the present study, an attempt was also made to study the association of the target traits *viz.,* kernel iron and zinc concentrations with yield and its related traits. So the available literature pertaining to the present study in different crops is reviewed below:

Chakraborti *et al.* (2009) conducted experiments in maize to understand the association of kernel iron and zinc concentration with yield and other yield attributing traits and revealed the existence of negative significant association between kernel iron concentration (-0.25) and kernel yield and no significant association between kernel zinc concentration and yield. They also reported the existence of negative significant association between kernel zinc concentration and days to 50 % anthesis (-0.27) and days to 50 % silking (-0.25).

Govindaraj *et al.* (2009) observed the existence of a strong positive correlation among grain iron and zinc concentrations (0.87) and their significant association with 100-grain weight (0.64) in pearl millet. They further found that these grain micronutrient concentrations were not associated with grain yield.

Velu *et al.* (2011b) carried out research in several thousand accessions of wheat to understand the association and variation of grain iron and zinc concentrations and

found a positive significant correlation between grain iron and zinc concentrations (0.416) and a positive association of grain iron and zinc concentrations with thousand kernel weight (0.332). In this study, protein content also showed positive significant association with grain iron and zinc concentrations. These results were in accordance with the findings of Ghanbari and Mameesh (1971) and Badakhshan *et al.* (2013) in wheat where a positive significance was noticed in the association between grain iron and zinc concentrations and their association with protein content.

Bekele *et al.* (2013a) carried out experiments in rice using sixty four genotypes to study the correlation between grain zinc concentration and other yield parameters and found a positive significant association of grain yield per plant with grain zinc concentration and 100-kernel weight (0.268). On the contrary, Bekele *et al.* (2013b) while, working with one hundred seventy six Recombinant Inbred Lines (RILs) observed a negative significant correlation (-0.24) of grain zinc concentration with kernel yield per plant and days to flowering and a positive correlation with 100-kernel weight (0.15). These results were partially in accordance with the findings of Gande *et al.* (2014) while handling RIL populations of rice where significant negative correlation between grain zinc concentration and grain yield per plant and a positive significant correlation between grain zinc concentration and days to flowering and days to maturity was observed. However a positive significant correlation (0.487) between grain iron and zinc concentrations and non-significant association of grain iron and zinc concentrations with grain yield were noticed by Nagesh *et al.* (2012) while working with forty six rice hybrids.

Ribeiro *et al.* (2013a) carried out experiments in common bean using fourteen advanced breeding lines to understand the association of kernel zinc concentration with yield and selected mineral elements and observed a significant positive correlation between kernel zinc concentration and kernel yield per plant (0.348). However, non-significant association was observed between grain zinc concentration and other mineral element. Similar kind of study was made by Ribeiro *et al.* (2013b) in common bean and a negative significant association between grain iron concentration and grain yield and a positive significant association of grain iron with grain calcium concentration.

To improve the kernel iron and zinc concentrations in groundnut a study was conducted by Janila *et al.* (2014) to initiate breeding strategy for the same using sixty four genotypes for two years in eight different environments and observed that the association of kernel iron concentration was positively significant with the kernel zinc (0.535) concentration, protein concentration (0.166) and oil concentration (0.228)

whereas negative and non-significant with pod yield and hundred kernel weight. With respect to kernel zinc concentration, it had positive significant association with protein content (0.678), pod yield (0.168) and hundred kernel weight (0.153) and non-significant association with oil content.

Kanatti *et al.* (2014) studied character associations in pearl millet and revealed the existence of significant positive correlation of grain iron and zinc concentrations with 1000-grain weight (0.42 and 0.43, respectively) and a significant negative association with grain yield (-0.29 and -0.26, respectively) and a strong correlation between grain iron and zinc concentrations (0.88).

Ravikiran *et al.* (2014) reported the existence of a strong positive correlation (0.538) between grain iron and zinc concentrations in sorghum. Susmita and Selvi (2014) also recorded strong positive association between kernel iron and zinc concentrations (0.853) along with a positive significant association between grain yield and kernel iron (0.374) and zinc (0.27) concentrations in sorghum.

Material and Methods

Chapter III

MATERIAL AND METHODS

The present investigation is divided into two major experiments and was carried out at ICRISAT, Patancheru, Hyderabad, located at an altitude of 545 m above mean sea level and at 17.53° N latitude and 78.27° E longitude, with an objective to identify the markers associated with the trait of interest and to understand the gene action involved in the inheritance of kernel iron and zinc concentrations in groundnut.

EXPERIMENT-I:

3.1 QTL ANALYSIS FOR KERNEL IRON AND ZINC CONCENTRATION:

The present investigation was carried out in M. S. Swaminathan Applied Genomics Laboratory at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Telangana, India. The details of the experiments conducted in the laboratory are given below.

3.1.1. Mapping Population

One hundred and eighty four $F_{2:3}$ mapping population derived from a cross ICGV 06099 × ICGV 93468, along with respective parents were used in the present investigation.

3.1.2 Phenotyping

The $F_{2:3}$ phenotyping population along with parental lines were sown in Alfisols (Alfisol-Patancheru Soil Series); fields at ICRISAT, Patancheru, India during rainy season, 2013. The experiment was laid out in alpha lattice design with two replications. The plot size consisted of twenty six blocks each having ten 1 m rows which are 60 cm apart in every broad bed. Seeds were planted on ridges of those 1 m rows. The plant to plant distance within a row was 10 cm. Standard agronomic management practices were followed in each season *viz.*, 60 kg phosphorus pentoxide (P_2O_5) as a basal application, seed treatment with mancozeb (2 g per kg seed), pre emergence application of pendimethalin (1 kg active ingradient per ha.), irrigation soon after planting,

subsequently as and when needed or as per the requirement of the treatment, gypsum (400 kg ha^{-1}) at peak flowering and protection against insect pests and diseases.

Soil analysis to estimate the iron and zinc status of the experimental block was conducted in both the replications. Samples were collected from a depth of 15 cm using an auger at multiple locations in a replication which were further bulked; thoroughly mixed and foreign material such as roots, stones, pebbles and gravel were removed. After this, soil sample of 100-150 g was prepared by using quartering method which was used to estimate the micronutrient status of the soil. The samples were analysed at the Charles Renard Analytical laboratory (CRAL) at ICRISAT.

Protocol for estimation of iron and zinc concentration in groundnut kernels using Inductively Coupled Plasma- Optical Emission Spectrometry (ICP-OES) method was explained under sub-heading 3.1.2.1.11 in experiment I.

3.1.2.1 Observations Recorded

Observations on the following quantitative traits were recorded as per the procedure explained below:

3.1.2.1.1 Days to emergence: Number of days counted from the date of sowing (irrigation) to the date when seedling emergence was observed.

3.1.2.1.2 Days to 75 % flowering: Number of days counted from the date of sowing (irrigation) to the date when flowering observed in 75 % of the plants in a plot.

3.1.2.1.3 Days to maturity: This was determined by examining the foliage, internal pericarp colour and colour of pods. The pods of the groundnut from several plants in the field were picked randomly and cracked or cut open to determine maturity. The percentage of pods with tan to brown colour inside the hull and pink to dark pink seed coats was worked out. Harvesting was done when mature pods range from 75 to 85 %, depending on the variety and environmental factors.

3.1.2.1.4 100-kernel weight (g): A random sample of 100 kernels was taken from each genotype and weighed.

3.1.2.1.5 Single plant yield (g): From the plant, mature pods were stripped, dried, cleaned and shelled then kernel yield was recorded in grams.

3.1.2.1.6 Pod yield (g plot^{-1}): It was calculated by multiplying single plant yield with total number of plants in a given plot.

3.1.2.1.7 Kernel yield (g plot^{-1}): It was calculated by multiplying kernel yield per plant with total number of plants in a given plot.

3.1.2.1.8 Sound mature kernel percentage (%): Sound mature kernel percentage was computed by the ratio of weight of the perfectly filled kernels to the total weight of the kernels after shelling expressed in percentage.

$$\text{Sound mature kernel percentage} = \frac{\text{Weight of the perfectly filled kernels (g)}}{\text{Total kernel weight (g)}} \times 100$$

3.1.2.1.9 Shelling percentage (%): After shelling known weight of pods and weighing the kernels obtained, shelling percentage was calculated as per the formula given below:

$$\text{Shelling percentage} = \frac{\text{Kernel yield after shelling (g)}}{\text{Pod weight (g)}} \times 100$$

3.1.2.1.10 Oil, Protein, Oleic acid, Linoleic acid, Palmitic acid and Stearic acid content (%): The data pertaining to all these parameters were estimated by scanning the samples using Near Infra-Red Spectroscopy (NIRS) system.

3.1.2.1.11 Kernel iron (Fe) and zinc (Zn) concentration (mg kg^{-1}): After harvesting and shelling kernels were cleaned without any metal contamination. The cleaned kernels were collected in packets and the defatted kernel iron and zinc concentrations in them were measured with Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES), the details of which are briefly given below:

Principle of ICP-OES:

ICP, abbreviation for Inductively Coupled Plasma, is one method of optical emission spectrometry. When plasma energy is given to an analysis sample from outside, the component elements (atoms) are excited. When the excited atoms return to low energy position, emission rays (spectrum rays) are released and the emission rays that correspond to the photon wavelength are measured. The element type is determined based on the position of the photon rays and the concentration of each element is determined based on the rays' intensity. To generate plasma, first, argon gas is supplied to torch coil, and high frequency electric current is applied to the work coil at the tip of the torch tube. Using the electromagnetic field created in the torch tube by the high

frequency current, argon gas is ionized and plasma is generated. This plasma has high electron density and temperature (10000 K) and this energy is used in the excitation-emission of the sample. Solution samples are introduced into the plasma in an atomized state through the narrow tube in the centre of the torch tube.

ICP-OES determination:

Determinations were carried out using a Prodigy High Dispersion Inductively Coupled Plasma (ICP) Spectrometer equipped with a dual view torch and 60 position auto sampler. The Prodigy is a compact bench-top simultaneous ICP-OES featuring an 800 mm focal length echelle optical system coupled with a mega-pixel Large Format Programmable Array Detector (L-PAD). At 28 × 28 mm, the active area of the L-PAD is significantly larger than any other solid-state detector currently used for ICP-OES. This combination allows Prodigy to achieve significantly higher optical resolution than other solid-state detector based ICP systems. The detector also provides continuous wavelength coverage from 165 to 1100 nm permitting measurement over the entire ICP spectrum in a single reading without sacrificing wavelength range or resolution. This detector design is inherently anti-blooming and is capable of random access, non–destructive readout that results in a dynamic range of more than 6 orders of magnitude.

3.1.3 Genotyping of Parents and Mapping Population

Although several procedures for genomic DNA isolation were available (Dellaporta *et al.,* 1983; Murray and Thompson, 1984 and Tai and Tanksley, 1990), genomic DNA isolation was done by Cityl Tri methyl Ammonium Bromide (CTAB) method (Mace *et al.,* 2003) with slight modifications.

DNA was obtained from approximately 30 mg sample of each F_2 progeny and parental lines by using CTAB method (Mace *et al.,* 2003) with slight modifications. DNA was further purified by RNase digestion followed by extraction with phenol : chloroform : Iso-amyl alcohol (25 : 24 : 1) and ethanol precipitation as described by Mace *et al.* (2003). The reagents required for DNA extraction are listed in Appendix I and the adopted procedure of 96-well plate mini DNA extraction is described here under.

3.1.3.1 DNA Extraction Procedure

A) Preparation: Two chrome-plated grinding balls (4 mm in diameter), pre-chilled at –20°C for about 30 minutes, were dispensed by an automatic ball dispenser to 12 × 8 well polypropylene strip extraction tubes with strip caps that were kept on ice. 3%

CTAB buffer was pre-heated at 65°C in water bath (Precision Scientific Model: Shaking Water Bath 50) before starting DNA extraction.

Leaves were collected from one week-old seedlings of parents and F_2 progeny and cut into small pieces (approximately 30 mg), which were then transferred to an extraction tube fitted in a box. This was repeated for all 184 F_2 progenies and parental lines.

B) Grinding and extraction: 450 µl of pre-heated 3 % CTAB buffer was added to each extraction tube containing leaf sample and tightly capped with polyethylene strip caps. Grinding was carried out using a Sigma Geno-Grinder (Spex Certiprep, USA) at 500 strokes per minute for 2 minutes. Grinding was repeated until the colour of the solution became pale green and leaf strip pieces were sufficiently macerated. After the first round of grinding, the boxes were checked for leakage by taking them out from the Geno-Grinder and were shaken for proper mixing of leaf tissues with buffer.

After grinding, the box with the tubes was fixed in a locking device and incubated at 65°C in a water bath for 10 minutes with occasional manual shaking.

C) Solvent extraction: 450 µl of chloroform : Iso-amyl alcohol (24 : 1) mixture was added to each tube, tubes were inverted twice for proper mixing and the samples were centrifuged at 6200 rpm for 10 minutes. After centrifugation, the aqueous layer (approximately 300 µl) was transferred to a fresh tube (Marsh Biomarket).

D) Initial DNA precipitation: To each tube containing the aqueous layer, $7/10^{th}$ volume (approximately 210 µl) of cold Isopropanol (kept at –20°C) was added. The solution was carefully mixed and the tubes were kept at -20°C for 10 minutes. The samples were centrifuged at 6200 rpm for 15 minutes. The supernatant was decanted under the fume hood and pellets were allowed to air dry for about 30 minutes.

E) RNase A treatment: In order to remove co-isolated RNA, pellets were dissolved into 200 µl of TE buffer (T_1 $E_{0.1}$) and 3 µl of RNase A. The solution was incubated at 37°C for 30 minutes or overnight at room temperature.

F) Solvent extraction: After incubation, 200 µl of phenol : chloroform : Iso-amyl alcohol (25 : 24 : 1) was added to each tube, mixed and centrifuged at 5000 rpm for 10 minutes.The aqueous layer in each tube was transferred to a fresh tube (Marsh Biomarket) and 200 µl of chloroform : Iso-amyl alcohol (24 : 1) was added to each tube, mixed and centrifuged at 5000 rpm for 10 minutes. The aqueous layer was transferred to fresh tube (Marsh Biomarket).

G) DNA precipitation: 15 µl (approximately $1/10^{th}$ volume) of 3 M sodium acetate (pH 5.2) and 300 µl (2 volumes) of absolute ethanol (kept at –20°C) were added to each of

the tubes and the mixture was subsequently incubated in a freezer (–20°C) for 5 minutes. Following the incubation at –20°C, the tubes were centrifuged at 6200 rpm for 15 minutes.

H) Ethanol wash: After centrifugation, the supernatant was carefully decanted from each tube in order to ensure that, the pellet remained inside the tube. Subsequently, 200 µl of 70% ethanol was added to each of the tubes and it was followed by centrifugation at 5000 rpm for 5 minutes.

I) Final re-suspension: The supernatant was carefully decanted and the pellet was allowed to air dry for one hour. Dried pellets were re-suspended in 100 µl of $T_{10}E_1$ buffer and kept overnight at room temperature to dissolve completely. The re-suspended DNA samples were stored at 4°C.

3.1.3.2 Quantification of DNA Concentration and Quality Check

To determine the quantity and quality of genomic DNA using agarose gel, an aliquot of 1 µl of DNA from each sample along with 5 ng of molecular weight marker (λ DNA, Amersham Biosciences) were initially analyzed by electrophoresis on 0.8 % agarose gels containing ethidium bromide (0.5 µl per 10 ml of gel) and run in 0.5X TBE (Tris Borate EDTA) buffer at a constant voltage (100 V) for one hour. The gel was viewed under UV illumination and recorded using an UVi Tech gel documentation system (DOL-008.XD, England). A smear of DNA indicated poor quality whereas a clear band indicated good quality DNA. In the present study, the quality of genomic DNA was examined by using agarose (0.8 %) gel electrophoresis and quantity was accurately quantified by using Nanodrop (Nanodrop 8000 Spectrophotometer). After quantification of DNA, working stock of DNA with 5 ng/µl concentration was made by diluting with sterile double distilled water.

3.1.3.3 Parental Polymorphism and Genotyping the F_2 Population

According to Caetano-Anolles *et al*. (1997), the parameters of DNA amplification *viz.*, specificity, efficiency and fidelity are strongly influenced by the different components of the reaction and by thermal cycling. Therefore careful optimization of these parameters will ultimately result in reproducible and efficient amplification.

To identify SSR primer pairs detecting polymorphism between parents, initial screening of parental lines was conducted. For this, DNA was extracted from ICGV 06099 (taken as first parent *i.e.* P_1) and ICGV 93468 (taken as second parent *i.e.* P_2). A total of 200 SSR primers were used to screen the parents. From this screening, 33 SSR primers detecting scorable polymorphism between the parents were noted and out of

which twenty eight markers shown clear amplification in the mapping population and were used for genotyping of the $F_{2:3}$ mapping population of these parents. The sequence information of forward and reverse primers used for genotyping is presented in Table.3.1.

3.1.3.4 Polymerase Chain Reaction (PCR)

PCR was carried out in 96 and 384-well plates in a GeneAmp PCR system PE 9700 (Applied Biosystem, USA) DNA thermal cycler in volumes of 5 µl. A touchdown PCR program was used to amplify the DNA fragments. Reaction conditions were as follows:

Initial denaturation for 5 minutes at 94°C (to minimize primer - dimer formation and to activate the *Taq* polymerase), subsequently 10 cycles of denaturation for 15 seconds at 94°C, annealing at 61°C to 52°C for 20 seconds, the annealing temperature for each cycle was reduced by 1°C and extension at 72°C for 30 seconds followed by 40 cycles of denaturation at 94°C for 10 seconds, annealing at 54°C for 30 seconds and extension at 72°C for 30 seconds followed by final extension at 72°C for 20 min. PCR amplification was checked on 1.2 % agarose gels and PCR products of direct labelled primers and M_{13} tailed primers were separated by capillary electrophoresis on an ABI3730xl sequencer and their sizes were determined using GeneMapper® Version 4.0 software (Applied Biosystems, USA) and PCR products of unlabelled primers were separated on Agarose gel.

3.1.3.5 Genotyping Using Capillary Electrophoresis

The PCR products amplified using fluorescence-labeled primers were separated by capillary electrophoresis using an ABI Prism 3700 automatic DNA sequencer (Applied Biosystems Inc.). This has the ability to detect size differences of 1 bp using a fluorescence-based detection system, thus dispensing with the need for radioactivity or laborious manual Polyacrylamide gel techniques.

For this purpose, forward primers were labelled with 6-FAM™ (Blue), VIC™ (Green), NED™ (Yellow) or PET™ (Red) fluorophores (Applied Biosystems). PCR products of primers labelled with different dyes or same flourophore-labelled primers with non-overlapping amplicons (in terms of size) were pooled.

The products of different flourophore–labelled primers were pooled in different proportion (1.0 µl of 6-FAM–labeled product, 0.8 µl of VIC-labeled product, 1.4 µl of NED–labeled product, and 1.0 µl of PET-labeled product). The pooled PCR products were then mixed with 0.2 µl of GeneScan 500™ LIZ® internal size standard (Applied

Biosystems) and 7.0 µl of Hi-Di™ Formamide (Applied Biosystems). The final volume was made up to 15 µl with sterile double-distilled water. DNA fragments were denatured for 5 minutes at 95ºC (Perkin Elmer 9700, Applied Biosystem) and cooled immediately on ice.

3.1.3.5.1 Fragment size fractionation: The PCR products with denatured DNA were electrophoresed and the capillary run was performed using the "Genscan2 POP6 Default" run module and "G5" filter-set. The analysis module used was "GS500 analysis". The fragments were separated in a 50 cm capillary array using POP6 (Performance Optimized Polymer, Applied Biosystems) as separation matrix.

3.1.3.5.2 Data processing: GeneMapper® version 4.0 software (Applied Biosystems, USA) was used to size the peak pattern in relation to the internal size standard, GeneScan 500™ LIZ®. The principle behind this is that standards are run in the same lane or capillary injection as the samples, which contain fragment of unknown sizes labelled with different flourophores. GeneMapper® version 4.0 software automatically calculates the size of unknown DNA fragments by generating a calibration sizing curve based upon the migration times of the known fragments in the standard. The unknown fragments are mapped on to the curve and the sample data is converted from migration times to fragments size. The peaks are displayed with base pair values and height (amplitude) in a chromatogram. The height of the chromatogram peaks (representing the alleles) obtained through capillary electrophoresis is directionally proportionate to the signal strength, which in turn is determined by the amount of amplified product in the sample.

3.1.3.6 Scoring of Amplified Bands

The banding pattern of each of amplified PCR products of various marker systems were scored as follows:

A = Homozygote for the allele for high iron and zinc parent at a locus.

B = Homozygote for the allele for low iron and zinc parent at a locus.

H = Heterozygote carrying the alleles from both parents.

- = Missing data for the individual at a locus.

After scoring individual progenies, the data set was assembled in Microsoft Excel spreadsheet in a format suitable for linkage analysis by JoinMap and Mapmaker (*i.e.* rows = genotype score at a given locus; columns = individual $F_{2:3}$ progenies).

In this experiment, construction of Linkage map was exempted due to lack of sufficient number of markers for twenty linkage groups in groundnut.

3.1.4 Quantitative Trait Loci (QTL) Analysis

The phenotypic data sets of 184 F_2 individuals and their genotyping data from 33 SSR markers were used to identify genomic regions associated with the traits using Single Marker Analysis (SMA).

3.1.4.1 Analysis of Variance

In trials with high treatment numbers, e.g. variety trials, complete blocks are too large to give a good control of the experimental error due to soil heterogeneity. In these cases designs with incomplete blocks are useful. Every block only contains a fraction of the total number of treatments and is therefore *incomplete*. Several incomplete blocks form one complete replication. One type of such designs is the *lattice design*. The blocks of an incomplete block design can be arranged in any way that is useful for controlling soil heterogeneity (Büchse *et al.,* 2007). Though there are different kinds of lattice designs, in the present study alpha-lattice design was used because of its flexibility in grouping the number of genotypes in to different blocks (Patterson and Williams 1976, Patterson *et al.*, 1978).

Table 3.1. List of SSR markers found polymorphic between the parents, ICGV 06099 and ICGV 93468 in the study along with their sequence information

S. No.	Marker	Sequence		Linkage Group	Position (cM)	Size (bp)	Reference
		Forward	Reverse				
1	GM1954	GAGGAGTGTGAGGTTCTGACG	TGGTTCATTGCATTTGCATAC	A03	114.43	115	Nagy *et al.* (2010)
2	GM1991	GAAAATGATGCCGAGAAATGT	GGGGAGAGATGCAGAAAGAGA	B06	92.89	122	
3	GM1742	GCCTTGTTGCAATCATCACA	ACCTCCAACAGGAACATTGC	B10	38.69	270	
4	GM2536	AGCCTCCACCTTCTCCTATTG	GATGCAGTGGAGGGATAACAA	A06	115.72	336	
5	GM1577	GCGGTGTTGAAGTTGAAGAAG	TAACGCATTAACCACACACCA	A05	53.75	278	
6	GM2032	GCCGATGATGTACGTTTCTTC	GAGACGGCATGTCAAAAGAAT	B10	24.30	149	
7	TC3B05	GGAGAAAACGCATTGGAACT	TTTGTCCCGTTGGGAATAGT	A08	23.09	248-270	Moretzsohn *et al.* (2005)
8	GM2053	ACAAGGAAAACCCATCCAATC	ACGTGATGGATTCTTGTGGAG	B03	74.42	405	Guo *et al.* (2012)
9	GM2301	GTAACCACAGCTGGCATGAAC	TCTTCAAGAACCCACCAACAC	B03	113.75	137	
10	GM2120	TCCACTGCCACCTCTATCATC	TCCACCCACATAGACAGAAGC	B09	90.39	139	Nagy *et al.* (2010)
11	TC9F10	ATCACAATCACAGCTCCAACAA	GGCAAGTCTAATCTCCTTTCCA	A08	73.94	286-320	Moretzsohn *et al.* (2005)
12	GM2638	ATGCTCTCAGTTCTTGCCTGA	CAGACATAACAGTCAGTTTCACC	A04	86.55	107	Nagy *et al.* (2010)
13	IPAHM245	CCCAAGGACCTAGTGACCAA	GGACCCTTAGCACATTCCAA	A06	55.16	290	Cuc *et al.* (2008)
14	GM2746	TCAACCTCAAGGGTGATTGTC	ACACAAACCCGCTCACTCTAA	B08	60.30	120	Nagy *et al.* (2010)
15	IPAHM103	GCATTCACCACCATAGTCCA	TCCTCTGACTTTCCTCCATCA	A03	133.84	160	Cuc *et al.* (2008)
16	IPAHM524	GCCATGGATAAGAACCTGAAA	CAGTAAGCTGAGCTGGCAGA	B02	46.11	300	
17	PM36	ACTCGCCATAGCCAACAAAC	CATTCCCACAACTCCCACAT	A05	54.89	190-240	He *et al.* (2003)
18	SEQ19B01	TTGGTGATGGTGTTGGAGAA	TTAAACCAGGCCAAAAGTGG	A09	54.44	198	Ferguson *et al.* (2004)
19	TC7E04	GAAGGACCCCATCTATTCAAA	TCCGATTTCTCTCTCTCTCTCTC	A03	127.20	300	Moretzsohn *et al.* (2005)
20	S109	AAGGGAGCACAATCATA	GAGCACGAGTTCATACAC	A04	55.62	370-430	Wang *et al.* (2007)
21	SEQ2B09	GCAACATGCTCTGAATTTTGAC	TGTGCAACCCAATTCAATAACTT	B09	82.55	259	Ferguson *et al.* (2004)
22	SEQ5D01	TGGCCAAAACAACTGATTGA	TCCCAACTTTTCCGTTCTTG	A01	65.76	264	
23	SEQ17E03	TTTCCTTTCAACCCTTCGTG	AATGAGACCAGCCAAAATGC	A09	85.93	193	
24	SEQ19G07	ATTCAATTCCTCTCTCCCCC	TCAATCAATCAATCGCAGGA	A03	106.08	149	
25	SEQ1B09	CGTTCTTTGCCGTTGATTCT	AGCACGCTCGTTCTCTCATT	A02	38.49	282	
26	SEQ3A08	ATACGTGACTTGGGCCAGAC	AGTGAAAAATACACCCAACGAA	A08	53.56	152	
27	SEQ9G05	CAAATTGTGCAGCCAAGAGA	CATATGCCCAGGAAGAGGAA	B05	32.05	273	
28	GM2079	GGCCAAGGAGAAGAAGAAAGA	GAAGGAGTAGTGGTGCTGCTG	B03	115.71	418	Guo *et al.* (2012)
29	TC1B02	AACATGCATGCAAATGGAAA	GCCAAAGTCACTTGTTTGCTT	B02	55.56	220-270	Moretzsohn *et al.* (2005)
30	TC4G02	GATCCAACTGTGAATTGGGC	CACACCAGCAACAAGGAATC	B03	88.70	130-166	
31	TC4F12	GATCTTTCCGCCATTTTCTC	GGTGAATGACAGATGCTCCA	A02	34.51	230	
32	IPAHM689	GATGACAATAGCGACGAGCA	GTAAGCCTGCAGCAACAACA	A06	52.22	240	Cuc *et al.* (2008)
33	TC1E05	GAAGGATAAGCAATCGTCCA	GGATGGGATTGAACATTTGG	A08	60.27	215-260	Moretzsohn *et al.* (2005)

The analysis of variance was usually presented in the following format (Table 3.2).

Table 3.2. Analysis of variance of phenotyping material using alpha-lattice design

Source of variation	**Degrees of freedom**	**Sums of squares**	**Mean squares**	**F-value**
Replicates	r-1	SSr	MSr	
Blocks (within replicates, ignoring treatments)	rs-r	SSb	MSb	
Treatments (adjusted for blocks)	t-1	SSt	MSt	F_{cal}
Error	rt-rs-t+1	SSe	MSe	
Total	n-1	SSc	-	-

Where,

r = number of replications

s = number of blocks

t = number of treatments / genotypes

n = number of entries

SSr = sum of squares due to replications

MSr = Mean sum of squares due to replications

SSb = sum of squares due to blocks

MSb = Mean sum of squares due to blocks

SSt = sum of squares due to treatments

MSt = Mean sum of squares due to treatments

SSe = sum of squares due to error

MSe = Mean sum of squares due to error

SSc = Total sum of squares

F_{cal} = calculated F value

3.1.4.2 Single Marker Analysis (SMA)

Single marker analysis can be conducted using a variety of statistical analyses, including t-tests, ANOVA, regression, maximum likelihood estimation and log likelihood ratios. The fact that molecular markers classifies the genotypes into groups, means that marker genotypes can be used as classifying variables for a t-test or ANOVA, or as variables for regression analysis. The null hypothesis tested is that genotypic classes do not differ in phenotype for a given molecular marker. Single

marker analysis calculates whether phenotype values differ among genotypes for a given molecular marker.

$$Y = \mu + f(\text{marker}) + \text{error}$$

Where,

Y = Trait value
μ = Population mean
f (marker) = Function of the molecular marker

Analysis of R^2 value was calculated by STATISTICA 4.5 software.

3.1.5 Principal Component Analysis (PCA)

Associations among the traits were also determined by Principal Component Analyses (PCA) (Hatcher, 1994) using R version 3.0.2 (R Project for Statistical Computing, http://www.r-project.org/).

EXPERIMENT-II:

3.2 ESTIMATION OF GENE EFFECTS BY GENERATION MEAN ANALYSIS:

The technique adopted in carrying out the present investigation to generate data and the statistical procedures adopted for analyzing the data are described in the following sub-heads:

1. Generation of breeding material
2. Evaluation of experimental material
3. Statistical analysis

3.2.1 Generation of Breeding Material

The material comprised of six basic generations *viz.*, P_1, P_2, F_1, F_2, B_1 and B_2 generated during rainy season, 2013 derived from two crosses (ICGV 06040 × ICGV 87141 and ICGV 06099 × ICGV 93468) involving four genotypes of groundnut with variation in kernel characteristics (Fig 3.1) including the kernel iron and zinc concentration. The above six generations were evaluated during post-rainy season, 2013-14 to understand the gene action involved in the inheritance of the traits of interest. The details of the parental lines used in this experiment are furnished in Table 3.3.

3.2.2 Evaluation of Experimental Material

3.2.2.1 Development of F_2 and Back Cross (B_1 and B_2) Generations

Four parental lines, *viz*., ICGV 06040, ICGV 87141, ICGV 06099 and ICGV 93468 and two resultant F_1 hybrids (ICGV 06040 × ICGV 87141 and ICGV 06099 × ICGV 93468), which were already developed, were planted in the field during rainy season, 2013 to produce F_1, F_2, B_1 and B_2 generations. F_2 generation of each cross was produced by selfing the F_1 plants, while B_1 and B_2 generations were developed by crossing back each F_1 hybrids with their female (ICGV 06040 and ICGV 06099) and male (ICGV 87141 and ICGV 93468) parents, respectively.

3.2.2.2 Field Evaluation

The six basic generations *viz*., parents, (P_1 and P_2), first and second filial generations (F_1 and F_2), first and second backcrosses (B_1 and B_2) of each combination of crosses were evaluated in a compact family block design (Fig 3.2) with three replications at ICRISAT, Patancheru during post-rainy season, 2013-14 for yield, its contributing characters, kernel iron and zinc concentrations. Each block comprised of one row each of P_1, P_2 and F_1, two rows each of B_1 and B_2 and eight rows of F_2. Each row was of 2 m length with a spacing of 30 cm between rows and 10 cm between the plants. Sowing was done on red precision soils at ICRISAT in broad-bed and furrow system and recommended package of practices were adopted for optimum crop growth and protective measures were applied to control insects and diseases.

3.2.2.3 Observations Recorded

Data on days to emergence, days to flowering, days to maturity, 100-kernel weight, pod yield per plant, shelling percentage, sound mature kernel percentage and kernel iron and zinc concentrations (fatted) were recorded on individual plant basis in all the generations in each replication as per the procedure explained under the sub head of 3.1.2.1 in experiment I.

3.2.3 Statistical Analysis

The data recorded on different traits were subjected to the following statistical analyses.

- Analysis of variance
- Estimation of genetic parameters like genotypic and phenotypic coefficients of variation, heritability in narrow sense and broad sense and genetic advance under selection, genetic advance as per cent of mean, degree of dominance, heterosis and inbreeding depression.
- Estimation of gene effects by Generation Mean Analysis
- Scaling tests of Mather (1949)
- Six parameter model of Hayman (1958)
- Correlation co-efficient analysis

3.2.3.1 Analysis of Variance

The data were subjected to analysis of variance for compact family block design as described by Panse and Sukhatme (1985). Here, crosses and generations within each cross were taken as families and progenies, respectively. The analysis was carried out in two stages.

(a) First from the data of main plots, the variance between crosses and the corresponding error was calculated by treating the experiment as one in simple randomized blocks. The structure of ANOVA between families is given below in Table 3.4a:

(b) The analysis for progenies under each family was done separately for each character using the data of sub plots to give the variance between different generations and the corresponding error. The structure of ANOVA for progenies within a family is given below in Table 3.4b:

Table 3.4a. Analysis of variance between crosses			
Sources of variation	**Degrees of freedom**	**Mean sum of squares**	**Expected mean square**
Replications	(r–1)	M_r	$\sigma^2_{e1} + f\sigma^2_r$
Families (crosses)	(f–1)	M_f	$\sigma^2_{e1} + r\sigma^2_f$
Error	(r–1) (f–1)	M_{e1}	σ^2_{e1}

Table: 3.4b. Analysis of variance among generations within a cross			
Sources of variation	**Degrees of freedom**	**Mean sum of squares**	**Expected mean square**
Replications	(r–1)	M_r	$\sigma^2_{e2} + p\sigma^2_r$
Progenies within family (generations)	(p–1)	M_p	$\sigma^2_{e2} + r\sigma^2_p$
Error	(r–1) (p–1)	M_{e2}	σ^2_{e2}

Where,

r = Number of replications

f = Number of families (crosses)

p = Number of progenies within each family (generations)

M_r = Mean square due to replications

M_f = Mean square due to families

M_p = Mean square due to progenies within each family

M_{e1} = Error mean square for families

M_{e2} = Error mean square for progenies within each family

3.2.3.2 Estimation of Components of Variances and Genetic Parameters

3.2.3.2.1. Phenotypic and genotypic co-efficient of variation: The components of variances were used to estimate genetic parameters like phenotypic and genotypic co-efficients of variation (PCV and GCV) as per the formulae given by Falconer (1981).

Phenotypic standard deviation, $(\sigma p) = \sqrt{\sigma^2 p}$

$$= \sqrt{\sigma^2 g + \sigma^2 e}$$

$$\text{PCV (\%)} = \frac{\text{Phenotypic standard deviation } (\sigma_p)}{\text{Grand mean } (\overline{X})} \times 100$$

$$\text{Genotypic standard deviation } (\sigma g) = \sqrt{\sigma 2g}$$

$$\text{GCV (\%)} = \frac{\text{Genotypic standard deviation } (\sigma_g)}{\text{Grand mean } (\overline{X})} \times 100$$

Categorization of the range of variation was followed as reported by Subramanian and Menon (1973).

Low : Less than 10 %
Moderate: 10 to 20 %
High : More than 20 %

3.2.3.2.2. Degree of dominance: The magnitude of variance due to dominance deviations, relative to that of additive genetic variance (Robinson *et al*. 1949) expressed as a square root of ratio of additive variance to dominance variance is known as degree of dominance. It is calculated as follows:

$$\text{Degree of dominance} = \sqrt{H/D}$$

Where,

H = Dominance variance

D = Additive variance

3.2.3.2.3. Heritability in broad sense [$h^2_{(b)}$]: It is the ratio of genotypic variance to the phenotypic variance. It is the heritable portion of phenotypic variance which responds to selection. It can be calculated using the formula (Hanson *et al*., 1956):

$$h^2_{(b)} = \frac{\text{Genotypic variance } (\sigma^2 g)}{\text{Phenotypic variance } (\sigma^2 p)} \times 100$$

3.2.3.2.4. Heritability in narrow sense [$h^2_{(n)}$]: Heritability in the narrow sense refers to the proportion of additive variance to the total observed variance in the total population. Heritability in narrow sense [$h2_{(n)}$] was calculated according to the formula given by Allard (1960):

Table 3.3. Pedigree and characteristics of the groundnut genotypes used as parents in the present investigation.

Parental line	Pedigree	Characteristics
ICGV 06040	[{(ICGS 35 x NC Ac 1705) x CS 16-B2-B2} x {(NC Ac 343 x (Dh. 3-20 x Robut 33-1)} x {(NC Ac 343 x (Dh. 3-20 x Robut 33-1)}]	Confectionary type, virginia bunch, medium duration, smooth pod, tan colour and medium size seed. Rich in iron (56.1 mg kg^{-1}) and zinc (80.1 mg kg^{-1}).
ICGV 87141	(TMV 10 x Chico)	Confectionary type, virginia bunch, medium duration, smooth pod, tan colour and medium size seed. Low in iron (44.1 mg kg^{-1}) and zinc (55.7 mg kg^{-1}).
ICGV 06090	[{(ICGS 35 x NC Ac 1705) x CS 16-B2-B2} x {(NC Ac 343 x (Dh. 3-20 x Robut 33-1)} x {(NC Ac 343 x (Dh. 3-20 x Robut 33-1)}]	Confectionary type, virginia bunch, medium duration, smooth pod, tan colour and medium size seed. Rich in iron (57.3 mg kg^{-1}) and zinc (81.0 mg kg^{-1}).
ICGV 93468	[(ICGS 44 x TG 2E) x {ICGS 30 x (TMV 10 x Chico)}]	Confectionary type, spanish bunch, medium duration, smooth pod, tan colour and medium size seed. Low in iron (45.2 mg kg^{-1}) and zinc (60.7 mg kg^{-1}).

$$h^2_{(n)} = \frac{\text{Additive variance } (\sigma^2 a)}{\text{Phenotypic variance } (\sigma^2 p)} \times 100$$

The range of heritability estimates were categorized as follows as suggested by Johnson *et al.* (1955):

Low : 0-30%

Medium : > 30-60%

High : > 60%

3.2.3.2.5 Genetic advance (GA): Genetic advance refers to the expected genetic gain or improvement in the next generation by selecting superior individuals under certain amount of selection pressure. From the heritability estimates, the genetic advance was estimated by the following formula given by Burton (1952):

$$GA = K.\ h^2_{(b)}.\ \sigma_p$$

Where,

GA = Expected genetic advance

K = Selection differential, the value of which is 2.06 at 5% selection intensity

σp = Phenotypic standard deviation

$h^2_{(b)}$ = Heritability in broad sense

3.2.3.2.6 Genetic advance as percent of mean (GAM): In order to visualize the relative utility of genetic advance among the traits, genetic advance as percent for mean (GAM) was computed as described by Johnson *et al*. (1955)

$$GAM = \frac{\overline{GA}}{\text{Grand mean } (X)} \times 100$$

The range of genetic advance as percent of mean was classified as suggested by Johnson *et al*. (1955).

Low : < 10 %

Moderate : 10-20 %

High : > 20 %

3.2.3.2.7 Heterosis and Inbreeding Depression Studies

3.2.3.2.7.1 Heterosis: Heterosis was estimated for two hybrids for eight traits using the following formulae. Estimates of heterosis were calculated according to Fonseca and Patterson (1968).

3.2.3.2.7.1.1 Heterosis over mid-parent (Relative heterosis): Heterosis was expressed as per cent increase or decrease observed in the F_1 over the mid-parent as per the following formula.

$$\text{Relative heterosis (\%) } (H_1) = \frac{\overline{F_1} - \overline{MP}}{\overline{MP}} \times 100$$

3.2.3.2.7.1.2 Residual heterosis over mid-parent: The heterosis over mid-parent or average / relative heterosis present in F_2 generation is calculated as residual heterosis over mid-parent. It is calculated as follows (Rao, 1980):

$$\text{Residual heterosis (\%) } = \frac{\overline{F_2} - \overline{MP}}{\overline{MP}} \times 100$$

3.2.3.2.7.1.3 Heterosis over better parent (Heterobeltiosis): It was expressed as per cent increase or decrease observed in F_1 over the better parent as per the formula of Liang *et al*. (1971).

$$\text{Heterobeltiosis (\%) } (H_2) = \frac{\overline{F_1} - \overline{BP}}{\overline{BP}} \times 100$$

(For the traits like days to flowering, earliness is desirable so the early parents are taken as better parents).

3.2.3.2.7.1.4 Residual heterosis over better parent: The heterosis over better parent or heterobeltiosis present in F_2 generation is calculated as residual heterosis over better parent. It is calculated as follows (Rao, 1980):

$$\text{Residual heterosis over better parent (\%)} = \frac{\overline{F_2} - \overline{MP}}{\overline{MP}} \times 100$$

The significance of heterosis, was tested by using ‘t’ test as suggested by Snedecor and Cochran (1989) and Paschal and Wilcox (1975).

$$\text{Heterosis } t = \frac{\overline{F_1} - \overline{MP}}{\sqrt{1.5EMS/r}}$$

$$\text{Heterobeltiosis } t = \frac{\overline{F_1} - \overline{BP}}{\sqrt{1.5EMS/r}}$$

Where,

EMS = Error Mean Sum of Square

r = Number of replications

$\overline{BP}$ = Mean of better parent

$\overline{MP}$ = Mean of mid parent

$\overline{F_1}$= Mean of F_1 generation

$\overline{F_2}$= Mean of F_2 generation

The calculated ‘t’ value was compared with table ‘t’ value at error degrees of freedom.

3.2.3.2.7.2 Inbreeding depression: The loss of fitness in the progenies or decline in trait expression with decreased heterozygosity arising from consanguineous mating is known as inbreeding depression or inbreeding decline. It can be calculated using the following formula given by Kempthrone (1957):

$$\text{Inbreeding depression} = \frac{\overline{F_1} - \overline{F_2}}{\overline{F_1}} \times 100$$

Where,

$\overline{F_2}$ = mean of F_2 population

$\overline{F_1}$ = mean of F_1

3.2.3.3 Generation Mean Analysis

The concept of Generation Mean Analysis (GMA) was developed by Hayman (1958) and Jinks and Jones (1958) for the estimation of genetic components of variation. This technique involves six different generations *viz.*, parents (P_1 and P_2), their F_1, F_2 and back crosses (B_1 and B_2). Accordingly, the means were computed for each generation of P_1, P_2, F_1, F_2, B_1 and B_2 for each cross. The variance and corresponding standard errors of the means were computed from the deviations of the individual values obtained from individual plants for each of the generation in each cross and were analyzed to estimate various genetic parameters and the type of gene action involved in the inheritance of various traits.

The biometrical analysis consists of two main steps, *viz.*, (i) testing for epistasis and (ii) estimation of gene effects and variances.

3.2.3.3.1 Scaling test: The test which provides information regarding presence / absence of gene interaction is termed as scaling test. The test of adequacy of scales is important because in most of the cases the estimation of additive and dominance components of variances were made assuming the absence of gene interactions. Mather (1949) and Hayman and Mather (1955) gave four scaling tests to test the adequacy of additive-dominance model. The different scales, variances and standard errors are computed by using the following formulae:

$$\text{Scale A} = 2\,B_1 - P_1 - F_1 = 0$$

$$\text{Scale B} = 2\,B_2 - P_2 - F_1 = 0$$

$$\text{Scale C} = 4\,F_2 - 2F_1 - P_1 - P_2 = 0$$

Where,

P_1, P_2, F_1, F_2, B_1 and B_2 are means of different generations over the replications.

The variances of the quantities A, B, C and D were calculated from respective variances of different generations as follows

$$V_A = 4V\,(B_1) + V\,(P_1) + V\,(F_1) = 0$$

$$V_B = 4V\,(B_2) + V\,(P_2) + V\,(F_1) = 0$$

$$V_C = 16V\,(F_2) + 4V\,(F_1) + V\,(P_1) + V\,(P_2) = 0$$

$$V_D = 16\,V\,(F_3) + 4V\,(F_2) + V\,(P_1) + V\,(P_2) = 0$$

Where,

V_A, V_B, V_C and V_D are the variances of the scale A, B, C and D;

V_{P1}, V_{P2}, V_{F1}, V_{F2}, V_{B1} and V_{B2} are the variances of means of P_1, P_2, F_1, F_2, B_1 and B_2 generations, respectively.

The variance of mean for each generation was calculated by dividing variance obtained from ANOVA table by the number of observations taken. The standard error of the scale A, B, C and D were worked out by taking the square root of respective variances.

$$\text{S.E. A} = \sqrt{V_A}$$
$$\text{S.E. B} = \sqrt{V_B}$$
$$\text{S.E. C} = \sqrt{V_C}$$
$$\text{S.E. D} = \sqrt{V_D}$$

The 't' values were calculated by dividing the scale effects of A, B, C and D by their respective standard error.

$$\text{t cal for A-test} = \text{Scale A} / \text{S.E. A}$$
$$\text{t cal for B-test} = \text{Scale B} / \text{S.E. B}$$
$$\text{t cal for C-test} = \text{Scale C} / \text{S.E. C}$$
$$\text{t cal for D-test} = \text{Scale D} / \text{S.E. D}$$

The calculated values of 't' were compared with 't' table values at 5% and 1% level of significance at their respective degrees of freedom. In each test, the degrees of freedom was taken as the sum of the degrees of freedom of various generations involved in that scaling test and the degrees of freedom for any generation was calculated as total number of observations minus number of replications. However, in case of un-replicated data the degrees of freedom will be the number of observations per generation minus one.

If the calculated value of these scales is higher than 't' table values (when *d.f.* = > 30, then 't' table values are 1.96 and 2.58 at 0.05 and 0.01 level of probability, respectively), it is considered significant and vice versa. The significance of any one of these scaling tests indicates the presence of epistasis. It is to be noted that,

(a) D provides a test largely of '*i*' type of interaction (additive × additive).

(b) C indicates '*l*' (dominance × dominance) type of gene interaction.

(c) Significance of C + D relates to '*i*' (additive × additive) and '*l*' (dominance × dominance) type of interaction.

(d) '*j*' (additive × dominance) type of interaction has no effect on C and D but it affects A and B. A and B tests provide an evidence on *i*, *j* and *l* type of gene interactions (Singh and Chaudhary, 1977).

3.2.3.3.2 Components of generation means: When the scales were significant, the mean values over replications were used for the estimation of the gene effects. Owing to the presence of six generations (P_1, P_2, F_1, F_2, B_1 and B_2) in each cross, six parameter model proposed by Hayman (1958) and Jinks and Jones (1958) was followed to estimate the genetic parameters *viz.*, mean (*m*), additive gene effects (*d*), dominance gene effects (*h*) and three types of non-allelic gene interactions *viz.*, additive × additive (*i*), additive × dominance (*j*) and dominance × dominance (*l*).

m = Mean = F_2

d = Additive effect = $B_1 - B_2$

h = Dominance effect = $F_1 - 4F_2 - (1/2)\ P_1 - (1/2)\ P_2 + 2B_1 + 2B_2$

i = Additive × Additive effect = $2B_1 + 2B_2 - 4F_2$

j = Additive × Dominance effect = $B_1 - (1/2)\ P_1 - B_2 + (1/2)\ P_2$

l = Dominance × Dominance effect = $P_1 + P_2 + 2F_1 + 4F_2 - 4B_1 - 4B_2$

Where,

P_1, P_2, F_1, F_2, B_1 and B_2 are the mean values of P_1, P_2, F_1, F_2, B_1 and B_2 generations, respectively.

3.2.3.3.3 Test of significance of various gene effects: The test of significance of the gene effects was done by 't' test for which variance and standard error of each estimates were calculated using following equations.

Calculation of variances:

$V_m = V(F_2)$

$V_d = V(B_1) + V(B_2)$

$V_h = V(F_1) + 16V(F_2) + (1/4)V(P_1) + (1/4)V(P_2) + 4V(B_1) + 4V(B_2)$

$V_i = 4V(B_1) + 4V(B_2) + 16V(F_2)$

$V_j = V(B_1) + 1/4V(P_1) + V(B_2) + 1/4\,V(P_2)$

$V_l = V(P_1) + V(P_2) + 4V(F_1) + 16V(F_2) + 16V(B_1) + 16V(B_2)$

Where,

$V(P_1)$, $V(P_2)$, $V(F_1)$, $V(F_2)$, $V(B_1)$ and $V(B_2)$ were the variances of P_1, P_2, F_1, F_2, B_1 and B_2 generations, respectively.

Calculation of standard error:

The standard error of each of the gene effects was estimated as follows

$$S.E.(m) = \sqrt{V_m}$$

$$S.E.(d) = \sqrt{V_d}$$

$$S.E.(h) = \sqrt{V_h}$$

$$S.E.(i) = \sqrt{V_i}$$

$$S.E.(j) = \sqrt{V_j}$$

$$S.E.(l) = \sqrt{V_l}$$

The 't' values were worked out using following formulae

$$t(m) = m / S.E.(m)$$

$$t(d) = d / S.E.(d)$$

$$t(h) = h / S.E.(h)$$

$$t(i) = i / S.E.(i)$$

$$t(j) = j / S.E.(j)$$

$$t(l) = l / S.E.(l)$$

The significance for the above genetic parameters were tested with the help of 't' test. First standard error (S.E.) is worked out for each component separately by taking the square root of the variance of the respective component. Significance of the genetic effects is tested using 't' test in a similar manner as in the case of scaling test. If the calculated value is greater than 't' table value, it is considered significant and vice versa (Singh and Chaudhary, 2001).

3.2.3.4 Correlation Co-Efficient Analysis

Trait association refers to a change in one trait accompanied by a change in the other trait. The data recorded on various traits were utilized for the computation of correlation coefficients to understand the association between them. The formulae suggested by Snedecor and Cochran (1967) were utilized for the computation of correlation coefficients.

$$r(xy) = \frac{Cov\ (xy)}{\sqrt{(Var\ x)\ (Var\ y)}}$$

$$Cov\ (xy) = 1/n\ (\sum xy - \sum x \sum y/n)$$

$$Var\ (x) = 1/n\ (\sum x^2 - (\sum x)^2/n)$$

Where,

r(xy) = Correlation between x and y
Cov(xy) = Covariance for traits x and y
Var(x) = Variance for x
Var(y) = Variance for y
r = Correlation coefficient
xy = Two independent variables

To test the significance of correlation coefficients, the estimated values were compared with the table values of correlation coefficients (Fisher and Yates, 1967) at 5 % and 1 % levels of significance with (n-2) degrees of freedom, where 'n' is the total number of observations used.

Results and Discussion

Chapter IV

RESULTS AND DISCUSSION

The World Health Organization (WHO) recognized iron, zinc and vitamin A as the critical micronutrients that are most limiting in diet. Iron and zinc are receiving global attention as their deficiencies are widespread, particularly in developing countries. If there is sufficient genetic variation for the density of micronutrients in edible parts of the crop, bio-fortification can be achieved through plant breeding (Mayer *et al*., 2008). Bio-fortification offers a cost effective and sustainable approach and has become an active goal of plant breeding programs in the developing world (Welch, 2002). In groundnut genetic variability is reported for Fe and Zn concentration (Upadhyaya *et al.*, 2012 and Janila *et al*., 2014) and thus bio-fortification is possible. Knowledge on genetics of kernel iron and zinc and association of these micronutrient concentrations with other important traits is essential to develop varieties with improved yield and nutritional quality. Besides, if markers linked to the traits of interest are available, they may be used for selection in the breeding program to accelerate the genetic gains for kernel iron and zinc concentrations. Hence the present study was carried out in two separate experiments.

4.1 Quantitative Trait Loci (QTL) analysis for kernel iron and zinc concentrations

4.2 Generation Mean Analysis

4.1 QUANTITATITVE TRAIT LOCI (QTL) ANALYSIS FOR KERNEL IRON AND ZINC CONCENTRATIONS

As compared to cereals, legume kernels contain higher iron and zinc concentrations which is retained during processing, unlike for milled cereal seeds (Beebe *et al.,* 2000 and Wang *et al.,* 2003). Plant roots take up iron and zinc from the soil which is then translocated to the kernels and other tissues of the plant through vascular transport and partitioning mechanisms. The process of uptake and translocation within the plant and subsequent accumulation in the kernel is influenced by transporters and storage reserves (Frossard *et al.,* 2000 and Grusak, 2002). Groundnut plant uses rhizosphere acidification process for uptake of the iron and zinc by

root hairs (Marschner and Römheld 1994 and Briat and Lobreaux 1997). Once iron and zinc are taken up into the plant root's epidermal cells, various metal transporters are involved in translocation throughout the plant (Grotz and Guerinot 2006). The minerals are then used for vegetative growth, where iron homeostasis is mediated by ferritin, an iron storage protein (Briat and Lobreaux 1997) and during reproductive phases minerals are remobilized to kernels (Frossard *et al.,* 2000). The inheritance of iron and zinc concentration in groundnut is reported to be mostly quantitative and influenced by the environment, but can also vary depending on the source genotype (Guzman-Maldonado *et al.,* 2003; Blair *et al.,* 2009 and Cichy *et al.,* 2009).

As compared to other traits, very little progress has been made to understand the genetic basis of iron and zinc concentration in groundnut due to difficulties involved in carrying out phenotypic studies for these traits. The Information on the genetic basis of accumulation of micro-nutrients in the kernels and mapping of the quantitative trait loci (QTL) will provide the basis for preparing strategies to improve kernel micronutrient concentration through marker assisted selection. QTL mapping employs genetic variation which exists between different accessions or segregating populations to identify polymorphic markers, which are then used to develop a linkage map and carry out QTL analysis. QTL analysis provides information on the chromosomal locations of the important loci without any prior knowledge on the genes involved and reveals their possible genetic effects leading to phenotypes of interest. DNA markers which are closely linked to the QTL region that governs desired traits allow the selection of plants possessing those traits prior to trait expression.

In the present study, the experimental material involving an F_2 population, consisting of 184 individual plants derived from the cross between ICGV 06099 and ICGV 93468, was used for QTL analysis using SSR markers. The results obtained are discussed under the following headings:

4.1.1 Parental polymorphism studies

4.1.2 Phenotyping of experimental material

4.1.3 Genotyping for identification of genomic regions associated with kernel iron and zinc concentrations

4.1.4 QTL (single marker) analysis

4.1.5 Principal Component Analysis

4.1.1 Parental Polymorphism Studies

Parental polymorphism survey between two parents, *viz.*, ICGV 06099, parent with high kernel iron and zinc and ICGV 93468, parent with low kernel iron and zinc using 200 SSR markers revealed that 33 SSR markers that amounts to about 16.5 % of tested markers were polymorphic (Fig. 4.1), 143 markers (71.5 %) were monomorphic (Fig. 4.2) and remaining 24 markers (12 %) were not amplified. Allo-polyploidy nature, with AABB genomes (2n = 4x = 40) and evolution from single hybridization event followed by chromosome doubling along with cross incompatibility of cultivars with wild species due to ploidy differences made groundnut cultivars less polymorphic compared to other crops. Polymorphism in the present study was checked using agarose gels and Genemapper version 4.0 software.

4.1.2 Phenotyping of Experimental Material

Phenotyping is the primary data that is required for QTL analysis and should be precise enough. The precision is extremely important and recorded with utmost care, as quantitative traits are often affected adversely by experimental errors which are further worsened by environmental effects. In the present study $F_{2:3}$ phenotyping population was developed by crossing ICGV 06099 and ICGV 93468 which were contrasting for kernel iron and zinc concentrations. Single plant progenies harvested from F_2 individual plants constituted the $F_{2:3}$ population that was phenotyped with two replications.

Initial soil analysis to estimate the iron and zinc status of the experimental block in both the replications at different sites, revealed that the iron and zinc concentrations were above critical limits in the soil.

4.1.2.1 Phenotyping

Plant phenotyping is the comprehensive assessment of complex plant traits. Valid and authentic phenotypic data is essential for successful identification of QTLs for a given trait. In the present study, phenotyping of $F_{2:3}$ population from the cross ICGV 06099 × ICGV 93468 was carried out during rainy season, 2013 at ICRISAT, Patancheru, India in alpha lattice design with two replications.

Fig.4.1. GeneMapper profile for an amplified SSR marker showing polymorphism between the parents, ICGV 06099 and ICGV 93468

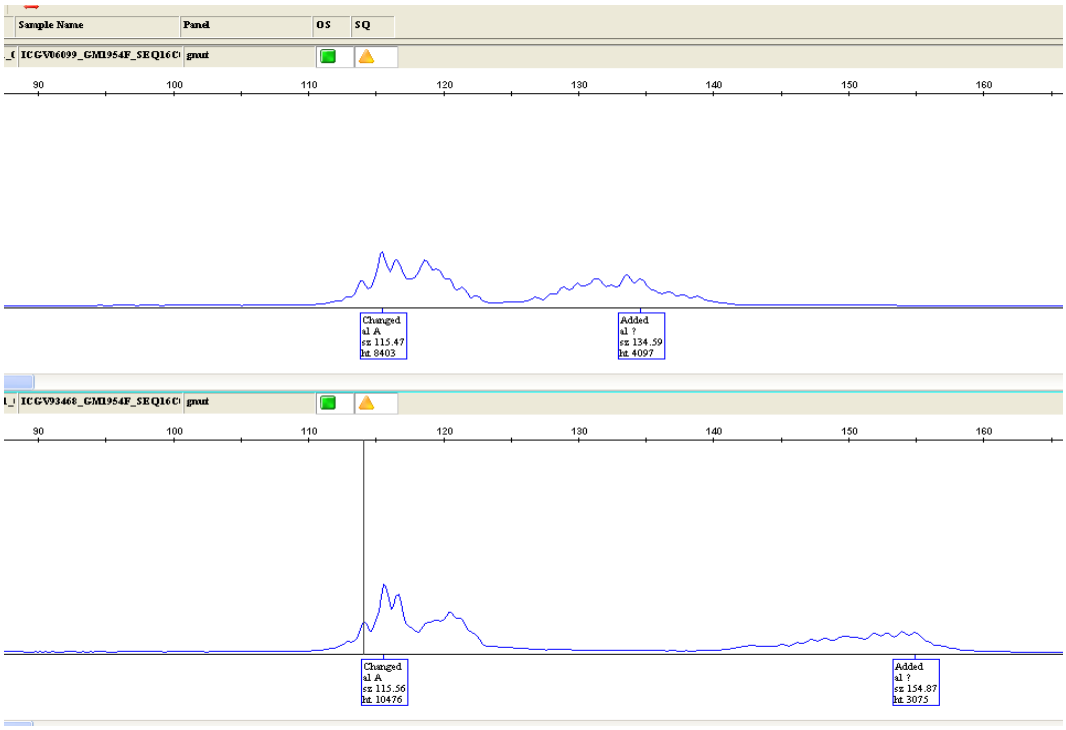
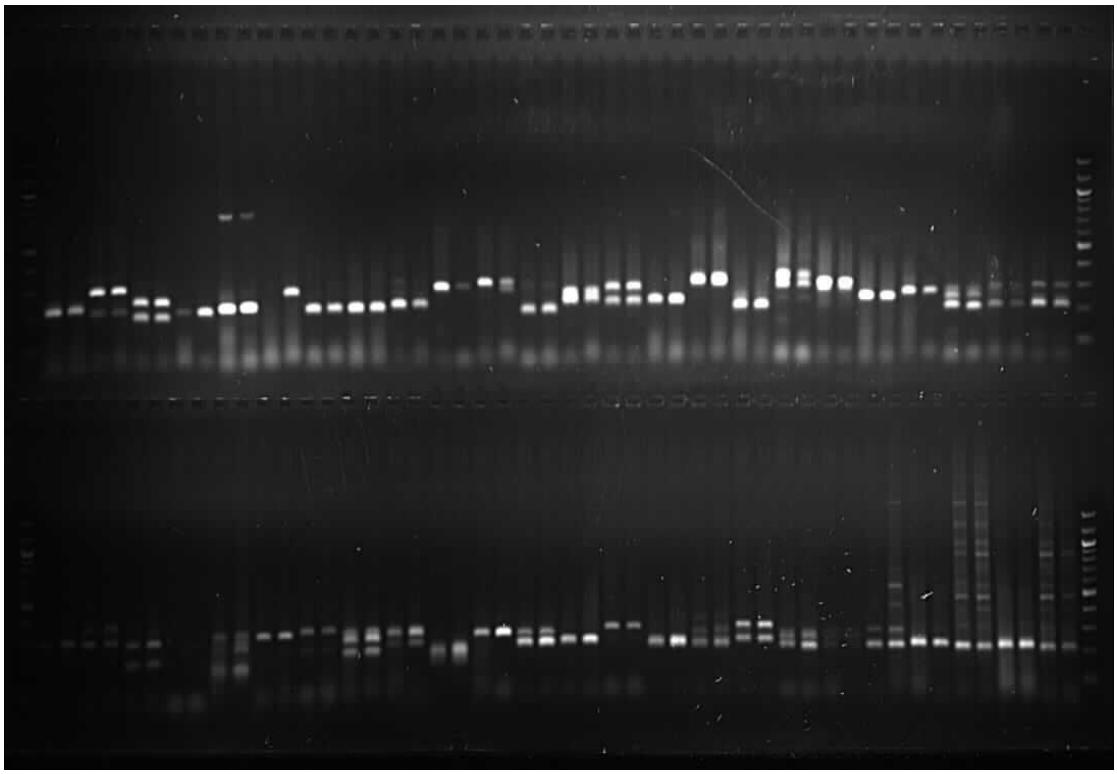


Fig.4.2. Agarose gel picture showing monomorphism between the parents, ICGV 06099 and ICGV 93468



Data were recorded on *viz.*, days to emergence, days to flowering, days to maturity, 100-kernel weight (g), single plant yield (g), pod yield (g $plot^{-1}$), kernel yield (g $plot^{-1}$), sound mature kernel percentage (%), shelling percentage (%), oil content (%), protein content (%), kernel iron and zinc concentrations (mg kg^{-1}), linoleic acid (%), oleic acid (%), palmitic acid (%) and stearic acid (%) content, and subjected to statistical analysis.

The analysis of variance (Table 4.1) revealed the existence of significant variation for all the traits except for days to emergence, days to maturity, protein content and palmitic acid content. Significant variability for kernel iron and zinc concentrations was found in the mapping population suggesting that QTL analysis for these traits can be carried out with the present population.

For kernel iron and zinc concentration, among the parents, ICGV 06099 recorded mean values of 52.5 mg kg^{-1} and 79.5 mg kg^{-1}, respectively whereas ICGV 93468 recorded mean values of 37.3 mg kg^{-1} and 64.6 mg kg^{-1}, respectively (Table 4.2) for the same. In the $F_{2:3}$ population, mean values of 45.4 mg kg^{-1} and 76.7 mg kg^{-1}, were observed for kernel iron and zinc concentration, respectively. Though the mean values were low compared to that of higher parent *i.e.,* ICGV 06099, presence of entries with high values for iron (>52.5 mg kg^{-1}) and zinc (>79.5 mg kg^{-1}) concentrations suggested the presence of transgressive segregants in the $F_{2:3}$ mapping population.

Table 4.2 Mean and standard deviation of the kernel iron zinc concentrations among parents and $F_{2:3}$ mapping population of the cross ICGV 06099 × ICGV 93468 in groundnut

Characters	Parents				$F_{2:3}$ Population (n = 184)		
	ICGV 06099 (n = 4)		ICGV 93468 (n = 4)				
	Mean	SD (±)	Mean	SD (±)	Mean	Range	SD
Kernel iron concentration (mg kg^{-1})	52.5	5.93	37.3	3.30	45.4	31.8 - 61.4	6.10
Kernel zinc concentration (mg kg^{-1})	79.5	4.67	65.0	4.14	76.7	59.6 - 90.4	5.60

Where,

n = No. of individuals; SD = Standard Deviation

4.1.2.1.1 Descriptive statistics of phenotyping population

The population was phenotyped for seventeen different traits which are described below. The details of the descriptive statistics of the population were presented in the Table 4.3 and their respective histograms showing normal distribution were depicted in Figure 4.3.

A histogram is a graphical representation of the distribution of numerical data. It is an estimate of the probability distribution of a continuous variable (quantitative variable) which gives a rough sense of the density of the data and was first introduced by Pearson (1895) whereas skewness is the measure of asymmetry of the probability distribution of a real-valued random variable. Direction of variation of the data can be known using skewness value. Histogram is a useful representation to understand that the data was skewed or normally distributed.

4.1.2.1.1.1 Days to emergence: It is the number of days taken from date of sowing to date when seedling emerges. This trait has direct influence on days to flowering, maturity duration and ultimately yield. The phenotyping population showed a range of 5 to 10 days for emergence with a mean of 7 days. Normal distribution was observed for the trait with a low skewness value of 0.385 (Fig 4.3a). Both the parental lines *viz.*, ICGV 06099 (P_1) and ICGV 93468 (P_2) were within the range of the population though P_2 was earlier (6 days) compared to P_1 (7 days).

4.1.2.1.1.2 Days to 75 % flowering: It is the number of days taken from the date of sowing to emergence of flower in 75 % of total plants in a line. Among the mapping population this trait ranged from 30 to 39 days with a mean of 34 days. P_1 (34 days) and P_2 (33 days) recorded almost same number of days to flower and both the parental lines were within the range of population. Normal distribution was observed with a low skewness value of -0.110 (Fig 4.3a).

4.1.2.1.1.3 Days to maturity: It is the number of days taken from date of sowing to final harvest which is determined by examining the foliage, internal pericarp colour and colour of pods for maturity indices. In groundnut, based on maturity duration, the genotypes are generally grouped in to very early (<90 days), early (90-100 days), medium (101-110 days) and late (111-120 days) maturing types (PPVFRA, 2009). Uniform maturity of the plants in the field will result in greater pod yield. The maturity duration of the population ranged from 103 to 114 days with a mean of 111 days. Since the entire population in the present study was grouped into only medium and late maturity types it exhibited skewed distribution with a skewness value of -1.158 (Fig 4.3a).

Among the parental lines, P_2 matured in 104 days (medium) while P_1 took 114 days to mature (late).

4.1.2.1.1.4 100-kernel weight (g): It is an important yield related parameter for estimating seed dry mass. Based on 100-kernel weight, the groundnut genotypes are generally categorized in to low (<36 g), medium (36-50 g), high (51-65 g) and very high (>65 g) seed mass (PPVFRA, 2009). For the population the 100-kernel weight varied from 19.20 to 43.00 g with a mean of 31.65 g, thus the entire population was grouped in to low to medium 100-kernel weight. Both the parental lines *viz.*, P_1 and P_2 had 100-kernel weights of 33.01 g and 31.36 g, respectively and were within the range of population. The trait showed normal distribution with a skewness value of -0.062 (Fig 4.3a).

4.1.2.1.1.5 Single plant yield (g): Data on single plant yield is useful in estimating the performance of individual plant so that transgressive segregants which were outperforming the parents can be isolated from the population. The population recorded a range of 4.08 to 78.60 g of single plant yield with a mean of 18.24 g. P_1 (23.67 g) recorded higher single plant yield than to P_2 (11.43 g) and both the parents were within the range of the population. The trait exhibited higher skewness value of 1.880 (Fig 4.3a).

4.1.2.1.1.6 Pod yield per plot (g plot^{-1}): Breeding objectives in groundnut must consider higher pod yield as prime objective. The mapping population recorded a range of 25.6 to 244.0 g plot^{-1} pod yield with a mean of 104.2 g plot^{-1}. Both the parental lines were within the range of mapping population though P_1 (133.58 g plot^{-1}) recorded higher pod yield per plant compared to P_2 (96.21 g plot^{-1}). Normal distribution was observed with a moderate skewness value of 0.614 for this trait (Fig 4.3b).

4.1.2.1.1.7 Kernel yield per plot (g plot^{-1}): Kernel yield per plot gives the actual yield of kernels after shelling in a given area. Kernel yield per plot in the population ranged from 13.9 to 158.5 g plot^{-1} for the trait with a mean of 59.67 g plot^{-1}. P_1 (74.87 g plot^{-1}) obtained higher kernel yield per plot compared to P_2 (51.91 g plot^{-1}) and both the parents were within the range of the mapping population. The trait distributed normally by recording a moderate skewness value of 0.802 (Fig 4.3b).

4.1.2.1.1.8 Sound mature kernel percentage (%): It is the percentage of perfectly filled kernels without any wrinkles out of a given volume of kernels. Sound mature kernel percentage varied from 28.81 to 95.72 % in the mapping population understudy with a mean of 69.47 %. Both the

parental lines were within the range of the population though P_1 (70.37 %) recorded higher sound mature kernel percentage compared to P_2 (62.64 %). The trait showed normal distribution by recording a low skewness value of -0.270 (Fig 4.3b).

4.1.2.1.1.9 Shelling percentage (%): It is measured by shelling known weight of pods and weighing the kernels obtained after shelling. Based on this trait, groundnut genotypes are categorized in to low (<66 %), medium (66-75 %) and high (>75 %) (PPVFRA, 2009). In the present population, this trait had a range of 30.49 to 84.80 % shelling percentage covering all categories of this trait with a mean of 58.06 %. Both the parental lines had low shelling percentage which was 55.22 % 53.22 % in P_1 and P_2, respectively. Normal distribution was observed for the trait by recording a low skewness value -0.278 (Fig 4.3b).

4.1.2.1.1.10 Oil content (%): Groundnut, being an important oilseed crop the percentage of oil in their kernels determines the oil yield per unit area. Both oil content and pod yield determine oil yield of a variety. Based on this trait groundnut genotypes are categorized in to low (<45 %), medium (45-48 %), high (49-52 %) and very high (>52 %) (PPVFRA, 2009). The present mapping population ranged from 43.49 to 59.61 % of oil content with a mean of 48.53 %. P_1 recorded high oil content (49.23 %) whereas medium oil content was observed in P_2 (46.09 %) but both the parents were within the range of the mapping population. This trait exhibited normal distribution by recording a moderate skewness value of 0.714 (Fig 4.3b).

4.1.2.1.1.11 Protein content (%): Being leguminous crop groundnut kernels are rich in protein. The protein content in the mapping population ranged from 24.89 to 29.75 % with a mean of 27.22 %. P_1 (27.08 %) and P_2 (27.36 %) recorded almost similar protein content and were within the range of mapping population. The population distributed normally for the trait under concern by recording a low skewness value of -0.221 (Fig 4.3b).

4.1.2.1.1.12 Kernel iron concentration (mg kg^{-1}): The mean kernel iron concentration among the mapping population was 45.29 mg kg^{-1} with a range of 31.77 to 61.41 mg kg^{-1}. Among the parental lines, P_1 (52.50 mg kg^{-1}) recorded higher kernel iron concentration than P_2 (37.30 mg kg^{-1}) and both the parents were within the range of mapping population. Normal distribution was observed for this trait with a moderate skewness value of 0.516 (Fig 4.3c).

4.1.2.1.1.13 Kernel zinc concentration (mg kg^{-1}): The population had a kernel zinc concentration ranged from 59.64 to 90.40 mg kg^{-1} with a mean of 76.74 mg kg^{-1}. Both the parents were within the range of the population though P_1 (79.5 mg kg^{-1}) recorded higher kernel

zinc concentration than P_2 (65.00 mg kg^{-1}). The population exhibited normal distribution with a low skewness value of -0.115 for this trait (Fig 4.3c).

4.1.2.1.1.14 Oleic acid content (%): Oleic acid content in the oil has got an important role in human diet as well as in industrial uses. Ratio of oleic to linoleic acid is important in determining the quality of groundnut oil. Oleic acid contributes nearly 46.8 % of total fatty acid composition of groundnut oil. The trait ranged from 31.66 to 53.93 % in the population understudy with a mean of 41.31 %. P_2 (44.75 %) recorded higher oleic acid content than P_1 (40.04 %) and both the parental lines were within the range of the mapping population. Normal distribution was noticed for the trait with a low skewness value of 0.377 (Fig 4.3c).

4.1.2.1.1.15 Linoleic acid content (%): Linoleic acid contributes nearly 34 % of total fatty acid composition of groundnut oil. The population showed a range of 24.04 to 42.17 % for linoleic acid content with a mean of 34.08 %. Both the parental lines were within the range of the mapping population though P_1 (35.50 %) recorded higher linoleic acid content than P_2 (30.67 %). A skewness value of -0.495 was obtained indicating normal distribution for this trait (Fig 4.3c).

4.1.2.1.1.16 Palmitic acid content (%): Palmitic acid content varied from 11.01 to 14.38 % in the population with a mean of 12.73 %. Both the parents were within the range of the population and recorded almost similar values for palmitic acid content though P_1 (12.63 %) recorded a little higher value than P_2 (12.55 %). Normal distribution was recorded for the trait with a low skewness value of -0.042 (Fig 4.3c).

4.1.2.1.1.17 Stearic acid content (%): In the mapping population stearic acid content varied from 1.54 to 3.65 % with a mean of 2.22 %. Almost same stearic acid content was observed in both the parents though P_1 (2.24 %) recorded slightly higher value compared to P_2 (2.13 %) and both of them were within the range of the mapping population. Normal distribution was observed for the trait though it has recorded a moderate skewness value of 0.912 (Fig 4.3c).

Overall perusal of descriptive statistics revealed that normal distribution was observed for almost all the traits except days to maturity and protein content. Transgressive segregants which were outperforming either of the parents for all the traits including kernel iron and zinc concentrations (except for days to maturity) were present in the population. This suggests that the present mapping population is perfect for QTL mapping for kernel iron and zinc concentrations.

4.1.2.2 Estimation of Genetic Parameters

Genetic variability is essential for initiating an effective and successful breeding programme and it becomes imperative to study the level of genetic variability available in the existing genotypes. Genetic improvement of a crop through breeding relies solely on the utilization of available or created genetic variability. Depending on the trait, variability in a population can arise from genotype or environment or genotype × environment interaction effects. If the variability in the population is largely due to genetic cause with least environmental effect, the probability of isolating superior genotypes through selection will be more (Nath and Alam, 2002). Breeding in such population is primarily conditioned by the magnitude and nature of genotype × environment interactions on plant characters. Thus, to improve selection efficiency it becomes necessary to have an understanding of parameters such as genotypic and phenotypic co-efficient of variation, genetic advance and heritability which helps to further clarify the nature of character. Heritability and genetic advance is a useful tool for breeders in determining the direction and magnitude of selection. Therefore, the present investigation was undertaken to study variability, heritability and genetic advance (Table 4.4) for various characters in $F_{2:3}$ population of the cross ICGV 06099 × ICGV 93468 in groundnut.

4.1.2.2.1 Phenotypic and genotypic coefficient of variation (%)

In the present population, phenotypic co-efficient of variation (PCV) varied from 2.30 % for protein content to 34.45 % for single plant yield (Table 4.4), whereas genotypic co-efficient of variation (GCV) varied from 1.68 % for days to flowering to 32.04 % for single plant yield. For almost all the traits the difference between PCV and GCV was moderate suggesting influence of environment on the expression of these traits. Vishnuvardhan *et al.* (2013) and Satish (2014) also reported influence of environment on several traits in groundnut.

For kernel iron and zinc concentrations, a moderate difference between PCV and GCV was observed suggesting moderate influence of environment. The estimates and the difference between PCV and GCV were higher for kernel iron concentration than that for kernel zinc concentration indicating that kernel iron concentration was more influenced by the environment than kernel zinc concentration in groundnut. In contrast to the present findings, Ravikiran *et al.* (2014) reported higher influence of environment on grain zinc concentration in sorghum.

Low PCV estimates were recorded for protein content (2.30 %), days to emergence (2.38 %), days to maturity (3.93 %), days to flowering (4.69 %), palmitic acid (5.05 %), kernel zinc

concentration (6.84 %), oleic acid (8.59 %) and linoleic acid content (9.06 %) whereas moderate PCV recorded for oil content (10.72 %), kernel iron concentration (12.60 %), stearic acid content (12.91 %), 100-kernel weight (13.22 %), shelling percentage (14.14 %) and sound mature kernel percentage (18.23 %). Higher PCV was recorded for pod yield per plot (28.40 %), kernel yield per plot (30.35 %) and single plant yield (34.45 %). Similarly, low GCV recorded for most of the characters except pod yield per plot (27.10 %), kernel yield per plot (26.10 %) and single plant yield (32.04 %) which had high GCV (Fig 4.4). The results pertaining to 100-kernel weight were partly in accordance with the findings of Nath and Alam (2002), Parameswarappa *et al.* (2005), Jonah *et al.* (2012) and Satish (2014) with respect to higher estimates of PCV but in contrast the difference between the estimates of PCV and GCV, which was very low in their findings. Contrasting results to the present study were reported by Parameswarappa *et al.* (2005) and Jonah *et al.* (2012) for shelling percentage and Vishnuvardhan *et al.* (2013) and Satyanarayan *et al.* (2014) for sound mature kernel percentage.

For oil content moderate PCV and low GCV were observed in the present study. Low GCV for oil content was also reported by Parameswarappa *et al.* (2005) and Shukla and Rai, (2014). In the present study, low PCV and GCV recorded for protein content which is in agreement with the findings of Channayya *et al.* (2011), but in contrast to the findings of Parameswarappa *et al.* (2005) where moderate estimates were observed.

Shukla and Rai (2014) reported higher estimates of PCV and GCV for oleic acid content which was in contrast with the present study where low PCV and GCV were recorded for this trait.

Satish (2014) reported narrow difference between PCV and GCV for pod yield, while Parameswarappa *et al.* (2005), Jonah *et al.* (2012) and Satyanarayan *et al.* (2014) reported moderate difference between these estimates for the same trait. However, in the present study large difference between PCV and GCV was observed for pod yield per plot.

4.1.2.2.2 Broad sense heritability and genetic advance

Heritability provides information about the extent of which a particular genetic character can be transmitted to the successive generations (Mangi *et al*., 2010). High heritability indicates less environmental influence in the observed variation (Mohanty, 2003 and Eid, 2009). However, heritability value alone cannot provide information on the amount of genetic progress that would result from selection of best individuals. Johnson *et al*. (1955) reported that heritability estimates

along with genetic advance would be more successful in predicting the effectiveness of selecting the best individuals. Genetic advance, which estimates the degree of gain in a trait obtained under a given selection pressure, is an important parameter that guides the breeder in choosing a selection programme (Hamdi *et al*., 2003). High heritability and high genetic advance for a given trait indicates that it is governed by additive gene action and, therefore, provides the most effective condition for selection (Tazeen *et al*., 2009).

In $F_{2:3}$ population of the cross, ICGV 06099 × ICGV 93468, estimates of broad sense heritability varied from 27.66 % for sound mature kernel percentage to 91.00 % for pod yield per plot (Table 4.4). The genetic advance varied from 0.09 % for stearic acid content to 55.50 % for pod yield per plot, whereas genetic advance as per cent of mean (GAM) ranged from 1.24 % for days to flowering to 61.37 % for single plant yield. Higher broad sense heritability coupled with higher GAM observed for pod yield per plot, single plant yield and kernel yield per plot indicating the easy transferability of the characters to the succeeding generations. Though higher heritability (broad sense) recorded for kernel iron (64.24 %) and zinc (62.21 %) concentrations, their genetic advance and GAM values were low. Moderate broad sense heritability (44.59 %) coupled with low GAM (3.22 %) observed for oil content whereas for protein content higher broad sense heritability (70.40 %) and low GAM (3.34 %) were recorded.

Overall, higher broad sense heritability (>60 %) was noticed for pod yield per plot (91.00 %), single plant yield (86.45 %), kernel yield per plot (73.91 %), protein content (70.40 %), days to maturity (64.44 %), kernel iron concentration (64.24 %), days to emergence (62.36 %) and kernel zinc concentration (62.21 %) indicating that these traits can be easily transferred to the succeeding generations. These results are in support with the findings of Nath and Alam (2002), Parameswarappa *et al.* (2005), Jonah *et al.* (2012), Satish (2014), Satyanarayan *et al.* (2014), Vishnuvardhan *et al.* (2013) and Janila *et al.* (2014). Moderate heritability (30-60 %) was observed for palmitic acid content (48.73 %), shelling percentage (47.98 %), oil content (44.59 %), linoleic acid content (39.42 %), stearic acid (37.74 %), 100-kernel weight (37.00 %), oleic acid content (31.81 %) and days to flowering (30.60 %) making these traits to be transferred to their progeny with little difficulty. These results are in agreement with the findings of Noubissie *et al.* (2012) for oil content, Alam *et al.* (2013) for 100-kernel weight, but in contrast to the findings of Jonah *et al.* (2012) for days to emergence, Vishnuvardhan *et al.* (2013), John *et al.* (2014) and Satish (2014) for days to flowering, Parameswarappa *et al.* (2005) and Jonah *et al.*

(2012) for shelling percentage, Nath and Alam (2002), Alam *et al.* (2013) and Janila *et al.* (2014) for 100-kernel weight and Shukla and Rai (2014) for oil content. Low broad sense heritability was recorded for sound mature kernel percentage (27.66 %) indicating difficulty in transfer of this trait to the progeny. However, these results are in contrast to the findings of Satyanarayan *et al.* (2014) in groundnut.

Higher genetic advance was obtained for pod yield per plot (55.50), while moderate level of genetic advance was recorded for kernel yield per plot (27.58) and single plant yield (11.19) suggesting that genetic gain can be expected for these characters in the succeeding generations (Table 4.4). These results were in support with the findings of Jonah *et al.* (2012) and Satish (2014) but in contrast to those reported by Parameswarappa *et al.* (2005) and Satyanarayan *et al.* (2014) where a higher genetic advance value was obtained. The remaining characters *viz.*, stearic acid content (0.09), days to flowering (0.41), oil content (0.58), palmitic acid content (0.64), days to emergence (0.83), protein content (0.91), oleic acid content (0.98), linoleic acid content (1.13), 100-kernel weight (1.41), kernel zinc concentration (3.83), kernel iron concentration (4.40), sound mature kernel percentage (7.21) and shelling percentage (8.11) recorded low genetic advance suggesting low increase in performance value up on selection in the next generation progeny. Similar results are obtained earlier by Vishnuvardhan *et al.* (2013), John *et al.* (2014), John and Reddy (2014) and Satish (2014) for days to 75 % flowering; Parameswarappa *et al.* (2005) and Jonah *et al.* (2012) for shelling percentage; Vishnuvardhan *et al.* (2013) and Satyanarayan *et al.* (2014) for sound mature kernel percentage; Parameswarappa *et al.* (2005), Noubissie *et al.* (2012) and Shukla and Rai (2014) for oil content, but contradictory results were recorded by Nath and Alam (2002), Parameswarappa *et al.* (2005), Jonah *et al.* (2012) and Satish (2014) for 100-kernel weight.

Genetic advance as per cent of mean (GAM) for the studied traits ranged from 1.24 % for days to flowering to 61.37 % for single plant yield (Table 4.4). Higher GAM was recorded for single plant yield (61.37 %) followed by pod yield per plot (53.26 %) and kernel yield per plot (46.22 %) whereas moderate levels of GAM was observed for shelling percentage (13.98 %), and sound mature kernel percentage (10.39 %). The remaining traits *viz.*, days to 75 % flowering (1.24 %), oleic acid content (2.38 %), days to emergence (3.06 %), oil content (3.22 %), linoleic acid content (3.32 %), protein content (3.34 %), stearic acid content (4.46 %), 100-kernel weight (4.46 %), kernel zinc concentration (4.99 %), palmitic acid content (5.07 %), days to maturity

(5.22 %) and kernel iron concentration (9.72 %) recorded low GAM indicating low improvement of these traits in the succeeding generations upon selection.

4.1.2.2.3 Correlation studies

An understanding of the characters associated with kernel yield and kernel mineral concentration is desirable for effective selection in the segregating populations. Correlation studies provide information on the nature and magnitude of association between pairs of traits, which is useful for the breeder in carrying out multiple trait improvements. Hence an attempt was made in the present investigation to explore correlation of kernel iron and zinc concentrations with agronomic traits and kernel nutrient parameters (Table 4.5).

Kernel iron concentration showed highly significant positive association with kernel zinc concentration (0.302) (Fig 4.5). In an earlier study by Janila *et al.* (2014) involving 64 diverse advance breeding lines of groundnut also showed positive association between iron and zinc concentration. Similar results were recorded by Govindaraj *et al.* (2009) and Kanatti *et al.* (2014) in pearl millet, Ravikiran *et al.* (2014) and Susmitha and Selvi (2014) in sorghum, Ghanbari and Mameesh (1971), Velu *et al.* (2011b) and Badakhshan *et al.* (2013) in wheat and Bekele *et al.* (2013a) in rice. However, Ribeiro *et al.* (2013a) reported non-significant association between kernel iron and zinc concentrations in common bean. Kernel iron concentration had highly significant positive association with sound mature kernel percentage (0.132). Kernel zinc concentration exhibited significant positive association with days to flowering (0.149). No significant association was found between kernel iron and zinc concentration for pod yield. These results are in support with the findings of Govindaraj *et al.* (2009) in pearl millet, Nagesh *et al.* (2012) in rice and Janila *et al.* (2014) in groundnut. However, Kanatti *et al.* (2014) observed negative significant association between kernel iron concentration and kernel yield per plant in pearl millet whereas, Ravikiran *et al.* (2014) and Susmitha and Selvi (2014) reported significant positive association of grain iron and zinc concentrations with yield per plant in sorghum. Absence of association between pod yield and kernel iron and zinc concentrations in groundnut suggests the feasibility of kernel improving iron and zinc without jeopardizing the pod yield.

Pod yield per plot recorded highly significant positive association with kernel yield per plot (0.915), 100-kernel weight (0.340), single plant yield (0.323) and sound mature kernel percentage (0.224) and linoleic acid content (0.239) whereas negative significant association was

observed for pod yield per plot with shelling percentage (-0.278), oleic acid content (-0.245) and oil content (-0.127). Kernel yield per plot also showed significant positive correlation with 100-kernel weight (0.352), single plant yield (0.298), linoleic acid content (0.266), sound mature kernel percentage (0.192) and shelling percentage (0.097) and negative significant association with oleic acid content (-0.267) and oil content (-0.132). Significant positive correlation of kernel yield per plot with 100-kernel weight, sound mature kernel percentage and shelling percentage was also reported earlier by Mahalakshmi *et al.* (2005), Kotzamanidis *et al.* (2006), Patil *et al.* (2006), Korat *et al.* (2010), Channayya *et al.* (2011) and Parmer *et al.* (2013) in groundnut.

Oil content had positive significant association with days to maturity (0.286) and single plant yield (0.263) and negative significant association with palmitic acid content (-0.226), pod yield per plot (-0.127) and kernel yield per plot (-0.132). No significant association was observed between oil content and kernel iron and zinc concentrations in the present study. Mahalakshmi *et al.* (2005) and Korat *et al.* (2010) found non-significant association of pod or kernel yield with oil content. Protein content showed negative significant association with palmitic acid content (-0.245) and oleic acid content (-0.176) (Fig 4.6). Parmer *et al.* (2013) observed that protein content have positive significant correlation with pod yield per plant and negative significant association with days to maturity.

Considering the other important traits 100-kernel weight showed strong positive association with single plant yield (0.158), sound mature kernel percentage (0.245), pod yield per plot (0.340) and kernel yield per plot (0.352). These results are in accordance with the findings of Korat *et al.* (2010), Channayya *et al.* (2011) and Thirumala Rao *et al.* (2014) in groundnut. Oleic acid content recorded negative significant association with linoleic acid (-0.970), pod yield per plot (-0.245), oil content (-0.176) and kernel yield per plot (-0.267). Negative association between oleic and linoleic acid can be explained by fatty acid biosynthetic pathway wherein, enzymatic activity of delta-12-desaturase enzyme catalyses the addition of double bond onto oleic acid to produce linoleic acid. Linoleic acid content exhibited positive significant association with palmitic acid content (0.622). Thus in breeding high oleate lines with high oleic and low linoleic acid content, it is possible to even achieve reduced palmitic acid content of the oil which is desirable for consumer health. Among earliness traits, days to emergence showed significant positive association with days to flowering (0.241) and days to maturity (0.132) which is similar to the findings of Makinde and Ariyo (2013), Parmer *et al.* (2013) and

Thirumala Rao *et al.* (2014). Days to maturity showed positive significant association with 100-kernel weight (0.202), single plant yield (0.307), pod yield per plot (0.164), kernel yield per plot (0.158), sound mature kernel percentage (0.157) and oil content (0.286). Channayya *et al.* (2011) earlier reported similar association of days to maturity with sound mature kernel percentage. Because of positive association of days to maturity and several yield related parameters, breeding for early maturity may possibly have penalty on pod yield in groundnut.

4.1.3 Genotyping for Identification of Genomic Regions Associated with Kernel Iron and Zinc Concentrations

Genotyping of 184 F_2 individuals was carried out using 33 SSR markers which were found polymorphic between the parents understudy. However, out of 33 markers, 28 SSR markers were clearly amplified in the mapping population.

Genotyping was performed using GeneMapper ver. 4.0 software in which scoring was given for each marker on every individual entry based on the base pair size difference *viz.*, score 'A' for parent A type *i.e.,* ICGV 06099, 'B' for parent B type *i.e.,* ICGV 93468 and 'H' for heterozygous individuals. Like this, all the 28 markers were scored on all 184 individuals of the F_2 population.

4.1.4 QTL (single marker) Analysis

QTL analysis was performed using both genotypic and phenotypic data of all the individuals of the population. In general, linkage map data is required for QTL analysis for any given trait, but it is not possible to construct a linkage map using 28 markers. Therefore, we proceeded to Single Marker Analysis (SMA) as it doesn't require prior linkage map information.

Markers identified through marker-trait association studies using one single mapping population has to be validated in different genetic backgrounds to determine the consistency of results (Miklas, 2007). Markers showing greater association and tighter linkage with the trait of interest can be used for marker assisted selection. The objective of this experiment was to identify the linked markers associated with the kernel iron and zinc concentrations in $F_{2:3}$ mapping population of the cross ICGV 06099 × ICGV 93468 and to validate the identified linked markers in alternate $F_{2:3}$ mapping population of cross ICGV 06040 × ICGV 87141 for their efficiency in contributing phenotypic variation to the kernel iron and zinc concentration.

4.1.4.1 Single Marker Analysis and Validation

The information on genetic basis of accumulation of micro-nutrients in the kernels and mapping of the QTLs will provide the means to devise strategies for improving kernel micronutrient concentration through marker assisted selection. DNA markers which are closely linked with desired traits allow the selection of plants possessing those traits prior to trait expression. Hence the present study was carried out to identify the molecular markers associated with the kernel iron and zinc concentrations using single marker analysis.

The most basic way of determining whether an association exists between a molecular marker and a trait is to do single marker analysis. It will help in identifying significant association between markers and trait of interest on individual marker basis by providing information on the amount of phenotypic variation contributed by a particular marker towards the traits of interest. More the contribution of marker to phenotypic variation, stronger will be the association between marker and trait.

In the present study, twenty eight out of 33 polymorphic SSR markers which were clearly amplified in the mapping population were used for single marker analysis to identify markers that are closely associated with kernel iron and zinc concentrations in groundnut. Results revealed that, three markers *viz.,* IPAHM245, SEQ1B09 and SEQ9G05 showed significant association with a phenotypic variation of 2.19, 0.23 and 6.24 % (Table 4.6), respectively for kernel iron concentration, while three other markers *viz.,* GM2638, IPAHM245 and SEQ9G05 showed significant association with a phenotypic variation of 1.75, 2.25 and 6.01 %, respectively for kernel zinc concentration. Among these, two markers *viz.,* IPAHM245 and SEQ9G05 were contributing for both kernel iron and zinc concentration suggesting the presence of QTLs governing kernel iron and zinc concentrations on the same location of the chromosome. This indicated that kernel iron and zinc concentrations were co-segregating with each other. These results are in agreement with the findings of Anuradha *et al.,* (2012) in rice, Shi *et al.,* (2008) and Tiwari *et al.,* (2009) in wheat, Jin *et al.,* (2013) in maize, Cichy *et al.,* (2009) and Blair *et al.,* (2009) in common bean and Klein and Grusak (2009) in clover. Therefore, these two markers can be used for further studies to identify the exact genomic regions (QTLs) associated with kernel iron and zinc concentrations in groundnut.

The correlation studies on kernel iron and zinc concentrations in $F_{2:3}$ population also revealed the existence of highly significant positive association between these two micronutrient

concentrations (Velu *et al.,* 2011b in wheat; Govindaraj *et al.,* 2009 and Kanatti *et al.,* 2014 in pearl millet and Ravikiran *et al.,* 2014 and Susmita and Selvi, 2014 in sorghum) as that observed during single marker analysis. Thus identifying genomic regions (QTLs) associated with either of the micronutrient concentration may be useful in simultaneous improvement of both the micronutrients in groundnut kernels.

Validation of putative markers is required to confirm the reproducibility of results by selected markers for marker aided breeding program (Miklas, 2007). So the markers which were found significant on $F_{2:3}$ population of the cross ICGV 06099 × ICGV 93468 were validated on alternate $F_{2:3}$ population derived from the cross ICGV 06040 × ICGV 87141. The kernel iron and zinc concentrations of the entries which scored similar to the entries of genotyping population having high iron and zinc parent type were biochemically analysed. The results revealed that most of the entries identified by the above mentioned markers were having higher iron and zinc concentrations in their kernels (Table 4.7) suggesting that all the four markers which were found significant in the genotyping population were actually linked to the traits of interest. Hence these markers can be efficiently utilised in marker aided breeding programmes aimed at the improvement of these two micronutrient concentrations in groundnut kernels.

Table 4.6. Results of Single Marker Analysis (SMA) for kernel iron and zinc concentrations using three significant markers each in $F_{2:3}$ mapping population of the cross ICGV 06099 × ICGV 93468 in groundnut

Marker No.	Marker	Probability	R^2 (%)
For kernel iron concentration			
11	IPAHM245	0.009 **	2.19
21	SEQ1B09	0.049 *	0.23
23	SEQ9G05	0.002 **	6.24
For kernel zinc concentration			
10	GM2638	0.038 *	1.75
11	IPAHM245	0.012 *	2.25
23	SEQ9G05	0.001 **	6.01

Where,

R^2 (%) = Phenotypic variation explained (%);

4.1.5 Principal Component Analysis (PCA)

Based on the phenotypic data of mapping population Principal Component Analysis (PCA) was performed for important traits *viz*., kernel iron and zinc concentrations, pod yield per plot, kernel yield per plot, oil content, protein content, oleic acid content and linoleic acid

content to understand the variation contributed by these traits (Fig 4.7) and to find out the association between them. Results revealed that first two principal components contributed to 49 % of total variance (Rao, 1964). Among which PC1 contributed 28.8 % and PC2 contributed 20.2 % of phenotypic variation (Kumar *et al.*, 2010b). The kernel iron and zinc concentrations formed one group (G_1), oil and protein content formed second group (G_2) and pod yield per plot and seed yield per plot formed another group (G_3). However oleic acid and linoleic acid contents were located away from all the observations and were 180° apart from each other.

The association between various traits using PCA is determined using the degree of angle between two variables. If the angle between two variables is less than 90° then they are positively correlated, if the angle is equal to 90° there is no correlation between variables and if the angle is more than 90° then the two variables in question are negatively correlated (Rad *et al.*, 2013). In the present study, positive correlation was observed between kernel iron and zinc concentrations, pod yield per plot, kernel yield per plot, and oil and protein contents. But a strong negative relation was observed between oleic and linoleic acid contents since the degree of angle was nearly 180°. This can be attributed to their biochemical pathway of conversion of oleic to linoleic acid. However no association of kernel iron and zinc concentrations with yield was observed as the angle between these traits was nearly 90°.

The present study was based on only two mapping populations (one population is for the genotyping and the other population is for validation) evaluated for only one season. Thus evaluating populations of more number of crosses at multiple locations may be done to test the validity and reproducibility of the present findings.

4.2 GENERATION MEAN ANALYSIS

Generation mean analysis (Mather and Jinks, 1982) is a simple but useful technique for estimating gene effects for polygenic traits. It provides information on the relative importance of average effects of the genes (additive effects), dominance deviations and effects due to non-allelic genetic interactions in determining genotypic values of the individuals and consequently, mean genotypic values of families and generations. In the present experiment generation mean analysis was conducted on six generations of two crosses *viz.* ICGV 06040 × ICGV 87141 and ICGV 06099 × ICGV 93468. The parents in each cross had contrasting kernel iron and zinc concentrations (Table 3.3). The results obtained are discussed under the following headings:

4.2.1 Analysis of variance

4.2.2 Mean performance

4.2.3 Genetic parameters

4.2.4 Heterosis and inbreeding depression

4.2.5 Gene effects using generation mean analysis

4.2.6 Correlation studies

4.2.1 Analysis of Variance

Analysis of variance was performed for nine characters as per the design of experiment for comparison of crosses as well as generations of each cross according to Panse and Sukhatme (1985). The mean squares from ANOVA, presented in Table 4.8 revealed significant differences among the crosses for five traits *viz.*, days to maturity, 100-kernel weight, pod yield per plant, kernel iron and kernel zinc concentrations which indicated that considerable amount of variability was present between the crosses for these traits. Likewise the mean sum of squares among the progenies (generations) for the nine characters studied in both the crosses revealed the existence of significant differences among the six generations for seven traits *viz.,* days to emergence, days to maturity, 100-kernel weight, shelling percentage, pod yield per plant, kernel iron and zinc concentrations in the first cross *i.e.* ICGV 06040 × ICGV 87141 and for six traits *viz.*, days to emergence, days to maturity, 100-kernel weight, pod yield per plant, kernel iron and zinc concentrations in the second cross *i.e.* ICGV 06099 × ICGV 93468. So, further genetic analyses of generation means was carried out for seven traits in the cross ICGV 06040 × ICGV 87141 and for six traits in the cross ICGV 06099 × ICGV 93468.

4.2.2 Mean Performance

The mean performance of six generations (P_1, P_2, F_1, F_2, B_1 and B_2) of two crosses for nine different characters including kernel iron and zinc concentrations was furnished in Table 4.9. The female and male parents were indicated as P_1 and P_2, respectively. The results obtained are discussed trait-wise for each cross below:

4.2.2.1 Days to Emergence

In the cross ICGV 06040 × ICGV 87141, among all generations, the female parent (P_1) had taken less number of days to emerge (11 days) compared to the male parent (P_2) which

recorded 15 days to emergence. The hybrid (F_1) had taken 12 days to emerge which was nearer to the mid parental value. Whereas, F_2, B_1 and B_2 generations recorded mean values of 14, 15 and 16 days, respectively to emerge. P_1 was statistically at par with F_1 and F_2 whereas P_2, F_2, B_1 and B_2 were on par with each other.

Among all the generations in the cross ICGV 06099 × ICGV 93468, P_1, P_2, F_1 and F_2 generations took 11 days to emerge whereas B_1 and B_2 generations recorded 14 and 12 days, respectively to emerge. All the generations, except B_1 were statistically at par with each other.

4.2.2.2 Days to Flowering

In the cross ICGV 06040 × ICGV 87141, the parent P_1 took 48 days to flower which was earlier compared to P_2 which recorded 50 days to flower. The hybrid (F_1) and B_2 generations recorded 49 and 48 days to flower, respectively whereas F_2 and B_1 generations have recorded 50 and 49 days, respectively to flower. As all the generations were at par with each other significant difference was not observed for this trait.

In the cross ICGV 06099 × ICGV 93468, both the parents, F_1, F_2, B_1 and B_2 recorded nearly same mean number of days (~47 days) to flower which indicated that there was no significant variation for this trait among the generations.

4.2.2.3 Days to Maturity

In the cross ICGV 06040 × ICGV 87141, P_1 and P_2 took 159 and 142 days to mature, respectively. Among generations B_2 matured in 156 days, B_1 and F_2 took 158 days to mature and F_1 required 159 days to attain maturity (Fig 4.8). All the generations with the exception of P_2 were statistically at par with each other.

In the cross ICGV 06099 × ICGV 93468, P_2 took less number of days (133 days) to mature followed by B_2 (137 days), F_1 (148 days), B_1 (156 days), F_2 (156 days) and P_1 (159 days) (Fig 4.8). P_2 and B_2 generations were observed to be on par with each other and were significantly different from rest of the generations which were at par among themselves.

4.2.2.4 100-kernel Weight (g)

In the cross ICGV 06040 × ICGV 87141, among generations, B_1 recorded highest 100-kernel weight (44.54 g) followed by P_1 (44.53 g), F_1 (43.94 g), F_2 (43.00 g), B_2 (36.49 g) and P_2 (34.82 g) generations (Fig 4.8). B_2 and P_2 generations were at par with each other and were significantly different from rest of the generations.

In the cross ICGV 06099 × ICGV 93468, the F_1 generation recorded highest 100-kernel weight of 46.37 g followed by P_1 (46.32 g), B_1 (45.03 g), P_2 (44.78 g), B_2 (44.33 g), and F_2 generations (36.43 g) (Fig 4.8). With the exception of F_2, no significant difference was observed among the other generations.

4.2.2.5 Shelling Percentage

In the cross ICGV 06040 × ICGV 87141, highest shelling percentage (75.4 %) was observed in F_1 generation followed by P_1 (72.54 %), F_2 (66.07 %), B_2 (65.51 %), B_1 (61.05 %) and P_2 (57.74 %) generations. P_1 and F_1 were at par with each other and were significantly different from rest of the generations, whereas F_2, B_1 and B_2 were at par with each other and P_2 generation was found significantly different from all the remaining generations.

In the cross ICGV 06099 × ICGV 93468, among all the generations F_2 recorded highest shelling percentage (65.32 %) followed by B_2 (62.77 %), B_1 (62.29), P_2 (61.26 %), P_1 (60.17 %) and F_1 (58.98 %) generation. There was no significant variation for this trait among the generations.

4.2.2.6 Sound Mature Kernel Percentage (%)

In the cross ICGV 06040 × ICGV 87141, among the generations, B_2 had highest sound mature kernel percentage (68.88 %) followed by F_2 (67.50 %), P_1 (64.70 %), F_1 (60.85 %), P_2 (55.24 %) and B_1 (54.38 %) generations.

Among all the generations in the cross ICGV 06099 × ICGV 93468, highest sound mature kernel percentage (71.79 %) was obtained in F_2 generation, followed by F_1 (67.88 %), B_1 (67.34 %), P_1 (65.18 %), B_2 (58.28 %) and P_2 (57.86 %) generations.

4.2.2.7 Pod Yield per Plant (g plant^{-1})

In the cross ICGV 06040 × ICGV 87141, significant variation was observed among the parents for pod yield per plant. The mean pod yield per plant for P_2 was 31.09 g in comparison to 24.82 g for P_1. Among generations, B_1 recorded highest pod yield per plant (39.99 g) followed

by B_2 (34.12 g) whereas, F_1 (31.67 g) and F_2 (31.40 g) generations recorded almost same pod yield per plant (Fig 4.9). P_1 was significantly different from B_2 and B_1 which were at par with each other and P_1 was at par with F_1, F_2 and P_2 for this trait.

In the cross ICGV 06099 × ICGV 93468, significant variation was observed among the parents with P_1 (30.71 g) recording higher yield than P_2 (26.77 g). Among the generations, highest pod yield per plant was recorded by F_1 (37.51 g), followed by B_2 (35.90 g), B_1 (33.50 g) and F_2 (30.82 g) generations (Fig 4.9). P_1, P_2 and F_2 were statistically at par with each other and were significantly different from F_1.

4.2.2.8 Kernel Iron Concentration (mg kg^{-1})

In the cross ICGV 06040 × ICGV 87141, this trait showed significant difference between the parents with P_1 recording higher kernel iron concentration (33.32 mg kg^{-1}) compared to the parent P_2 (25.54 mg kg^{-1}). Among the generations, F_1 (28.49 mg kg^{-1}) and F_2 (28.38 mg kg^{-1}) recorded almost similar concentration, whereas B_2 recorded higher concentration (31.49 mg kg^{-1}) than B_1 (29.42 mg kg^{-1}) (Fig 4.9). F_1, F_2 and B_1 were statistically at par with each other and were significantly different from P_1 whereas P_2 was significantly different from P_1 and B_2.

In the cross ICGV 06099 × ICGV 93468, significant difference was observed for kernel iron concentration between the parents with P_1 (25.49 mg kg^{-1}) recording higher concentration than P_2 (20.83 mg kg^{-1}). Among the generations, B_1 had higher concentration (26.25 mg kg^{-1}) followed by F_2 (25.19 mg kg^{-1}), B_2 (24.07 mg kg^{-1}) and F_1 (21.95 mg kg^{-1}) (Fig 4.9). P_1 was statistically on par with F_2, B_1 and B_2 and was significantly different from P_2 and F_1.

4.2.2.9 Kernel Zinc Concentration (mg kg^{-1})

In the cross ICGV 06040 × ICGV 87141, kernel zinc concentration varied significantly between the parents with P_1 (50.91 mg kg^{-1}) recording more concentration than P_2 (36.05 mg kg^{-1}). Among the generations, B_1 (42.46 mg kg^{-1}) recorded higher concentration than B_2 (41.98 mg kg^{-1}), whereas F_1 (40.27 mg kg^{-1}) and F_2 (39.80 mg kg^{-1}) generations had almost same concentration (Fig 4.9). F_1, F_2, B_1 and B_2 were statistically at par with each other and were significantly different from P_1 and P_2 which themselves were found to be significant.

In the cross ICGV 06099 × ICGV 93468, the trait showed significant difference between the parental generations with P_1 (36.58 mg kg^{-1}) having more concentration than P_2 (30.39 mg kg^{-1}). Among the generations, B_1 (37.27 mg kg^{-1}) recorded higher concentration than B_2 (32.91 mg kg^{-1}) and F_2 (35.80 mg kg^{-1}) recorded higher concentration than F_1 (32.01 mg kg^{-1}) (Fig 4.9).

The generations P_2, F_1, F_2 and B_2 were statistically at par with each other but were significantly different from P_1 and B_1 generations.

4.2.3 Genetic Parameters

The various genetic parameters *viz.*, phenotypic coefficient of variation (PCV %), genotypic coefficient of variation (GCV %), heritability (broad and narrow sense), genetic advance, genetic advance as per cent of mean and degree of dominance for nine characters (Table 4.10) were computed and the results are discussed below:

4.2.3.1 Phenotypic and Genotypic Coefficient of Variation

Genetic variability is an essential prerequisite for any crop improvement programme for developing high yielding varieties. The improvement in any trait requires a thorough knowledge of the existing genetic variation among cultivars which can be obtained through the estimation of different genetic parameters like genotypic and phenotypic coefficients of variability, heritability and genetic advance (Younis *et al*., 2008). The observed variability is a combined estimate of genetic and environmental causes, of which only the former one is heritable (Noubissié *et al.*, 2012). In genetic studies, characters with high genotypic coefficient of variation indicate the potential for an effective selection (Sadiq *et al.*, 1986).

In the present study, PCV for the cross ICGV 06040 × ICGV 87141 varied from 1.39 % for days to maturity to 52.23 % for days to emergence (Table 4.10) and genotypic coefficient of variation (GCV) ranged from 1.01 % for days to flowering to 32.52 % for days to emergence. In the cross ICGV 06099 × ICGV 93468, PCV ranged from 1.86 % for days to flowering to 16.70 % for pod yield per plant and GCV ranged from 0.86 % for days to flowering to 13.90 % for pod yield per plant. In general, the PCV was found to be higher than GCV in both the crosses for all the traits suggesting profound influence of environment on the expression of the traits.

For kernel iron and zinc concentrations, PCV values were moderately higher than GCV estimates in both the crosses suggesting moderate influence of environment on the expression of these traits. In both the crosses the difference between PCV and GCV values was higher for kernel iron concentration than that for kernel zinc concentration suggesting more influence of environment on kernel iron accumulation than that of kernel zinc. However, Ravikiran *et al.* (2014) reported more influence of environment on kernel zinc concentration in sorghum. The

difference between PCV and GCV was found to be higher for days to emergence in the cross ICGV 06040 × ICGV 87141, indicating considerable influence of environment on the trait. For the cross ICGV 06099 × ICGV 93468, though the difference between PCV and GCV was less, higher estimates of PCV indicated the existence of considerable environmental influence on the expression of this trait. Similar kinds of results were obtained earlier in groundnut by Jonah *et al.* (2012), Rai *et al.* (2014) and Satyanarayan *et al.* (2014).

For days to flowering and days to maturity, higher values of PCV over GCV was observed in both the crosses, which is in agreement with the reports of Zaman *et al.* (2011), Vishnuvardhan *et al.* (2013), Rai *et al.* (2014) and Satyanarayan *et al.* (2014) in groundnut. .

For 100-kernel weight, moderate PCV and low GCV estimates were recorded in both the crosses. Similar findings with respect to PCV were reported by Nath and Alam (2002), Parameswarappa *et al.* (2005), Jonah *et al.* (2012) and Satish (2014) in groundnut. However, in their studies the difference between PCV and GCV was low while it was moderate in the present study. For shelling percentage, moderate PCV and GCV values were observed in both the crosses, however the difference between PCV and GCV was low for the cross ICGV 06040 × ICGV 87141 and moderate for the cross ICGV 06099 × ICGV 93468. Similar findings with respect to the cross ICGV 06040 × ICGV 87141 were reported by Parameswarappa *et al.* (2005) and Jonah *et al.* (2012) in groundnut. For sound mature kernel percentage also, the PCV was higher than GCV suggesting profound influence of environment on the expression of this trait. Moderate PCV and low GCV estimates were observed which are in agreement with the findings of Vishnuvardhan *et al.* (2013). But Satyanarayan *et al.* (2014) reported less difference between PCV and GCV for this trait. For pod yield per plant, in both the crosses moderate estimates of PCV and GCV were obtained and the difference between PCV and GCV was moderate with higher value of phenotypic coefficient of variation suggesting a notable influence of environment on the expression of the this character. Similar kind of results were reported earlier by Parameswarappa *et al.* (2005), Jonah *et al.* (2012) and Satyanarayan *et al.* (2014).

4.2.3.2 Heritability (Broad Sense and Narrow Sense), Genetic Advance and Degree of Dominance

In the cross, ICGV 06040 × ICGV 87141, lowest and highest estimates of heritability (broad sense and narrow sense) were recorded for sound mature kernel percentage (20.18 % and 0.02 %) and days to maturity (88.88 % and 2.87 %), respectively. Genetic advance ranged from

0.51 for days to flowering to 12.71 for days to maturity, while genetic advance as per cent of mean (GAM) varied from 1.04 % for days to flowering to 23.01 % for pod yield per plant. Shelling percentage and kernel zinc concentration recorded lowest (0.45) and highest (3.67) degree of dominance, respectively in this cross (Table 4.10). High heritability coupled with moderate GAM was observed for shelling percentage, kernel iron and zinc concentrations and 100-kernel weight indicating easy transferability and genetic improvement of these traits is possible in the succeeding generations. Pod yield per plant recorded higher GAM (23.01 %) with moderate broad sense heritability (53.96 %) and kernel zinc concentration exhibited higher degree of dominance (3.67) along with higher broad sense heritability (71.15 %). Higher broad sense heritability was also reported by Janila *et al.* (2014) in groundnut suggesting that this trait can be easily transferred to succeeding generations upon selecting parental lines having higher zinc concentration in their kernels.

Low narrow sense heritability was recorded for all the traits indicating less contribution of additive gene action in governing these traits. In the cross ICGV 06040 × ICGV 87141, days to maturity (88.88 %), shelling percentage (82.56 %), kernel iron (72.43 %) and zinc (71.15 %) concentrations and 100-kernel weight (61.36 %) recorded higher heritability (broad sense), which indicates less influence of environment and easy transferability of above mentioned characters to the progeny (Table 4.10). These results were supported by Nath and Alam (2002), Parameswarappa *et al.* (2005), Jonah *et al.* (2012), Vishnuvardhan *et al.* (2013), Janila *et al.* (2014) and Satyanarayan *et al.* (2014) for the above traits. Moderate heritability (broad sense) was observed for pod yield per plant (53.96 %) and days to emergence (49.79 %) indicating a little difficulty in transfer of these traits to the succeeding progeny. Similar findings were also made by Jonah *et al.* (2012) and Rai *et al.* (2014) for days to emergence in groundnut but these results are in contrast with the findings of Jonah *et al.* (2012), Alam *et al.* (2013) Satish (2014) and Satyanarayan *et al.* (2014) for pod yield per plant in groundnut. Low heritability (broad sense) was recorded for days to flowering (24.94 %) and sound mature kernel percentage (20.18 %) indicating difficulty in improvement of these traits up on selection. In contrast high heritability (broad sense) for these two traits was reported by Vishnuvardhan *et al.* (2013) John and Reddy (2014), John *et al.* (2014), Satish (2014) and Satyanarayan *et al.* (2014) in groundnut.

Moderate genetic advance values were obtained for days to maturity (12.71) and shelling percentage (12.57) so that genetic gains can be expected for these characters in the succeeding

generations (Table 4.10). But these results were in contrast with the findings of Parameswarappa *et al.* (2005), Zaman *et al.* (2011), Jonah *et al.* (2012), Rai *et al.* (2014) and Satyanarayan *et al.* (2014). The remaining characters *viz.*, days to flowering (0.51), day to emergence (2.52), sound mature kernel percentage (3.66), kernel iron concentration (4.39), 100-kernel weight (6.43), pod yield per plant (7.45) and kernel zinc concentration (8.03) recorded low estimates of genetic advance suggesting low efficiency of selection for these traits in the next generation progeny. Similar results were also recorded earlier by Noubissie *et al.* (2012), Vishnuvardhan *et al.* (2013), Satyanarayan *et al.* (2014) and Shukla and Rai (2014). But these results are in contrast to the findings of Nath and Alam (2002), Parameswarappa *et al.* (2005), Jonah *et al.* (2012) and Satish (2014) for 100-kernel weight and pod yield per plant in groundnut.

GAM was high for pod yield per plant (23.01 %) and thus genetic gain can be expected through selection in the later generations. Moderate values of GAM was recorded for kernel zinc concentration (19.21 %), shelling percentage (18.95 %), days to emergence (17.86 %), 100-kernel weight (15.59 %) and kernel iron concentration (14.93 %) suggesting moderate transferability of these characters to the progeny (Table 4.10). The remaining traits including days to flowering (1.04 %), sound mature kernel percentage (5.89 %) and days to maturity (8.17 %) had low GAM indicating that selection might be ineffective in getting genetic gain in succeeding generations for these traits.

Degree of dominance indicates the magnitude of dominance deviation relative to the additive variance. Higher the degree of dominance value more will be the trait expressibility of the hybrid progeny. In the present cross, degree of dominance value was observed to be more than unity for most of the characters (Table 4.10) indicating over-dominance expression of these traits except for shelling percentage (0.45) and 100-kernel weight (0.86).

In the cross ICGV 06099 × ICGV 93468, the highest and lowest estimates of heritability (broad sense) were recorded for days to maturity (84.04 %) and sound mature kernel percentage (25.62 %), respectively. Narrow sense heritability ranged from 0.19 % for kernel zinc concentration to 24.68 % for days to maturity and genetic advance varied from 0.58 for days to flowering to 19.06 for days to maturity whereas GAM ranged from 1.22 % for days to flowering to 23.85 % for pod yield per plant. Degree of dominance ranged from 0.49 for 100-kernel weight to 5.19 for shelling percentage (Table 4.10). Higher heritability (broad sense) coupled with moderate genetic advance as per cent of mean was observed for days to maturity, days to

emergence and pod yield per plant indicating improvement of these traits in the succeeding progeny. Whereas days to maturity recorded higher genetic advance and shelling percentage showed higher degree of dominance along with higher heritability.

Days to maturity (84.04 %), days to emergence (74.83 %) and pod yield per plant (69.36 %) recorded high heritability (broad sense) which indicates easy transfer of above mentioned characters to the progeny (Table 4.10). Similar results with respect to days to emergence and pod yield per plant were reported by Jonah *et al.* (2012), Alam *et al.* (2013), Vishnuvardhan *et al.* (2013), Satish (2014) and Satyanarayan *et al.* (2014) in groundnut. But for days to emergence these results are in contrast with the findings of Rai *et al.* (2014) and Satyanarayan *et al.* (2014) in groundnut. Moderate heritability was observed for 100-kernel weight (51.52 %), kernel iron (49.85 %) and zinc (45.12 %) concentrations and days to flowering (36.68 %) which was in contrast with the findings of Parameswarappa *et al.* (2005), Jonah *et al.* (2012), Vishnuvardhan *et al.* (2013), Janila *et al.* (2014), John *et al.* (2014) and Satish (2014) in groundnut. Low broad sense heritability was recorded for shelling percentage (29.16%) and sound mature kernel percentage (25.62 %) making these traits difficult to improve in succeeding generations due to less transferability to the progeny. These results are similar with the findings of Vishnuvardhan *et al.* (2013) but are in contrast with the findings of Parameswarappa *et al.* (2005), Jonah *et al.* (2012) and Satyanarayan *et al.* (2014) in groundnut.

Maximum but moderate genetic advance estimate was obtained for days to maturity (19.06) so that genetic gain can be expected for this character in the succeeding generations of this cross (Table 4.10). But these results were in contrast with the findings of Parameswarappa *et al.* (2005), Zaman *et al.* (2011), Vishnuvardhan *et al.* (2013), Rai *et al.* (2014) and Satyanarayan *et al.* (2014) in groundnut. The remaining characters *viz.*, days to flowering (0.58), day to emergence (1.76), sound mature kernel percentage (2.83), kernel iron concentration (2.98), shelling percentage (3.82), kernel zinc concentration (4.05), 100-kernel weight (4.83) and pod yield per plant (8.08) recorded low genetic advance estimates suggesting low increase in performance in the next generation progeny upon selection. These results are supported by the earlier findings of Parameswarappa *et al.* (2005), Noubissie *et al.* (2012), Vishnuvardhan *et al.* (2013), Satyanarayan *et al.* (2014) and Shukla and Rai, (2014). But contradictory results were obtained by Nath and Alam (2002), Parameswarappa *et al.* (2005), Jonah *et al.* (2012) and Satish (2014) for 100-kernel weight and pod yield per plant in groundnut.

GAM was moderate for pod yield per plant (23.85 %) followed by days to emergence (14.94 %), days to maturity (12.84 %), kernel iron concentration (12.34 %), kernel zinc concentration (11.86 %), and 100-kernel weight (10.91 %) (Table 4.10), suggesting moderate improvement of these traits up on selection. Low GAM estimates were recorded for shelling percentage (6.05 %), sound mature kernel percentage (4.66 %) and days to flowering (1.22%) indicating low transferability of the characters and difficulties in selection.

Degree of dominance was more than unity for most of the studied characters indicating over dominance expression of these traits, except 100-kernel weight (0.49), kernel iron (0.78) and zinc (0.99) concentrations which had a value of less than unity suggesting partial dominance expression (Table 4.10). However, in self-pollinated crops like groundnut, over dominance expression of all the traits may not be supportive, thus further study is required to draw conclusions on the degree of dominance of various traits using more number of crosses.

4.2.4 Heterosis and Inbreeding Depression

The superiority of F_1 hybrid over the parents is termed as heterosis. Heterosis serves as a basic tool for improved production of crops in the form of F_1 hybrids. The phenomenon of heterosis of F_1 hybrids can reflect their own specific combining ability (SCA) and the general combining ability (GCA) of parental lines. The estimates of heterosis and inbreeding depression provide information about the nature of gene action involved in the expression of yield and related traits, which helps to formulate breeding programmes for the improvement of target traits.

In the cross ICGV 06040 × ICGV 87141, average heterosis ranged from -12.20 % for days to emergence to 44.44 % for pod yield per plant. Significant positive average heterosis was observed for days to maturity (5.64), shelling percentage (15.92) and pod yield per plant (44.44) revealing that the F_1 of this cross out performed mid-parental value significantly for the above mentioned traits (Table 4.11). However, for some traits such as days to maturity, days to flowering etc., negative significant heterosis is more preferred over positive significant heterosis. Residual heterosis over mid-parent varied from -7.90 % for kernel zinc concentration to 13.90 % for sound mature kernel percentage (Table 4.11). Positive significant residual heterosis over mid-parent was observed for days to maturity (5.21) which was not desirable as earliness was advantageous in most of the breeding experiments (Table 4.11).

Heterobeltiosis in the cross ICGV 06040 × ICGV 87141, varied from -18.57 % for kernel zinc concentration to 32.48 % for shelling percentage. Significant negative heterobeltiosis was observed for kernel iron (-14.14 %) and zinc (-18.57 %) concentrations indicating poor performance of hybrid over better parent, whereas pod yield per plant (27.50 %) and days to maturity (11.97 %) recorded significant positive heterobeltiosis suggesting better performance of F_1 over better parent for both these traits. However, positive significant heterobeltiosis was not desirable with respect to days to maturity as early maturity is an important breeding objective in groundnut. Residual heterobeltiosis over better parent ranged from -21.53 % for kernel zinc concentration to 26.59 % for days to emergence. Significant positive residual heterobeltiosis over better parent was observed for days to flowering (3.94 %) and days to maturity (11.50 %) which was not desirable as F_2 plants were taking more number of days than better parent to flower and to mature, respectively. Similar to heterobeltiosis values, significant negative residual heterosis over better parent was observed for kernel iron (-14.97) and zinc (-21.53) concentrations suggesting poor F_2 progeny performance over better parent (Table 4.11).

In the cross ICGV 06099 × ICGV 93468, average heterosis ranged from -9.88 % for shelling percentage to 32.63 % for pod yield per plant. Significant positive average heterosis was observed for pod yield per plant (32.63 %) suggesting superior performance of F_1 over mid-parent value for the trait concerned. Residual heterosis over mid-parent value varied from -20.02 % for 100-kernel weight to 20.47 % for sound mature kernel percentage. Significant positive residual heterosis was observed for days to maturity (6.90 %) which indicates delayed maturity of F_2 than the mid-parent value. Similarly sound mature kernel percentage (20.47 %) also recorded significant positive residual heterosis suggesting more amount of sound mature kernels in F_2 than the mid-parent value. Significant negative residual heterosis was observed for 100-kernel weight (-20.02 %) indicating smaller kernel size of F_2 individuals than mid-parent value.

Heterobeltiosis in the cross ICGV 06099 × ICGV 93468, varied from -16.75% for kernel zinc concentration to 14.86 % for pod yield per plant. Positive significant heterobeltiosis was observed for days to maturity (11.02 %), though negative significant heterosis was desirable for this trait (Table 4.11). Negative significant heterobeltiosis was observed for kernel zinc concentration (-16.75 %) indicating poor performance of F_1 individuals over better parent. Residual heterobeltiosis over better parent varied from -18.64 % for 100-kernel weight to 17.34 % for days to maturity. Significant positive residual heterobelteosis was observed for days to

maturity (17.34 %) and sound mature kernel percentage (13.31 %) suggesting delayed maturity and more number of good kernels of F_2 individuals than better parent in this cross (Table 4.11). However, 100-kernel weight recorded negative significant residual heterobeltiosis (-18.64 %) suggesting poor performance of F_2 individuals over better parent for this trait.

Groundnut being a self-pollinated crop, the role of inbreeding depression is very less. However, an attempt was made to understand the role of inbreeding depression in the present study. In the cross ICGV 06040 × ICGV 87141, inbreeding depression was ranged from -19.56 % for days to emergence to 24.51% for pod yield per plant (Table 4.11). Higher value of inbreeding depression was observed for pod yield per plant (24.51 %) and shelling percentage (12.26 %) which is not desirable since there was a reduction in yield and shelling percentage upon selfing. This can be attributed to the presence of considerable amount of heterosis in the hybrid progeny for the above mentioned traits which was removed up on selfing.

In the cross ICGV 06099 × ICGV 93468, inbreeding depression was ranged from -12.32 % for kernel iron concentration to 23.35 % for pod yield per plant. Higher quantum of inbreeding depression was observed for pod yield per plant (23.35 %) and 100-kernel weight (21.42 %) suggesting decrease in pod yield and kernel size upon selfing (Table 4.11). These results are in accordance with the findings of Gor *et al.* (2012) for 100-kernel weight and John *et al.* (2014) for pod yield per plant. Low and negative inbreeding depression was observed for days to maturity indicating earliness of the plants upon selfing which is desirable from a breeder's perspective.

Significant heterosis (either average heterosis or heterobeltiosis) coupled with higher inbreeding depression signifies non-additive gene action, whereas non-significant heterosis coupled with lower value of inbreeding depression implies additive gene action. For pod yield per plant in both the crosses and shelling percentage in the cross ICGV 06040 × ICGV 87141, the first situation was observed, which reveals the importance of non-additive gene action in their inheritance. But days to maturity showed significant positive heterosis along with lower value of inbreeding depression. However, it is highly unrealistic to come to a conclusion based on few parameters with only two crosses. Hence further study is required for making valid conclusions.

In all the cases of heterosis, days to maturity showed positive significance which is not desirable as it prolongs the duration of the crop and these results are in contrast with the findings of Gor *et al.* (2012), Waghmode *et al.* (2013) and John *et al.* (2014) who reported negative

significant heterosis for days to maturity in groundnut. Positive significant heterosis for pod yield per plant observed in the present study was also supported by the findings of Dwivedi *et al.* (1989), Vyas *et al.* (2001), Gor *et al.* (2012), Verma and Ranwah (2012), John *et al.* (2014) and Prabhu *et al.* (2014) in groundnut.

Negative significant heterosis estimates recorded by Gor *et al.* (2012) for 100-kernel weight and Venkateswarlu *et al.* (2007b), Gor *et al.* (2012) and Prabhu *et al.* (2014) for shelling percentage are in support with the findings in the present study suggesting that there would be reduction in kernel weight along with reduction in shelling percentage upon selfing.

In the present study, a negative significant heterobeltiosis was obtained for kernel iron and zinc concentrations which is in contrast with the findings of Aruselvi *et al.* (2006), Velu *et al*. (2011a) and Govindaraj *et al*. (2013) in pearl millet and Kumar *et al*. (2013) in sorghum where a significant positive heterosis and heterobelteosis were reported for these traits.

4.2.5 Gene Effects Using Generation Mean Analysis

To begin a breeding program aiming to obtain cultivars with improved yield, nutritional quality and other target traits, it is important to have information on the genetic control of the traits targeted for improvement (Silva *et al.*, 2013) as it has got direct bearing upon the choice of breeding procedures to be followed. Many traits of economic importance in groundnut are quantitatively inherited. The exploitation of genetic variability of these traits through hybridization and selection is the primary focus of most of the groundnut improvement programmes (Shobha *et al.*, 2010). In addition to additive and dominance variation, it has been suggested that epistasis may also be involved in the inheritance of many quantitative characters in groundnut (Hammons, 1973 and Wynne, 1976). The information on non-allelic interaction such as additive × additive epistatic variation is potentially useful, as it can be fixed in homozygous cultivars. But such information for quantitative traits in groundnut is very limited.

In the present study, the generation mean analysis was employed to separate the genetic variance into additive, dominance and epistatic components, which helps in formulating an effective and sound breeding programme. There is no published report till date on the detailed genetic dissection on the kinds and magnitude of epistatic gene action controlling the kernel iron and zinc concentrations in groundnut. Therefore, the present study was aimed at understanding the genetic components influencing kernel micronutrient concentrations through generation mean analysis in groundnut.

The mean data obtained from six generations of the two cross combinations *viz.,* ICGV 06040 × ICGV 87141 and ICGV 06099 × ICGV 93468 for seven characters in the first cross and six characters in the second cross including kernel iron and zinc concentrations were subjected to generation mean analyses using scaling tests to test the fitness of additive-dominance model and Hayman's six parameter model to find the significant inter-allelic interactions. The data on gene effects are presented in Tables 4.12 and 4.13 and described only for those traits which were having significant mean sum of square values (Table 4.8) among the generations within the family.

4.2.5.1 Days to Emergence

The scaling tests *viz*., A, B and C were found significant in the cross ICGV 06040 × ICGV 87141 (Table 4.12), while A and B scaling tests were found significant in the cross ICGV 06099 × ICGV 93468 (Table 4.13), indicating that simple additive-dominance model was inadequate to explain the observed variation and epistatic interactions were present. Hence a six parameter model was adopted to test the presence of non-allelic interactions in both the crosses understudy.

In the cross ICGV 06040 × ICGV 87141, dominance effect was found positively significant whereas in the cross ICGV 06099 × ICGV 93468, both additive and dominance effects were positively significant. Among the interactions, in both the crosses additive × additive interaction was found positively significant, while dominance × dominance interaction was observed to be negatively significant.

4.2.5.2 Days to Maturity

The three scaling tests *viz*., A, B and C were significant in the cross ICGV 06040 × ICGV 87141, while A and C were significant in the cross ICGV 06099 × ICGV 93468, indicating the inadequacy of simple additive-dominance model and the presence of epistatic interactions. Hence a six parameter model was adopted to test the presence of non-allelic interactions.

In both the crosses *viz.,* ICGV 06040 × ICGV 87141 and ICGV 06099 × ICGV 93468, additive effect and dominance × dominance component of epistasis were significant in positive direction in which dominance × dominance component recorded higher magnitude than additive effect, whereas dominance effect and additive × additive component of epistasis were negatively significant for the trait understudy. So besides additive gene action, dominance × dominance component of epistasis was found to be influencing the trait in both the crosses.

The results in both the crosses for days to maturity were similar indicating the significant influence of additive gene action and dominance × dominance component of epistasis in governing the trait understudy. The results are very much in line with the findings of Singh *et al.* (2001), Khattak *et al.* (2004), Singh *et al.* (2006), Akbari *et al.* (2013), Parmer *et al.* (2013) and Noorka *et al.* (2014) for this character in groundnut.

4.2.5.3 100-Kernel Weight (g)

Only one scaling test *i.e.,* B was significant in the cross ICGV 06040 × ICGV 87141, while scaling test C was significant in the cross ICGV 06099 × ICGV 93468, which indicated the failure of additive-dominance model to explain the inheritance of 100-kernel weight in both the crosses.

In both the crosses, only dominance × dominance component of epistasis was found positively significant whereas dominance effect and additive × additive component of epistasis were found negatively significant. So in the present study, dominance × dominance component was found to control the expression of 100-kernel weight in positive direction in both the crosses.

These results are in accordance with the findings of Singh *et al.* (2006), but in contrary, Azizi *et al.* (2006), Fatehi *et al.* (2008), Sundari *et al.* (2012), Mulugeta *et al.* (2013) and Santosh *et al.* (2014) reported an important role of additive gene action in governing the 100-kernel weight in groundnut. Hence further studies using more number of crosses are required for accurate prediction of nature of gene action in governing the 100-kernel weight in groundnut.

4.2.5.4 Shelling Percentage

Generation mean analysis for shelling percentage was carried out only in the cross ICGV 06040 × ICGV 87141, as it was found to be significant in analysis of variance. Scaling tests A and C were found significant for the trait thus indicating the presence of non-allelic interactions in governing this trait in this cross.

Positive significance was observed only for dominance × dominance component of interaction suggesting its influence in governing this trait. Additive effect and additive × additive epistatic component were negatively significant for the trait understudy.

4.2.5.5 Pod Yield per Plant (g)

In the cross ICGV 06040 × ICGV 87141, only scale A was found significant whereas only scale C was significant in the cross ICGV 06099 × ICGV 93468. This indicates the inadequacy of simple additive-dominance model to explain the inheritance of this character.

In both the crosses *viz.,* ICGV 06040 × ICGV 87141 and ICGV 06099 × ICGV 93468, positive significance was recorded by dominance and additive × additive component of epistasis but the magnitude was higher for the dominance parameter suggesting its pronounced contribution to the trait expression. Negative significant influence of dominance × dominance component of epistasis was observed only in the cross ICGV 06040 × ICGV 87141. These results are in support with the findings of Azizi *et al.* (2006) in maize, Sharmila *et al.* (2007) in sesame, Fatehi *et al.* (2008) in wheat, Sundari *et al.* (2012) in sesame, Biranvand *et al.* (2013) in chickpea and Jawahar *et al.* (2013) in sesame but in contrast to the findings of Kalia and Sood (2009) in pea and Akbari *et al.* (2013) in lentil suggesting the involvement of additive gene action in governing the concerned trait.

4.2.5.6 Kernel Iron Concentration (mg kg^{-1})

Only scaling test B was found significant in cross ICGV 06040 × ICGV 87141, while all three scales *viz*., A, B and C were significant in the cross ICGV 06099 × ICGV 93468 suggesting the inadequacy of simple additive-dominance model and the presence of epistatic interactions. So a six parameter model was adopted to test the presence of non-allelic interactions.

In the cross ICGV 06040 × ICGV 87141, positive significant values were recorded by additive effect, dominance effect and additive × additive epistasis whereas negative significant value was recorded by dominance × dominance component of epistasis. With regard to the magnitude, dominance gene action was higher followed by additive × additive component of epistasis.

In the cross ICGV 06099 × ICGV 93468, positive significant estimate was obtained only for additive component of variance suggesting maximum contribution of the same to the trait expression whereas negative significant value was recorded by dominance × dominance component of epistasis.

Up on observing the impact of various parameters additive component of variance was found to be common in both the crosses suggesting its role in governing the expression of kernel

iron concentration. The impact of additive gene action in controlling kernel iron concentration was found earlier in maize (Arnold *et al.*, 1977, Long *et al.*, 2004 and Chakraborti *et al.*, 2011), rice (Zhang *et al.*, 2004), pearl millet (Rai *et al.*, 2012), sorghum (Kumar *et al.*, 2013) and common bean (Silva *et al.*, 2013).

4.2.5.7 Kernel Zinc Concentration (mg kg^{-1})

The three scaling tests *viz*., A, B and C were significant in the cross ICGV 06040 × ICGV 87141, while A and C were significant in the cross ICGV 06099 × ICGV 93468, indicating the inadequacy of simple additive-dominance model and the presence of epistatic interactions. Hence a six parameter model was adopted to test the presence of epistatic interactions.

In the cross ICGV 06040 × ICGV 87141, additive effect and additive × additive component of epistasis were found positively significant for kernel zinc concentration suggesting strong influence of these components for trait expression which indicates that the trait can be fixed in the succeeding generations by selection.

In the cross, ICGV 06099 × ICGV 93468, only additive component was found positively significant emphasizing the influence of additive gene action in governing kernel zinc concentration.

The higher influence of additive gene action observed for kernel iron and zinc concentrations in the present study indicated that the lines or progenies with higher micronutrient concentrations can be developed by selecting parents with high concentration of these micronutrients and using them in crossing programs.

Comparison of Gene Effects over Traits in Two Crosses

The comparison of gene effects over traits in both the crosses (Table 4.14) revealed that, mean values were highly significant for all the traits studied except pod yield per plant suggesting the presence of variability among the generations for these traits.

In the cross ICGV 06040 × ICGV 87141, additive component was found to be positively significant for days to maturity and kernel iron and zinc concentrations and negatively significant for shelling percentage whereas, dominant component was positively significant for days to emergence, pod yield per plant and kernel iron concentration and negatively significant for days to maturity and 100-kernel weight. Among the interactions, additive × additive component was significant for all the characters among which days to emergence, pod yield per plant and kernel iron and zinc concentrations had recorded positive significance, whereas days to maturity, 100-

kernel weight and shelling percentage showed negative significance. Additive × dominance component of epistasis was non-significant for all the characters understudy. Dominance × dominance component was positively significant for days to maturity, 100-kernel weight and shelling percentage and negatively significant for days to emergence, pod yield per plant and kernel iron concentration.

Additive gene effect was found to be positively significant for days to emergence, days to maturity, kernel iron and zinc concentrations in the cross ICGV 06099 × ICGV 93468. Dominance component was significant for days to emergence and pod yield per plant in positive direction and days to maturity and 100-kernel weight in negative direction. Among the epistatic interactions, additive × additive component was positively significant for days to emergence and pod yield per plant and negatively significant for days to maturity and 100-kernel weight. Additive × dominance component had non-significant impact on the expression of all the traits. Dominance × dominance component was significant positively for days to maturity and 100-kernel weight and negatively significant for days to emergence and kernel iron concentration.

For all the traits in both the crosses *viz.,* ICGV 06040 × ICGV 87141 and ICGV 06099 × ICGV 93468, the gene interaction was considered to be of duplicate type, since the estimates of dominance (*h*) and dominance × dominance (*l*) had opposite signs. For days to maturity and 100-kernel weight in both the crosses and shelling percentage in the cross ICGV 06040 × ICGV 87141, the dominance (*h*) component had negative sign and dominance × dominance (*l*) had positive sign which showed duplicate interaction between decreasing genes (Hayman and Mather, 1955). Whereas for days to emergence, pod yield per plant and kernel iron and zinc concentrations in both the crosses dominance (*h*) component had positive sign and dominance × dominance (*l*) had negative sign which showed duplicate interaction between increasing genes.

This duplicate epistasis caused a higher degree of reduction of the positive effects of dominant genes, leading to low yield. Thus, for improving such traits it is better to defer selection till later generations until a high level of gene fixation is attained. Subsequent intermatings between promising lines may be important in accumulating favourable genes.

Comparison of gene actions revealed that both additive and dominance components are equally important for the studied traits. However, for the target traits *viz.,* kernel iron and zinc concentrations, the additive gene effects were prominent in both the crosses, though dominant component was also positively significant for kernel iron concentration in the cross ICGV 06040

× ICGV 87141. Importance of additive gene action in governing kernel iron and zinc concentrations was also reported by Zhang *et al.* (2004) in rice, Long *et al.* (2004) and Chakraborti *et al.* (2011) in maize, Velu *et al.* (2011a) and Rai *et al.* (2012) in pearl millet, Silva *et al.* (2013) in common bean and Kumar *et al.* (2013) in sorghum. So to improve the kernel iron and zinc concentrations, in groundnut it is suggested to use pedigree method of breeding and pureline selection to exploit both additive and non-additive components in breeding program. Such a strategy will help in increasing the frequency of favourable alleles while maintaining the genetic variation in the breeding population (Doerksen *et al*., 2003).

For reliable estimates of genetic effects using generation mean analysis, genes of like effects must be completely associated with the parents. Therefore, selection of parents contrasting for the trait being measured is crucial for this type of investigation. Any dispersal of like genes among the two parents may cause cancelling of genes of like effects resulting in the underestimation of additive (*d*), additive × additive (*i*) and additive × dominance (*j*) effects. Since the study was undertaken using parents contrasting mainly for kernel iron and zinc concentrations, estimates of additivity for other traits might be underestimated. The positive values of dominance (*h*) observed for pod yield per plant in both the crosses and kernel iron and zinc concentrations in cross ICGV 06040 × ICGV 87141 indicated that the alleles responsible for high value of the trait were dominant over the alleles controlling low value.

The results also indicated the important role of digenic non-allelic interactions (epistasis), among which additive × additive component was found to be influencing more number of traits especially kernel iron and zinc concentrations in the cross ICGV 06040 × ICGV 87141 compared to dominance × dominance component of epistasis. However, for 100-kernel weight the dominance × dominance component was significantly positive in both the crosses. For such traits, reciprocal recurrent selection might be useful to improve kernel size in groundnut.

From the results obtained in the present investigation it can be concluded that there was significant influence of additive gene action on the expression of kernel iron and zinc concentrations along with the contribution of dominance component for the control of pod yield per plant. The present study used a digenic interaction model to partition the genetic and epistatic effects into its different components and estimate the magnitude for each component for different traits in both the crosses. For better reliable information on such effects and interactions it is suggested to include more number of crosses involving contrasting parents to fit a trigenic

interaction and linkage model. This could also be useful in understanding the association levels between target traits with other important agronomic traits so that improvement of multiple traits could be achieved simultaneously to develop desirable genotypes.

4.2.6 Correlation Studies

Correlation analysis describes the mutual relationship between different pairs of characters. Most of the characters of breeder's interest are complex and are the result of interactions between several components. Understanding the relationships among various traits is of paramount importance for making the best use of these relationships in selection (Korat *et al.,* 2010). Therefore information derived from the correlation studies between two desirable traits will be useful for plant breeder in improving both the traits simultaneously. Pod yield and quality traits like kernel iron and zinc concentrations are end products of numerous genetically controlled traits which singly or jointly influence those traits (Khan *et al.,* 2000). Based on the information obtained from correlation studies it might be possible to devise a suitable strategy for improving both pod yield and mineral concentrations in groundnut kernels.

In the present study correlation coefficients were calculated for both the crosses *viz.*, ICGV 06040 × ICGV 87141 (Table 4.15) and ICGV 06099 × ICGV 93468 (Table 4.16) separately to understand the association of kernel iron and zinc concentrations with other traits under study.

In the cross ICGV 06040 × ICGV 87141, days to emergence had shown negative significant association with days to maturity (-0.226) whereas positive significant association with sound mature kernel percentage (0.172). Days to flowering exhibited positive significant association with days to maturity (0.277) and negative significant association with kernel iron concentration (-0.163) (Fig 4.10). These results were in agreement with the findings of John *et al.* (2007), Korat *et al.* (2010), Channayya *et al.* (2011), Makinde and Ariyo (2013) and Thirumala Rao *et al.* (2014) with respect to days to maturity in groundnut.

Days to maturity registered positive significant association with 100-kernel weight (0.158) and negative significant association with sound mature kernel percentage (-0.176). Karikari (1972) and Makinde and Ariyo (2013) also reported positive significant association between days to maturity and 100-kernel weight in groundnut.

The trait 100-kernel weight exhibited significant positive correlation with pod yield per plant (0.196), shelling percentage (0.142), sound mature kernel percentage (0.197) and kernel

zinc concentration (0.134) whereas negative significant association (-0.225) was observed between 100-kernel weight and kernel iron concentration. These results are in agreement with the findings of Mahalakshmi *et al.* (2005) and Patil *et al.* (2006) for sound mature kernel percentage, Zaman *et al.* (2011) for yield and shelling percentage, Kotzamanidis *et al.* (2006) and Korat *et al.* (2010) for yield per plant in groundnut and Govindaraj *et al.* (2009) for kernel zinc concentration in pearl millet.

Present study revealed significant positive association of pod yield per plant with 100-kernel weight (0.196). These results are in agreement with the findings of Kotzamanidis *et al.* (2006), Korat *et al.* (2010), Zaman *et al.* (2011), Sadeghi and Niyaki (2012) and Satish (2014) in groundnut.

Significant positive association was observed between kernel iron and zinc concentrations in both the crosses *viz.,* ICGV 06040 × ICGV 87141 and ICGV 06099 × ICGV 93468 (0.590 and 0.549, respectively). Similar kind of results were obtained by Kanatti *et al.* (2014) and Govindraj *et al.* (2009) in pearl millet, Ravikiran *et al.* (2014) and Susmitha and Selvi (2014) in sorghum, Ghanbari and Mameesh (1971), Badakhshan *et al.* (2013) and Velu *et al.* (2011) in wheat, Bekele *et al.* (2013a) in rice but in contrary, Ribeiro *et al.* (2013a) reported non-significant association between kernel iron and zinc concentrations in common bean.

In the cross ICGV 06040 × ICGV 87141, kernel iron concentration showed negative significant association with days to flowering (-0.163), 100-kernel weight (-0.225) and shelling percentage (-0.180). While in both the crosses, negative non-significant association was observed between kernel iron concentration and pod yield per plant. The results are in support with the findings of Janila *et al.,* (2014) in groundnut, Govindaraj *et al.* (2009) in pearl millet and Nagesh *et al.* (2012) in rice but in contrast Kanatti *et al.* (2014) reported negative significant association between grain iron concentration and grain yield per plant in pearl millet. Ravikiran *et al.* (2014) and Susmitha and Selvi (2014) recorded significant positive association of grain iron and zinc concentration with yield per plant in sorghum.

Kernel zinc concentration registered significant positive association with 100- kernel weight (0.134). Similar kind of association was reported in wheat by Velu *et al.* (2011). The association of kernel zinc concentration with pod yield was non-significant and similar to the findings of Chakraborti *et al.* (2009) in maize. But in rice (Bekele *et al.*, 2013a), common bean

(Ribeiro *et al.*, 2013a) and groundnut (Janila *et al.*, 2014) positive significant association between yield and kernel zinc concentration was observed.

In the cross ICGV 06099 × ICGV 93468, days to emergence showed positive significant association with days to maturity (0.153) and kernel iron concentration (0.104) and negative significant association with 100-kernel weight (-0.122).

For days to flowering, positive significant association was recorded with days to maturity (0.151) and shelling percentage (0.118). These results are in agreement with the findings of John *et al.* (2007), Makinde and Ariyo (2013) and Thirumala Rao *et al.* (2014) with respect to days to maturity in groundnut. However, no significant association was reported by John *et al.* (2007) and Korat *et al.* (2010) between days to flowering and shelling percentage.

Days to maturity was significantly and positively associated with 100-kernel weight (0.190), shelling percentage (0.112), kernel iron (0.158) and kernel zinc concentrations (0.220). Karikari (1972) and Makindo and Ariyo (2013) also reported positive association of days to maturity with 100-kernel weight in groundnut. Significant positive association between days to maturity and kernel zinc concentration was also observed by Gande *et al.* (2014) in rice. However, Govindaraj *et al.* (2009) and Sushmitha and Selvi (2014) observed non-significant association between days to maturity and kernel iron and zinc concentration. For 100-kernel weight, significant positive association was reported with shelling percentage (0.299), sound mature kernel percentage (0.286) and kernel zinc concentration (0.175). These results are in agreement with the findings of Mahalakshmi *et al.* (2005) and Patil *et al.* (2006) for sound mature kernel percentage, Zaman *et al.* (2011) for yield and shelling percentage in groundnut and Govindaraj *et al.* (2009) for kernel zinc concentration in pearl millet. But Susmitha and Selvi (2014) observed non-significant association between 100-seed weight and grain zinc concentration in sorghum.

Pod yield per plant was found to be significantly and negatively associated with shelling percentage (-0.207) which was in contrast with the findings of John *et al.* (2007) and Korat *et al.* (2010) where non-significant association was observed between pod yield per plant and shelling percentage.

Highly significant positive correlation between kernel iron and zinc concentrations in both the crosses of groundnut indicated the possibility of simultaneous improvement of both the traits. This might be due to co-segregation of tightly linked genetic elements governing the

physiology of these micronutrients or might be due to the pleotropic effect of genes. Days to maturity showed significant positive association with kernel iron and zinc concentrations in one cross but not in other cross thus there is a need to confirm the results of present study in this aspect using more number of crosses.

In the present study, pod yield per plant did not show any significant association with kernel iron and zinc concentrations indicating that breeding for improved kernel iron and zinc concentrations will not affect the yield. Similar results were earlier obtained in pearl millet (Govindaraj *et al.,* 2009) and groundnut (Janila *et al.,* 2014). 100-kernel weight recorded positive significant association with kernel zinc concentration suggesting that increased kernel size may also contribute to increased kernel zinc concentration. However, kernel iron concentration and 100-kernel weight had negative significant association in one cross and non-significant negative association in another cross which indicates that further confirmation might be required to understand the association between these two traits.

Summary and Conclusions

Chapter V

SUMMARY AND CONCLUSIONS

Groundnut is an important oil, food and fodder legume crop grown mainly in arid and semi-arid regions of the world. Groundnut kernels are highly nutritious with about 25 % protein, several minerals, micronutrients and high-energy contributed by 45-50 % oil. Of the 20 minerals necessary for normal body growth and maintenance, seven, including iron and zinc are present in groundnut. Bio-fortification, a process of enhancing the micronutrient concentrations by genetic means, is an effective approach to combat micronutrient malnutrition prevailing in the world. Most of the countries which are suffering from micronutrient malnutrition are cultivating groundnut crop. Thus, groundnut can contribute significantly towards reduction of protein-energy and micronutrient malnutrition. Bio-fortified groundnut will be of immediate use to make ready to use therapeutic food products. Besides, confections and other food products made from bio-fortified groundnut will contribute to enhanced nutrition of consumers.

Knowledge on genetics of kernel iron and zinc is necessary to begin a breeding program for the improvement of kernel iron and zinc. Besides, if markers linked to the traits of interest are available, they may be used for selection in the breeding program to accelerate the genetic gains for kernel iron and zinc. Association of kernel iron and zinc concentrations with other important traits is also essential to develop varieties with improved yield and nutritional quality. Hence, keeping all these points in view, the present investigation was carried out to identify the molecular markers associated with the kernel iron and zinc concentrations and to study the gene action involved in the inheritance of the traits under concern using generation mean analysis by conducting two separate experiments at ICRISAT, Patancheru.

In the first experiment, an attempt was made to identify the molecular markers associated with the kernel iron and zinc concentrations in groundnut using $F_{2:3}$ mapping population. The $F_{2:3}$mapping population was derived by crossing a high iron and zinc containing parent, ICGV 06099, with a low iron and zinc containing parent, ICGV 93468.Phenotyping of $F_{2:3}$population was carried out during rainy season, 2013 using alpha lattice design with two replications.

The analysis of variance revealed the existence of significant variation for all the traits except for days to emergence, days to maturity, protein content and palmitic acid content. Overall perusal of descriptive statistics revealed that normal distribution was observed for almost all the traits except days to maturity and protein content. Transgressive segregants which were outperforming either of the parents for all the traits including kernel iron and zinc concentrations (except for days to maturity) were present in the population.

Estimation of genetic parameters in the phenotyping material *i.e.*, $F_{2:3}$ population of the cross ICGV 06099 × ICGV 93468 revealed that the phenotypic coefficient of variation was moderately higher than the genotypic coefficient of variation for all the traits including kernel iron and zinc concentrations suggesting the influence of environment on the expression of these traits. Heritability estimates were moderate to higher for all the traits in the population. Pod yield per plot (91.00 %) along with kernel iron (64.24 %) and zinc concentrations (62.21 %) recorded higher heritability (broad sense) compared to all the other traits. Higher genetic advance as a per cent of mean (GAM) was observed for single plant yield (61.37 %), pod yield per plot (53.26 %) and kernel yield per plot (46.22 %), whereas kernel iron (9.72 %) and zinc (4.99 %) concentrations had low GAM values. High heritability along with high GAM was recorded by single plant yield, pod yield per plot and kernel yield per plot which indicated the easy transferability and genetic improvement of these traits in the succeeding generations.

Correlation studies revealed the presence of significant positive association between kernel iron and zinc concentrations. Sound mature kernel percentage and kernel zinc concentration exhibited positive association suggesting possible higher accumulation of zinc in fully matured kernels. However, kernel iron and zinc concentrations did not show any significant association with pod yield. The results indicated that it is feasible to select simultaneously for kernel iron and zinc concentrations in groundnut without any penalty on pod yield. Kernel iron and zinc were not associated with protein and oil contents suggesting the possible improvement of these quality parameters without concomitant change in other characters. Among yield parameters, significant positive association of pod yield with seed yield per plant and sound mature kernel percentage was observed and therefore seed yield per plant and sound mature kernel percentage can be targeted to improve pod yield. Shelling percentage and oil content showed significant negative association with pod yield. Both pod yield and shelling outturn are economically important traits and hence need to be considered together for selection.

Parental polymorphism survey between these two parents using 200 SSR markers revealed that 33 SSR markers (16.5 %) were polymorphic. Out of 33 polymorphic SSR markers, three markers *viz.*, SEQ1B09, IPAHM245 and SEQ9G05 showed significant association with the kernel iron concentration and explained a phenotypic variation of 0.23, 2.19 and 6.34 %, respectively towards the trait and three markers *viz.*, GM2638, IPAHM245 and SEQ9G05 showed significant association with phenotypic variation of 1.75, 2.25 and 6.01 %, respectively towards kernel zinc concentration. Validation of these markers in another $F_{2:3}$population derived from a cross between ICGV 06040 (high kernel iron and zinc containing parent) and ICGV 87141 (low kernel iron and zinc containing parent)also showed their strong association with the traits of interest.

Principal Component Analysis (PCA) results revealed that first two principal components contributed to 49 % of total variance. Among which PC1 contributed 28.8 % and PC2 contributed 20.2 % of phenotypic variation. A positive correlation was observed between kernel iron and zinc concentrations (G1), pod yield and kernel yield per plot (G2) and oil and protein contents (G3). But a strong negative association was observed between oleic and linoleic acid contents. This can be attributed to the biochemical pathway of conversion of oleic and linoleic acid. However no association of kernel iron and zinc concentrations with yield was observed.

In the second experiment, generation mean analysis was carried out in two crosses (ICGV 06040 × ICGV 87141 and ICGV 06099 × ICGV 93468) using parents having contrasting kernel iron and zinc concentrations with the aim of obtaining information on gene action governing kernel iron and zinc concentrations, estimating the genetic parameters, determining the correlation of kernel iron and zinc concentrations with kernel yield and other important traits in groundnut. From each cross six generations *viz.*, P_1, P_2, F_1, F_2, B_1 and B_2 were generated during rainy season, 2013, which were evaluated in compact family block design during post-rainy season, 2013-14 with three replications. Data were recorded on days to emergence, days to flowering, days to maturity, 100-kernel weight, shelling percentage, sound mature kernel percentage, pod yield per plant and kernel iron and zinc concentrations.

Genetic parameters like phenotypic coefficient of variation, genotypic coefficient of variation, heterosis, residual heterosis, inbreeding depression, heritability in broad sense and narrow sense, genetic advance as a percentage of mean and degree of dominance were estimated.

Generation mean analysis was carried out using the scaling tests given by Mather (1949) and Hayman and Mather (1955) and six parameter model given by Hayman (1958). Correlation coefficients were also calculated using the formulae suggested by Falconer (1981).

The analysis of variance showed significant differences between the crosses for five traits *viz.*, days to maturity, 100-kernel weight, pod yield per plant, kernel iron and zinc concentrations, whereas within crosses among the generations significance of difference was observed for days to emergence, days to maturity, 100-kernel weight, shelling percentage (only in ICGV 06040 × ICGV 87141), pod yield per plant, kernel iron and zinc concentrations for which generation mean analysis was carried out.

In both the crosses phenotypic coefficient of variation was moderately higher than genotypic coefficient of variation for all the traits. In both the crosses, kernel iron and zinc concentrations recorded moderate PCV % values and low GCV % values which emphasized the influence of environment on the accumulation of these micronutrients.

In the cross, ICGV 06040 × ICGV 87141, higher broad sense heritability coupled with moderate genetic advance as per cent of mean was obtained for100-kernel weight (61.36 % and 15.59), shelling percentage(82.56 % and 18.95) and grain iron (72.43 % and 14.93) and zinc (71.15 % and 19.21) concentrations. Whereas pod yield per plant recorded moderate broad sense heritability (53.96 %) and high genetic advance as per cent of mean (23.01). Since the heritability for most of the traits was high, selection in early generations can be carried out for the improvement of such traits. In the cross ICGV 06099 × ICGV 93468, higher broad sense heritability coupled with moderate genetic advance as per cent of mean was recorded for days to emergence (74.83 % and 14.94), days to maturity (84.04 % and 12.84) and pod yield per plant (69.36 % and 23.85) indicating the preponderance of additive gene action and effectiveness of selection. However, kernel iron and zinc concentrations recorded moderate heritability (broad sense) and GAM values. Moderate to high broad sense heritability and moderate genetic advance as per cent of mean were observed for kernel iron and zinc concentrations in both the crosses. Thus it can be concluded that genetic improvement for these traits can be possible through selection in early generations.

In both the crosses, kernel iron and zinc concentrations recorded negative, non-significant values for average heterosis and heterobelteosis (except for kernel zinc concentration in the cross ICGV 06099 × ICGV 93468)suggesting poor performance of F_1 over mid-parent and better

parent, respectively. In the cross ICGV 06040 × ICGV 87141, residual heterosis over mid parent was also negative and non-significant whereas significant negative residual heterosis over better parent was observed for kernel iron and zinc concentrations. In the cross ICGV 06099 × ICGV 93468, residual heterosis estimates over mid and better parents for kernel iron and zinc concentrations were non-significant in either directions indicating the lack of inherent heterotic substance in F_2 progeny over mid and better parents in both the crosses.

In both the crosses higher value of inbreeding depression was observed for pod yield per plant which indicates a reduction in yield up on selfing which is not desirable in case of self-pollinated crops like groundnut. In the cross ICGV 06040 × ICGV 87141, low inbreeding depression was observed for kernel iron (0.95) and zinc (3.63) concentrations, while in the cross ICGV 06099 × ICGV 93468, low (-12.32) and high (32.84) estimates of inbreeding depression were observed for kernel iron and zinc concentrations, respectively. Thus further studies are required to have better understanding of inbreeding depression for kernel micronutrient concentration in groundnut.

Degree of dominance was more than unity for most of the characters indicating over dominance expression of these traits. However, kernel iron and zinc concentrations recorded degree of dominance, which was more than unity in the cross ICGV 06040 × ICGV 87141 and less than unity in the cross ICGV 06099 × ICGV 93468 suggesting that further confirmation is required for degree of dominance for these traits understudy.

Correlation analysis involving six generations each of two crosses revealed the strong positive significant association between kernel iron and zinc concentrations and their non-significant association with pod yield per plant indicating that no penalty would be there on yield while selecting for kernel iron and zinc concentrations. Positive significant association between 100-kernel weight and kernel zinc concentration was observed indicating the chance that increase in seed size may improve zinc concentration in groundnut kernel. Though significant association was observed between kernel iron and zinc concentrations and days to maturity in the cross ICGV 06099 × ICGV 93468 further confirmation needs to be done by analyzing more number of crosses to understand the real association between these traits.

Generation mean analysis revealed that at least one of the scaling tests to a maximum of three scaling tests *viz.,* A, B and C were significant for the traits *viz.*,days to emergence, days to maturity, 100-kernel weight, shelling percentage (only in ICGV 06040 X ICGV 87141), pod

yield per plant, kernel iron and zinc concentrations which indicated the presence of non-allelic interactions. Hayman's six parameter model showed high significance of the mean effects (*m*) except for pod yield per plant indicating that all the studied traits in both the crosses were quantitatively inherited. Additive component was positively significant for days to maturity, kernel iron and zinc concentrations in both the crosses and for days to emergence in the cross ICGV 06099 × ICGV 93468 and negatively significant for shelling percentage in the cross ICGV 06040 × ICGV 87141. Dominance effect was found to be negatively significant for days to maturity and 100-kernel weight in both the crosses and positively significant for days to emergence and pod yield per plant in both the crosses and for kernel iron concentration only in the cross ICGV 06040 × ICGV 87141. Among the interactions, additive × additive component was significant in negative direction for days to maturity and 100-kernel weight in both the crosses and for shelling percentage in the cross ICGV 06040 × ICGV 87141 and positively significant for days to emergence and pod yield per plant in both the crosses and for kernel iron and zinc concentrations in the cross ICGV 06040 × ICGV 87141. Dominance × dominance component was found positively significant for days to maturity and 100-kernel weight in both the crosses and for shelling percentage in the cross ICGV 06040 × ICGV 87141 and negatively significant for days to emergence and kernel iron concentration in both the crosses and for pod yield per plant only in the cross ICGV 06040 × ICGV 87141.However, additive × dominance interaction was found non-significant for all the traits in both the crosses. Both direct effects and interaction effects were almost of equal magnitude in both the crosses whereas for kernel iron and zinc concentrations additive effect along with additive × additive interaction was found responsible for trait expression. The signs of dominance (*h*) and dominance × dominance (*l*) were opposite in all the cases indicating duplicate type of epistasis.

Conclusion and future strategy:

In the first experiment, due to lack of sufficient number of polymorphic markers single marker analysis was followed to identify the genomic regions associated with kernel iron and zinc concentrations. Though it is difficult to get large number of polymorphic markers, it facilitates the construction of good quality linkage map by covering all twenty linkage groups and it is useful in identifying QTLs responsible for the traits in question in groundnut. Hence further study is required with large number of molecular markers to exactly identify the QTL responsible for the kernel iron and zinc concentrations.

The PCV% and GCV% differed by moderate values for almost all the traits including kernel iron and zinc concentrations which depict the moderate role of environment in trait expression. High heritability was observed for kernel iron and zinc concentrations indicating the predominant role of additive gene action. Highly significant positive correlation was observed between kernel iron and zinc concentration suggesting that improvement in one micronutrient concentration will lead to simultaneous improvement in other micronutrient as well. However, no association of kernel micronutrient concentrations with pod yield was observed suggesting the possibility of improvement in kernel micronutrient concentrations without yield penalty.

In general, both additive and non-additive gene effects appear to be effective for all the traits studied. However, for kernel iron and zinc concentration, the involvement of additive effect in both the crosses and additive × additive interaction along with additive effect in one cross was observed. Thus, superior lines with higher kernel mineral concentrations can be developed by applying simple selection in early generations.

However, in the present study the conclusions drawn from the gene effects for different traits are based on digenic interaction model with the use of two crosses only. But, possibilities of trigenic or higher order interaction and/or linkages among the interacting genes cannot be ruled out. Hence, there is a need for further study with more number of generations to fit a trigenic interaction and linkage model using crosses involving parents contrasting for the respective traits.

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*- Review of literature is presented as per the thesis writing guidelines of the university

Appendices

Appendix B. Mean values of various traits in the $F_{2:3}$ mapping population of the cross ICGV 06099 × ICGV 93468 in groundnut

ENTRY	DE	DFL	DMt	HKW	SPYD	PY	SEED Y	SMK	SH	OIL	PRO	Fe	Zn	Linoleic	Oleic	Palmitic	Stearic
1	6.50	33.00	114.00	33.45	15.15	146.20	65.90	74.98	45.09	49.18	26.40	45.83	76.95	35.50	40.27	12.54	2.30
2	7.50	32.50	108.50	30.85	11.30	92.60	60.45	65.41	65.24	46.31	25.95	43.99	71.08	34.55	42.05	12.95	1.76
3	5.50	33.50	114.00	34.95	18.80	137.75	71.60	83.82	52.00	48.52	27.85	50.01	74.66	33.15	42.26	12.16	2.35
4	7.00	33.00	103.00	27.95	12.45	118.85	64.45	60.15	54.15	48.58	27.04	35.84	69.84	34.18	40.90	12.47	2.14
5	7.00	34.50	114.00	29.15	10.55	89.70	53.45	52.66	59.58	47.36	25.99	39.38	79.05	34.66	41.35	13.13	1.89
6	6.50	34.50	114.00	28.40	17.30	88.00	51.00	61.50	57.94	50.83	27.30	39.44	68.24	31.13	43.24	12.49	2.59
7	7.00	34.00	114.00	33.55	19.55	138.70	80.90	49.81	58.33	48.97	27.32	48.33	83.56	36.76	38.83	12.82	2.26
8	6.00	32.00	114.00	25.70	22.35	92.30	58.95	44.49	63.81	50.16	27.99	38.66	71.47	35.35	39.67	13.19	2.36
9	7.50	34.50	114.00	30.50	22.45	115.75	71.95	69.63	62.22	47.19	27.37	41.14	83.08	38.19	36.16	13.21	2.18
10	6.00	31.50	108.50	30.50	17.55	112.00	65.40	73.20	58.34	48.61	25.64	50.70	73.16	37.57	38.20	13.55	2.04
11	8.00	34.00	114.00	31.90	18.40	98.80	60.50	69.12	61.27	47.69	25.31	59.09	80.80	34.79	41.42	12.99	1.95
12	6.50	34.50	114.00	31.05	29.05	105.95	67.10	46.41	63.34	50.76	27.57	36.11	63.99	35.19	39.28	12.83	2.43
13	7.00	35.50	114.00	32.65	17.10	85.25	52.00	79.50	61.00	47.35	27.46	42.64	81.86	36.29	38.64	13.12	2.15
14	6.00	34.00	114.00	32.65	21.10	143.40	89.55	69.12	62.41	47.47	26.26	51.71	84.78	34.57	41.56	12.94	1.96
15	8.00	34.00	114.00	30.35	16.80	71.35	46.40	81.04	64.99	47.70	28.47	41.86	84.32	33.01	41.99	12.35	2.41
16	6.50	35.00	114.00	33.05	23.25	186.35	108.95	85.98	58.34	48.91	27.62	42.11	86.45	37.08	37.09	12.75	2.52
17	7.00	32.50	114.00	36.50	19.05	127.30	69.85	83.30	54.82	49.86	27.00	39.52	71.07	35.19	40.23	12.85	2.37
18	7.00	34.00	108.50	32.65	17.80	138.30	86.90	74.41	62.79	45.41	26.78	44.82	80.86	36.41	38.29	13.29	1.98
19	7.00	36.00	103.00	24.50	18.10	72.40	42.00	45.95	58.01	47.44	24.50	53.41	81.91	38.25	38.68	14.22	1.69
20	6.00	34.00	108.50	33.50	16.00	82.00	52.15	43.41	63.54	49.18	28.37	38.02	75.57	34.38	40.29	12.50	2.42
21	8.00	35.50	114.00	34.50	17.40	130.00	63.65	74.07	48.99	50.23	26.60	52.13	85.03	34.72	40.47	12.55	2.27
22	6.50	34.50	114.00	37.15	27.80	96.50	65.85	89.97	68.14	49.03	28.14	41.62	75.79	32.02	43.54	12.02	2.48
23	8.00	36.00	114.00	28.10	22.80	78.60	51.20	31.25	65.14	50.37	28.65	42.99	71.71	31.12	44.79	11.79	2.44
24	6.00	36.00	114.00	36.60	14.00	111.50	69.20	81.50	62.06	50.31	26.09	41.82	77.23	32.94	42.66	12.27	2.32
25	8.00	35.50	114.00	30.15	31.35	90.70	51.20	69.91	56.25	52.78	25.89	48.23	79.38	30.82	43.88	12.62	2.82
26	7.00	35.00	114.00	33.35	28.50	121.10	67.40	86.68	55.62	50.59	27.39	50.62	80.30	36.55	38.00	12.38	2.52

Appendix B. cont.

ENTRY	DE	DFL	DMt	HKW	SPYD	PY	SEED Y	SMK	SH	OIL	PRO	Fe	Zn	Linoleic	Oleic	Palmitic	Stearic
27	6.50	35.50	114.00	35.40	19.05	86.45	45.30	69.93	52.29	49.81	28.00	47.41	81.62	36.87	38.93	13.21	2.41
28	6.50	36.00	108.50	34.20	16.90	108.30	49.45	68.56	45.47	48.24	27.29	47.39	88.42	32.42	43.61	12.37	2.18
29	6.00	31.50	114.00	31.80	11.30	123.25	52.35	80.35	42.44	48.90	27.82	41.12	78.12	36.86	37.21	13.31	2.44
30	8.00	35.00	114.00	29.10	9.50	86.10	53.10	77.02	61.67	51.83	25.97	50.30	83.70	38.96	37.00	13.11	2.25
31	6.00	33.50	114.00	31.55	14.40	137.50	76.60	56.92	55.04	48.10	27.57	39.11	70.44	37.94	35.63	13.14	2.40
32	6.50	34.50	108.50	32.25	13.85	137.70	61.50	72.26	44.42	49.47	27.60	57.16	75.28	35.75	38.84	12.62	2.35
33	7.50	35.50	114.00	34.55	31.75	89.55	58.10	73.74	64.85	49.19	28.93	56.15	82.82	35.57	38.63	12.87	2.45
34	7.50	34.00	108.50	31.30	14.10	120.75	55.40	68.25	45.62	48.32	27.20	41.48	72.71	32.65	42.54	12.31	2.10
35	8.00	34.00	114.00	30.35	18.15	109.25	61.95	66.77	56.31	48.96	26.76	44.01	77.38	36.20	39.55	12.87	2.20
36	7.00	32.00	108.50	30.80	20.00	112.65	54.65	75.93	48.26	48.90	27.53	58.26	84.85	34.23	41.47	12.66	2.12
37	8.00	36.00	114.00	30.05	24.15	107.00	60.25	79.34	56.58	49.95	26.08	40.96	70.06	35.14	40.30	12.76	2.28
38	7.00	33.00	103.00	33.95	15.70	99.00	50.85	82.35	51.20	49.17	26.61	43.05	75.19	33.27	42.68	12.61	2.08
39	8.00	35.00	114.00	36.70	15.15	89.85	45.65	70.85	50.73	46.95	28.28	42.39	75.17	34.69	39.93	12.79	2.18
40	7.00	36.00	114.00	30.70	17.30	69.20	42.60	83.10	61.56	50.30	26.33	47.42	80.92	34.54	39.85	12.56	2.46
41	6.50	35.50	114.00	33.50	27.80	91.20	43.30	65.99	46.86	49.61	27.46	49.54	76.95	30.07	46.46	12.32	2.13
42	6.50	35.00	114.00	29.70	16.95	66.65	32.15	66.26	48.17	52.78	27.15	48.90	74.50	26.78	49.15	11.90	2.64
43	6.50	33.50	114.00	29.40	23.60	124.50	64.40	77.89	51.77	48.18	26.10	48.09	82.99	29.71	45.91	12.32	2.07
44	6.50	34.50	114.00	32.70	34.95	165.05	92.55	82.59	56.71	47.58	26.94	52.28	81.08	33.92	41.43	12.60	2.19
45	7.00	34.00	114.00	26.80	10.20	83.30	51.15	53.10	61.14	47.94	25.20	44.46	79.52	30.81	46.12	12.63	1.84
46	6.00	34.50	103.00	27.35	12.35	133.90	77.15	65.15	57.57	45.82	25.94	42.60	74.20	30.77	45.01	12.78	1.80
47	7.00	34.00	108.50	26.25	20.25	92.30	57.15	54.51	61.82	48.66	25.39	41.47	74.76	31.38	45.57	12.92	1.89
48	7.00	34.50	114.00	34.05	15.05	105.60	60.20	77.67	57.10	48.54	27.91	46.66	76.37	38.10	35.58	12.92	2.55
49	7.00	33.00	108.50	32.45	14.30	101.80	59.05	61.96	58.02	48.33	26.34	40.93	74.95	31.99	43.36	12.15	2.18
50	6.50	34.00	114.00	32.00	15.35	92.10	47.90	84.07	51.86	47.07	27.08	48.62	80.01	34.84	39.84	13.41	2.22
51	7.00	32.50	114.00	39.65	27.50	132.65	81.20	69.03	61.10	48.36	26.53	44.91	83.38	35.98	39.83	12.79	2.21
52	7.00	31.50	103.00	31.30	12.20	89.40	59.00	72.61	65.94	48.67	27.23	41.67	88.86	36.56	38.59	12.79	2.27

Appendix B. cont.

ENTRY	DE	DFL	DMt	HKW	SPYD	PY	SEED Y	SMK	SH	OIL	PRO	Fe	Zn	Linoleic	Oleic	Palmitic	Stearic
53	7.50	35.00	108.50	33.30	19.90	156.90	80.80	82.49	51.48	46.39	26.76	38.04	74.92	37.83	36.61	12.85	2.08
54	7.00	34.00	114.00	33.40	22.60	113.40	55.70	58.71	49.12	49.31	27.16	43.76	68.15	26.22	50.23	11.61	2.20
55	7.50	33.00	114.00	32.85	21.25	80.15	57.75	59.53	71.97	49.43	27.84	50.94	80.88	28.81	46.69	12.17	2.54
56	8.00	33.00	114.00	31.70	11.00	94.80	55.25	51.35	58.47	47.86	26.29	49.02	68.62	35.81	39.97	13.37	2.03
57	7.50	33.00	108.50	30.25	13.05	159.30	89.35	61.63	56.16	48.73	28.07	46.24	78.78	34.44	40.09	12.67	2.28
58	7.50	35.00	114.00	32.20	23.75	142.65	64.40	76.57	45.07	48.67	28.66	47.51	80.20	36.04	38.29	12.43	2.25
59	7.00	33.50	108.50	33.20	13.80	134.05	78.75	82.59	58.74	48.27	27.41	53.58	79.66	37.15	38.03	12.44	2.12
60	6.00	32.00	108.50	34.00	13.85	60.70	30.85	81.58	51.09	47.32	26.33	52.68	73.62	35.64	39.87	13.23	2.04
61	7.50	33.00	114.00	37.25	20.70	196.70	116.30	84.61	59.02	47.63	26.55	38.22	73.29	36.18	39.61	12.78	2.13
62	7.50	36.00	108.50	27.25	10.55	75.10	44.05	51.51	58.15	49.31	26.73	44.41	75.73	34.43	41.83	13.00	2.03
63	7.00	35.50	114.00	33.65	19.70	174.35	76.45	64.91	44.74	48.65	27.44	38.34	71.49	35.08	39.23	12.64	2.29
64	8.00	36.00	114.00	32.10	18.40	110.80	64.80	82.72	58.48	49.53	26.79	57.67	76.95	34.90	40.16	13.03	2.21
65	7.00	32.00	108.50	33.95	17.00	148.65	95.20	77.02	63.98	50.14	27.92	53.96	78.38	36.22	38.51	12.71	2.43
66	8.00	32.00	114.00	36.95	15.90	124.85	75.90	72.47	60.72	47.20	26.80	42.78	82.30	31.58	44.07	12.59	2.18
67	7.00	33.00	114.00	35.00	13.95	107.65	66.80	70.89	61.38	46.15	26.38	42.46	74.49	34.87	41.21	13.03	1.87
68	7.00	32.00	114.00	31.20	23.75	153.30	87.05	80.52	56.75	48.51	26.10	41.30	68.75	34.80	40.51	12.68	2.33
69	7.00	33.50	108.50	32.70	27.00	105.45	67.75	82.12	64.17	47.63	25.73	49.52	72.25	31.45	45.47	12.95	1.99
70	8.00	37.00	108.50	33.45	9.55	84.35	46.25	76.87	54.91	47.04	26.61	40.36	80.60	33.29	42.70	12.93	1.95
71	7.00	34.00	108.50	32.65	16.50	83.20	58.15	66.82	69.53	49.25	27.58	45.30	76.28	36.07	39.14	12.58	2.35
72	7.00	32.00	108.50	34.45	16.20	129.85	73.00	61.86	56.24	48.52	26.44	50.31	71.35	33.87	41.70	12.30	2.21
73	8.00	34.00	114.00	30.90	13.50	135.00	78.20	75.58	57.93	48.79	26.44	48.84	81.07	35.45	40.18	12.58	2.35
74	8.00	34.00	103.00	31.20	14.20	79.70	49.60	52.62	62.23	49.25	29.75	43.84	78.34	33.25	42.05	12.27	2.54
75	6.50	32.50	114.00	34.80	20.75	113.15	60.30	69.38	53.33	48.01	27.35	35.42	68.99	34.56	41.53	12.54	2.13
76	7.00	32.00	108.50	34.85	22.75	150.40	71.45	74.40	46.11	49.10	26.55	44.08	77.70	34.98	40.99	12.79	2.15
77	7.00	34.50	114.00	35.25	22.55	83.90	41.05	64.04	48.77	50.00	26.65	40.90	74.38	35.80	39.42	12.64	2.41
78	7.00	33.50	114.00	32.40	16.25	148.00	69.65	83.61	47.07	55.08	26.24	49.65	80.80	34.42	41.91	12.16	3.09
79	7.00	33.50	108.50	28.05	11.15	92.35	51.25	61.98	55.34	47.80	27.66	42.31	71.09	39.24	35.44	13.32	1.98

Appendix B. cont.

ENTRY	DE	DFL	DMt	HKW	SPYD	PY	SEED Y	SMK	SH	OIL	PRO	Fe	Zn	Linoleic	Oleic	Palmitic	Stearic
80	7.00	33.00	114.00	27.95	18.65	124.20	73.30	59.72	59.01	47.50	26.39	45.20	75.55	37.84	38.19	13.35	1.82
81	7.50	33.50	108.50	25.25	24.10	70.05	35.90	67.05	51.41	48.29	27.07	37.96	83.42	37.86	37.64	13.40	2.01
82	6.50	32.00	108.50	25.35	21.75	103.40	61.15	70.17	59.31	46.55	28.39	43.14	77.42	34.90	39.89	12.79	2.03
83	7.00	32.00	103.00	29.80	12.80	64.00	44.10	61.90	68.91	45.80	25.68	61.06	81.74	31.72	45.15	13.57	1.70
84	8.00	36.00	108.50	32.70	9.90	79.95	46.30	83.84	57.98	45.07	26.58	41.82	74.67	30.25	46.21	12.51	1.87
85	7.50	34.00	114.00	36.85	23.55	99.40	51.90	71.92	52.18	47.23	27.33	41.72	75.80	30.81	45.00	12.24	2.12
86	7.00	31.50	108.50	37.15	22.00	140.85	81.90	86.07	58.11	45.67	27.68	39.06	77.62	30.71	45.65	12.31	2.03
87	6.00	32.00	114.00	39.30	20.30	203.00	122.10	93.28	60.15	46.57	26.60	53.14	79.59	31.79	44.60	12.48	2.10
88	7.50	34.00	114.00	29.60	19.15	96.05	48.65	67.42	50.77	50.71	25.99	46.27	69.85	30.72	45.26	12.05	2.54
89	8.50	35.00	114.00	30.40	15.50	131.70	78.50	65.38	59.52	51.18	26.01	50.17	77.56	36.45	38.72	12.90	2.34
90	8.00	35.50	114.00	31.85	22.65	157.35	81.35	77.89	51.67	48.22	26.97	54.82	70.90	34.54	40.95	12.74	2.26
91	7.00	31.50	108.50	30.55	12.60	94.65	51.45	53.18	54.36	48.45	27.94	48.07	73.34	33.21	42.03	12.59	2.22
92	6.50	32.00	114.00	35.75	15.30	131.30	71.80	79.86	54.59	48.22	27.09	51.79	74.74	32.12	42.77	12.72	2.32
93	6.50	33.00	108.50	26.45	12.65	51.40	29.65	65.40	58.07	47.90	26.50	47.04	68.44	33.80	42.18	12.90	2.11
94	6.50	34.00	114.00	28.20	12.95	57.65	28.15	70.25	48.83	49.85	27.28	49.73	79.52	32.64	42.87	12.54	2.37
95	6.50	33.00	103.00	33.95	13.70	101.30	72.50	56.49	71.52	48.71	28.02	41.46	81.20	28.50	46.58	12.19	2.32
96	7.00	34.50	114.00	36.80	25.65	131.30	90.55	65.24	69.01	49.16	26.23	41.28	78.05	35.58	38.98	12.64	2.39
97	8.00	35.00	108.50	35.15	15.20	84.50	51.65	61.56	61.16	47.23	27.73	42.90	65.78	33.58	41.50	12.74	2.04
98	7.50	33.50	114.00	30.50	19.85	108.05	56.70	74.35	52.47	48.88	27.20	50.82	79.14	32.85	41.71	12.54	2.28
99	8.00	35.00	114.00	28.25	21.85	139.15	68.85	65.94	49.45	48.99	27.38	39.85	85.83	37.37	37.73	12.84	2.26
100	6.50	32.50	108.50	30.50	18.10	100.90	58.70	64.49	58.20	47.51	25.32	44.96	68.63	36.71	38.35	12.87	2.16
101	7.50	33.00	108.50	29.45	16.00	138.55	69.35	69.34	49.96	46.57	24.94	44.57	80.48	32.60	43.31	12.74	1.99
102	7.50	33.00	114.00	35.00	27.75	119.50	65.00	83.18	54.40	50.20	26.11	39.19	71.26	32.91	42.09	12.46	2.32
103	8.00	31.50	114.00	34.85	15.05	141.65	67.30	64.44	47.46	49.82	25.89	56.81	78.38	33.70	42.02	12.89	2.21
104	7.00	33.50	114.00	30.85	13.90	116.95	48.80	76.97	41.65	47.66	27.70	41.01	71.68	31.93	43.64	12.67	2.11
105	7.00	33.00	114.00	27.70	22.95	109.75	70.30	65.47	64.08	47.13	27.99	53.71	78.17	32.81	41.69	12.36	2.21
106	7.00	34.00	114.00	33.10	15.75	133.90	72.45	80.43	54.06	49.39	28.08	48.11	79.18	37.28	36.95	12.81	2.47

Appendix B. cont.

ENTRY	DE	DFL	DMt	HKW	SPYD	PY	SEED Y	SMK	SH	OIL	PRO	Fe	Zn	Linoleic	Oleic	Palmitic	Stearic
107	6.00	33.50	108.50	40.70	18.50	76.00	43.10	49.92	52.38	48.69	26.86	36.33	79.83	33.90	41.44	12.64	2.39
108	6.50	34.50	114.00	36.50	15.05	88.55	44.95	68.01	50.83	46.47	27.27	41.24	82.98	32.49	42.49	13.31	1.97
109	7.00	31.50	114.00	32.95	10.40	104.25	60.10	85.15	57.59	48.10	26.95	47.93	77.61	32.91	42.60	12.66	2.08
110	6.00	35.00	103.00	31.60	12.50	112.60	71.10	70.89	63.14	46.55	26.21	53.91	72.95	32.98	42.52	12.98	1.86
111	7.00	33.00	114.00	37.80	23.50	94.00	51.20	58.01	54.47	46.16	27.29	47.62	78.27	36.93	36.04	13.48	2.17
112	7.00	34.50	114.00	31.25	21.00	144.70	79.40	55.92	54.85	48.66	27.33	38.58	77.94	34.42	40.56	12.73	2.24
113	6.00	32.00	114.00	36.05	17.90	97.80	52.00	78.46	53.06	48.60	26.84	39.26	73.15	36.83	37.46	13.11	2.37
114	6.50	33.50	103.00	35.30	16.90	154.25	75.65	68.97	48.96	47.68	27.30	43.82	79.25	32.51	42.72	12.79	2.27
115	7.50	34.00	108.50	34.95	15.35	140.60	74.35	67.82	52.88	47.88	28.03	47.61	77.68	32.09	42.19	12.26	2.34
116	7.50	33.50	114.00	35.90	19.70	176.90	90.30	88.43	51.06	49.32	27.19	43.93	73.42	35.38	39.63	12.59	2.36
117	7.00	32.00	108.50	33.50	14.65	45.55	27.00	68.61	59.32	50.46	26.38	43.83	82.52	30.44	44.90	12.60	2.71
118	7.00	33.00	108.50	28.55	15.80	78.25	51.15	83.30	65.32	52.35	26.75	42.90	80.56	31.81	42.78	12.26	2.86
119	8.00	36.00	108.50	28.65	16.30	83.05	44.55	89.09	53.52	49.24	25.78	48.23	72.37	34.89	41.09	12.90	2.20
120	8.50	34.00	114.00	29.85	18.40	173.85	78.45	85.14	45.10	48.97	27.19	51.77	73.90	34.14	40.80	12.99	2.19
121	8.00	35.50	114.00	30.25	15.55	129.10	82.80	74.34	64.09	52.00	27.14	43.58	80.84	32.68	42.61	12.32	2.59
122	7.00	34.00	103.00	28.20	13.00	77.50	45.80	65.28	59.10	46.16	26.04	38.32	75.75	32.51	44.47	12.66	1.91
123	7.50	34.00	114.00	30.60	18.10	132.85	70.75	76.52	54.09	49.56	26.05	44.05	75.23	33.45	43.07	12.68	2.15
124	7.00	31.00	103.00	28.35	14.60	86.20	50.20	65.12	58.06	48.73	26.53	52.83	75.49	33.31	42.27	12.85	2.14
125	6.50	33.00	114.00	29.40	9.95	84.50	53.25	65.23	62.64	47.28	27.48	42.53	74.64	37.03	38.12	13.10	2.13
126	7.50	33.00	103.00	32.35	14.40	125.10	78.60	63.88	62.81	46.10	25.67	49.49	79.40	35.40	40.60	13.36	1.88
127	7.50	34.50	114.00	32.10	28.05	124.20	73.85	74.70	59.57	49.27	27.32	41.62	75.16	33.76	41.74	12.45	2.35
128	7.00	32.50	114.00	30.35	14.50	120.90	68.85	64.87	56.93	50.08	28.04	45.11	72.06	33.78	41.49	12.67	2.29
129	5.50	35.50	114.00	28.45	15.20	154.85	76.20	90.38	49.14	51.04	27.24	50.65	80.59	31.92	43.05	12.57	2.63
130	7.00	33.00	108.50	32.90	14.20	106.50	65.35	66.10	61.25	48.57	27.72	31.77	64.66	30.22	45.50	12.12	2.29
131	6.50	34.00	114.00	35.35	25.30	95.40	59.25	75.86	62.08	48.77	28.15	38.34	71.26	35.65	38.66	12.67	2.47
132	6.50	35.00	103.00	32.70	12.00	119.30	65.80	58.67	55.02	48.47	26.70	60.86	84.12	33.08	43.13	12.29	2.16

Appendix B. cont.

ENTRY	DE	DFL	DMt	HKW	SPYD	PY	SEED Y	SMK	SH	OIL	PRO	Fe	Zn	Linoleic	Oleic	Palmitic	Stearic
133	6.00	36.00	114.00	43.00	17.80	89.10	47.60	89.92	53.42	46.46	26.71	48.24	82.45	35.20	40.62	13.04	2.17
134	7.50	34.50	114.00	32.50	14.55	134.70	90.55	76.99	67.17	51.22	26.25	49.97	66.45	35.34	40.84	12.72	2.36
135	9.00	37.00	114.00	32.20	18.00	143.40	81.20	92.12	56.62	49.78	25.81	44.38	77.65	31.94	42.71	12.74	2.31
136	7.50	34.00	114.00	30.45	11.10	83.10	59.45	70.68	71.36	48.19	26.23	57.08	81.43	31.08	44.67	12.38	2.10
137	7.00	34.00	114.00	33.40	24.00	144.00	77.10	52.79	53.54	46.78	26.55	44.50	75.50	29.21	46.07	12.57	1.98
138	7.00	34.50	108.50	25.25	8.90	66.95	38.20	67.86	57.14	47.33	26.45	49.14	87.20	32.61	44.79	12.85	1.89
139	6.50	33.00	114.00	33.20	15.05	99.15	60.50	78.27	60.76	46.80	27.11	39.54	84.90	34.32	40.25	13.14	2.07
140	6.50	32.50	108.50	34.15	11.35	94.95	45.70	76.16	47.95	47.50	27.69	42.67	71.39	30.92	44.51	12.48	2.14
141	7.00	34.00	114.00	30.75	14.85	97.45	55.70	72.93	57.08	47.91	26.66	46.01	78.43	33.35	41.72	12.94	2.20
142	7.00	31.00	114.00	39.60	19.40	175.00	103.10	70.51	58.91	49.38	27.11	45.98	70.95	36.95	37.81	13.43	2.37
143	7.00	32.00	108.50	37.55	17.03	148.65	71.80	42.29	48.22	48.31	26.42	44.44	67.92	34.48	40.74	13.11	2.31
144	10.00	35.00	114.00	37.20	6.30	31.50	24.40	49.59	77.46	50.67	27.76	41.29	70.12	29.62	45.79	12.38	2.44
145	7.00	32.50	108.50	36.45	14.90	129.80	40.30	77.79	31.07	50.51	27.32	48.93	71.21	32.04	43.64	12.54	2.35
146	7.00	35.50	114.00	31.80	17.00	172.70	66.80	79.74	38.64	48.06	26.71	46.27	84.54	36.18	38.43	13.30	2.08
147	7.00	34.50	114.00	30.30	10.60	150.60	54.00	73.69	35.97	47.64	26.61	46.16	75.50	33.85	40.47	12.78	2.21
148	6.50	36.00	114.00	29.50	20.30	97.65	58.80	60.80	60.23	48.79	27.18	50.20	71.41	37.14	37.83	12.96	2.33
149	7.00	34.50	114.00	28.85	14.45	151.90	83.10	54.30	54.71	47.34	26.19	43.59	81.77	33.37	42.52	12.92	1.97
150	8.00	34.00	114.00	30.30	20.15	128.10	76.35	81.60	59.55	49.61	26.53	51.14	85.07	36.37	38.28	12.90	2.41
151	7.00	33.00	114.00	29.00	17.70	69.75	36.25	66.61	52.10	46.80	27.25	46.90	79.39	31.40	44.73	12.27	2.10
152	7.00	32.50	108.50	30.35	17.50	94.85	52.00	85.11	54.66	46.95	26.92	49.59	75.25	33.23	42.87	12.80	2.13
153	8.00	35.00	103.00	20.10	5.40	54.60	35.80	58.10	65.57	44.84	27.71	41.16	71.20	33.43	42.88	13.12	1.73
154	7.00	36.00	114.00	29.20	12.00	83.60	45.50	54.51	54.43	46.81	27.20	42.91	79.48	31.32	44.24	12.86	2.16
155	7.00	33.50	114.00	34.40	14.40	76.50	48.55	83.29	63.41	49.34	27.31	35.87	72.69	34.88	39.71	13.09	2.45
156	6.50	32.00	108.50	34.20	16.80	114.60	65.85	67.27	57.41	47.05	26.74	42.69	75.63	33.21	43.01	12.57	2.08
157	6.00	32.50	108.50	29.15	14.30	111.45	57.50	60.26	51.45	46.44	25.98	40.58	77.92	32.92	43.22	12.62	1.89
158	6.50	33.50	108.50	27.60	13.60	87.75	46.15	66.79	52.42	47.85	26.32	48.33	84.01	33.83	41.86	13.17	2.03
159	7.50	34.00	108.50	30.45	13.00	89.10	44.05	77.71	49.47	48.28	27.70	42.22	75.71	33.61	41.58	12.87	2.12

Appendix B. cont.

ENTRY	DE	DFL	DMt	HKW	SPYD	PY	SEED Y	SMK	SH	OIL	PRO	Fe	Zn	Linoleic	Oleic	Palmitic	Stearic
160	8.00	33.00	114.00	33.60	11.10	89.00	55.00	89.09	61.80	50.80	29.45	41.44	78.41	33.26	40.59	12.21	2.83
161	7.50	33.00	114.00	29.85	15.80	91.00	45.00	77.93	49.36	48.62	26.88	53.22	81.91	32.47	43.71	12.74	2.00
162	7.00	36.50	114.00	32.05	17.10	131.65	70.20	69.33	53.86	48.58	26.91	41.61	78.14	34.44	41.25	12.50	2.26
163	7.50	34.00	114.00	29.40	23.25	148.50	59.40	74.04	40.11	48.93	27.84	43.13	78.73	37.22	37.38	12.91	2.33
164	6.00	34.00	114.00	31.20	6.80	61.50	41.70	86.33	67.80	46.95	26.94	54.89	59.64	37.38	37.80	12.71	2.36
165	7.50	35.00	108.50	31.20	17.20	69.55	45.05	45.21	64.75	53.75	27.66	41.12	71.12	38.82	35.35	12.73	2.78
166	6.50	35.00	114.00	34.10	21.95	133.50	77.30	72.09	58.09	48.69	27.29	54.57	83.74	39.11	34.81	13.04	2.50
167	8.00	32.50	114.00	37.05	13.35	116.85	62.15	76.57	53.20	46.90	25.45	42.93	74.71	32.37	44.34	13.01	1.94
168	7.00	34.00	108.50	26.15	8.90	62.35	28.25	66.48	46.49	46.64	26.10	41.73	75.67	31.01	45.31	12.52	1.99
169	7.50	31.50	103.00	34.85	16.70	136.60	80.55	63.74	59.15	48.14	26.57	35.18	66.54	28.72	47.04	12.17	2.25
170	6.00	32.50	114.00	37.30	25.10	159.10	104.00	74.14	65.32	49.22	28.29	39.44	72.34	38.29	36.07	12.83	2.48
171	8.00	34.50	114.00	32.60	18.35	116.90	56.10	75.68	48.07	47.68	27.54	54.03	76.00	34.32	40.97	12.40	2.33
172	7.00	33.00	114.00	35.45	18.70	109.80	70.95	73.50	64.46	49.48	26.61	47.05	78.37	32.82	42.60	12.18	2.41
173	6.50	33.00	114.00	28.50	18.00	141.60	75.50	73.76	53.32	48.43	26.37	41.86	77.65	33.70	42.50	13.20	2.19
174	7.00	35.50	114.00	34.95	14.50	125.30	83.25	90.72	66.40	51.10	26.86	42.23	73.82	33.86	41.58	12.31	2.34
175	7.50	34.00	114.00	35.25	15.75	110.50	66.85	68.68	60.49	49.47	27.64	40.50	82.05	35.98	38.97	12.47	2.41
176	6.50	32.50	108.50	31.60	17.75	126.95	50.65	56.57	39.89	47.98	28.09	39.53	79.58	36.35	38.77	12.49	2.31
177	7.00	32.00	108.50	33.75	19.80	129.50	67.95	68.25	52.36	46.47	26.31	41.42	73.50	35.84	40.19	13.10	1.89
178	6.50	31.00	114.00	32.05	17.80	140.35	86.35	60.95	61.51	47.42	27.22	42.97	66.97	35.48	39.79	13.07	2.11
179	6.00	34.50	114.00	31.40	22.95	151.45	91.65	57.30	60.50	48.28	26.43	48.38	74.85	32.06	43.56	12.86	2.08
180	7.00	34.00	103.00	30.70	11.50	80.80	46.90	89.13	58.04	45.78	27.35	51.79	75.38	33.54	41.74	13.03	2.06
181	6.00	33.50	103.00	29.90	12.70	81.75	50.00	63.59	59.73	46.25	27.43	42.50	82.22	32.58	42.54	13.04	2.02
182	6.50	34.50	108.50	33.10	17.00	93.10	59.25	61.94	63.79	45.47	26.30	34.81	66.43	30.38	45.57	12.34	1.98
183	7.00	32.50	114.00	32.10	28.50	162.00	75.65	60.21	46.80	49.11	27.89	48.35	81.28	32.28	43.01	11.69	2.45
184	7.00	35.00	114.00	34.10	11.15	60.30	38.30	64.41	63.15	51.03	27.25	50.20	82.65	35.92	38.86	12.89	2.47
185	6.50	34.00	108.50	31.40	19.70	127.90	81.90	72.20	64.22	50.08	27.18	41.34	80.08	35.89	40.20	12.44	2.21
186	8.00	34.00	114.00	32.35	17.80	84.30	53.25	69.29	63.19	49.72	27.36	43.81	73.44	38.52	36.72	12.46	2.36

Appendix B. cont.

ENTRY	DE	DFL	DMt	HKW	SPYD	PY	SEED Y	SMK	SH	OIL	PRO	Fe	Zn	Linoleic	Oleic	Palmitic	Stearic
187	6.50	33.00	114.00	31.65	26.15	89.45	48.00	71.70	52.92	48.82	26.76	45.74	69.41	34.82	40.26	12.77	2.31
188	6.00	35.00	108.50	29.50	16.85	145.70	66.10	91.55	45.40	47.35	27.46	49.46	75.67	32.58	42.34	12.39	2.15
189	6.00	33.00	103.00	33.80	10.00	80.70	48.90	63.80	60.59	48.12	26.90	45.51	78.90	35.17	40.58	12.63	2.13
190	7.50	34.50	114.00	35.65	23.35	113.05	69.90	60.30	61.82	48.63	26.11	48.72	76.07	36.37	38.25	13.68	2.38
191	7.50	33.00	108.50	27.85	16.80	67.95	46.60	52.43	68.72	51.41	27.59	40.88	77.66	29.37	45.79	12.61	2.84
192	8.00	32.00	114.00	28.30	15.30	61.20	47.10	51.59	76.96	51.96	27.38	49.21	80.31	35.40	39.97	12.55	2.56
193	6.50	34.00	114.00	34.75	17.30	113.40	74.90	80.63	66.04	46.86	27.29	48.87	87.47	31.14	44.55	12.77	2.01
194	6.50	32.00	114.00	31.85	22.65	73.65	42.65	68.88	57.79	48.95	27.14	41.12	76.32	28.62	46.92	11.91	2.44
195	7.50	34.50	114.00	29.65	24.30	150.90	91.50	70.25	60.75	49.26	27.62	39.85	82.85	37.00	38.78	13.00	2.09
196	5.50	33.50	103.00	25.55	15.50	148.80	90.25	67.76	60.71	49.90	27.61	39.57	80.67	34.16	41.15	12.58	2.28
197	7.50	35.00	114.00	34.60	17.70	186.80	109.25	70.02	58.45	48.02	26.93	44.64	71.18	31.30	44.80	12.52	2.23
198	7.50	37.00	114.00	33.95	21.20	99.45	47.60	83.40	48.05	50.39	27.32	45.24	75.33	37.53	38.07	12.95	2.33
199	7.50	35.50	114.00	28.05	9.15	51.15	29.40	70.90	59.22	50.54	26.46	43.76	80.93	38.80	37.80	13.34	2.09
200	6.50	34.50	114.00	29.55	16.75	113.20	80.75	90.83	70.78	47.73	26.37	38.66	66.48	39.14	36.34	13.49	2.07
201	7.00	33.50	114.00	35.50	27.70	108.50	66.90	67.19	61.59	50.47	27.50	42.98	69.26	33.35	42.66	12.83	2.27
202	6.50	33.50	108.50	35.75	18.45	112.25	71.15	75.72	63.32	49.19	27.48	57.24	81.55	34.84	40.92	12.74	2.35
203	8.00	36.50	114.00	26.35	16.55	108.10	46.35	63.48	42.78	48.53	26.23	40.56	78.35	36.06	39.46	13.06	2.07
204	7.00	35.00	108.50	27.55	18.55	150.65	93.00	52.86	61.74	46.73	26.13	40.56	69.04	32.60	42.28	12.66	2.12
205	7.50	33.50	108.50	32.15	24.50	153.50	78.50	73.62	51.53	48.66	26.82	48.25	79.26	33.60	42.52	12.55	2.18
206	7.00	32.00	114.00	39.55	20.80	144.95	86.70	89.23	59.76	49.66	27.95	36.53	68.45	31.67	44.08	11.96	2.39
207	6.50	31.50	114.00	31.65	23.40	145.00	87.30	78.76	59.92	48.16	27.32	39.75	79.07	32.94	42.53	12.58	2.17
208	7.50	33.50	108.50	30.55	19.00	92.80	62.30	65.96	67.17	47.11	26.92	51.53	77.50	36.97	37.99	12.91	2.15
209	6.00	36.00	114.00	27.45	12.35	77.15	44.60	78.71	58.07	47.59	26.84	42.86	83.32	34.09	41.26	12.72	2.09
210	7.00	32.00	114.00	22.70	18.70	56.10	39.60	48.23	70.59	50.33	26.99	48.63	75.25	37.79	37.22	12.91	2.19
211	7.50	33.00	108.50	29.90	29.65	105.35	62.10	75.42	59.14	49.45	27.57	38.49	80.77	31.45	44.50	12.76	2.07
212	8.00	34.00	114.00	38.00	14.10	98.70	51.80	93.82	52.48	47.52	28.55	38.78	72.63	28.39	47.08	11.39	2.28
213	7.00	33.00	114.00	23.20	20.10	100.80	62.80	73.25	62.30	48.63	27.93	48.63	82.38	36.67	37.85	13.17	2.19

Appendix B. cont.

ENTRY	DE	DFL	DMt	HKW	SPYD	PY	SEED Y	SMK	SH	OIL	PRO	Fe	Zn	Linoleic	Oleic	Palmitic	Stearic
214	7.00	35.00	114.00	34.80	26.00	103.60	50.10	73.45	48.36	49.24	26.85	48.88	76.36	35.40	40.06	13.04	2.27
215	6.50	33.50	114.00	32.00	17.75	145.25	87.45	90.84	60.19	47.88	26.32	43.40	79.14	32.37	43.22	12.64	2.05
216	7.00	35.00	114.00	31.15	18.75	146.85	77.20	81.57	52.54	51.56	26.77	45.18	70.54	35.86	40.41	12.41	2.29
217	7.00	32.00	114.00	28.30	42.00	167.80	102.80	68.97	61.26	49.42	27.53	39.22	70.94	35.72	39.66	12.35	2.44
218	8.00	34.00	114.00	26.70	17.00	68.30	37.90	57.52	55.49	49.25	27.26	44.69	76.02	35.73	39.30	12.64	2.39
219	7.50	34.00	114.00	23.95	14.25	54.60	30.35	64.38	55.96	49.03	27.00	46.52	80.14	31.63	44.15	13.02	2.10
220	7.50	32.50	108.50	26.85	21.25	55.75	23.65	69.81	42.32	49.45	26.92	39.56	75.05	30.30	45.70	12.75	2.33
221	7.00	36.00	114.00	29.40	17.70	81.95	44.95	58.73	54.76	49.87	26.63	43.46	69.62	28.01	48.16	11.99	2.31
222	7.50	36.00	108.50	27.80	18.30	125.80	74.00	58.02	58.75	49.09	27.34	42.84	79.15	31.94	43.93	12.55	2.34
223	8.00	34.00	114.00	30.55	17.05	146.35	94.90	86.36	64.82	48.83	27.22	55.30	80.45	35.87	38.93	12.85	2.32
224	7.00	33.00	108.50	29.80	22.05	83.05	49.95	77.79	60.10	48.71	27.54	44.55	78.45	35.20	39.80	13.19	2.14
225	6.50	34.00	108.50	27.70	26.50	131.70	72.35	74.19	54.91	48.57	27.54	47.52	73.36	35.95	39.62	12.80	2.11
226	8.50	36.00	114.00	28.95	17.10	127.60	78.20	69.31	61.38	47.52	26.89	47.33	81.80	35.80	39.32	12.81	2.21
227	6.00	30.00	114.00	33.70	9.30	74.70	42.40	84.20	56.76	48.58	26.54	42.57	73.45	33.51	42.13	12.72	2.21
228	6.50	33.00	114.00	29.50	12.75	93.75	43.75	95.32	46.67	49.52	27.00	52.42	73.06	31.81	43.46	12.39	2.23
229	7.50	33.50	108.50	26.55	11.90	79.60	47.65	52.63	59.85	47.63	26.73	36.07	71.94	34.90	39.60	12.78	1.97
230	7.50	34.50	103.00	29.80	17.55	129.90	84.25	54.63	64.88	46.93	26.43	38.22	72.85	36.29	39.08	12.97	1.94
231	8.00	34.00	114.00	26.40	7.20	50.80	34.30	87.76	67.52	50.26	27.05	43.50	76.53	29.79	46.08	12.48	2.14
232	8.00	33.00	114.00	29.00	11.30	45.50	30.20	60.60	66.37	51.34	28.20	42.92	79.55	28.43	46.05	11.67	3.46
233	7.00	33.00	103.00	23.80	13.30	66.60	45.90	56.86	68.92	47.72	26.47	50.69	78.91	40.21	35.53	13.93	1.87
234	7.00	35.00	108.50	23.20	11.65	75.25	40.30	67.43	52.87	44.96	27.77	40.88	77.50	35.64	39.84	13.13	1.92
235	7.00	33.50	108.50	26.75	12.65	92.70	48.70	87.71	52.45	48.15	27.83	53.03	80.64	32.49	42.80	12.48	2.30
236	8.00	33.50	108.50	31.05	12.90	116.15	75.65	69.47	65.08	51.92	28.39	48.67	83.01	36.28	39.34	12.86	2.51
237	6.00	33.00	108.50	31.70	15.30	106.90	65.35	69.80	61.39	48.27	27.05	39.76	72.60	37.42	37.18	12.99	2.31
238	7.00	33.00	114.00	27.55	18.60	94.45	65.75	61.95	69.57	47.05	27.05	39.52	73.46	36.22	38.89	13.30	2.05
239	7.00	32.50	108.50	31.00	11.90	106.85	72.30	69.75	67.69	45.93	25.51	40.57	75.75	37.26	38.80	13.09	1.79
240	6.50	35.00	103.00	33.05	11.60	106.15	67.55	70.11	63.53	47.72	27.00	56.41	71.36	35.67	39.57	12.76	2.12

Appendix B. cont.

ENTRY	DE	DFL	DMt	HKW	SPYD	PY	SEED Y	SMK	SH	OIL	PRO	Fe	Zn	Linoleic	Oleic	Palmitic	Stearic
241	7.00	35.00	114.00	36.90	25.75	166.65	106.45	80.18	64.10	48.79	27.02	41.45	74.04	35.38	40.06	12.76	2.22
242	7.00	33.50	114.00	30.60	17.95	184.65	131.50	75.26	71.50	50.46	26.54	58.93	79.07	31.96	43.64	12.50	2.26
243	7.50	35.00	114.00	31.55	18.35	158.90	87.40	87.46	55.03	48.42	27.41	43.93	81.78	35.93	39.03	12.72	2.36
244	7.50	36.00	108.50	26.75	12.40	67.30	28.35	60.11	42.65	47.12	26.91	57.49	86.93	33.48	42.65	13.47	1.80
245	7.50	34.00	114.00	34.70	23.60	165.15	82.60	64.33	47.93	48.65	27.52	51.40	81.41	36.33	38.67	12.75	2.35
246	7.00	31.50	108.50	32.55	14.05	111.70	71.00	69.46	64.08	48.67	26.44	39.58	73.43	35.55	40.33	12.86	2.05
247	8.00	36.00	103.00	21.00	5.80	55.40	37.20	73.66	67.15	45.33	27.88	52.54	75.00	34.85	39.54	13.04	1.96
248	8.00	34.00	114.00	23.60	9.00	63.10	41.20	81.31	65.29	48.86	26.73	45.40	72.51	34.70	40.41	12.81	2.14
249	6.00	33.00	103.00	30.80	13.60	89.30	40.40	54.21	45.24	46.64	25.87	37.16	75.30	33.83	42.18	13.15	1.92
250	6.50	32.50	103.00	36.45	9.00	91.15	56.80	74.54	62.22	45.32	27.76	40.68	76.67	29.37	45.90	12.37	2.10
251	6.50	33.00	103.00	33.75	12.70	115.45	67.25	53.30	58.22	44.91	26.41	40.62	78.62	32.27	43.92	12.68	1.85
252	5.50	32.00	108.50	27.70	9.00	84.40	32.50	64.88	38.43	48.05	28.23	34.46	64.50	27.65	46.98	12.30	2.61
253	7.00	33.43	114.00	31.69	22.54	130.04	69.43	74.51	52.56	49.37	27.07	47.22	79.97	35.44	40.07	12.61	2.24
254	6.25	32.50	104.38	31.36	11.43	96.21	51.91	62.64	53.22	46.09	27.36	38.90	74.05	30.67	44.75	12.55	2.13

Where,

DE : Days to Emergence
DF : Days to Flowering
DMt : Days to Maturity
HKW : Hundred Kernel Weight
SPYD : Single Plant Yield
PY : Pod Yield per plot
SEED Y : Seed Yield per plot
SMK : Sound Mature Kernel Percentage
SH : Shelling Percentage
OIL : Oil Content
PRO : Protein Content
Fe : Kernel iron concentration
Zn : Kernel zinc concentration
Linoleic : Linoleic acid content
Oleic : Oleic acid content
Palmitic : Palmitic acid content
Stearic : Stearic acid content

Appendix A. Reagents required for DNA extraction

S.No.	Chemicals/Reagents	Chemical composition / Remark
1	3% CTAB (Cetyl Trimethyl Ammonium Bromide) buffer	10 mM Tris 1.21 g 1.4 M NaCl 8.18 g 20 mM EDTA 0.745 g 3% CTAB 3.0 g Distilled water 100 ml Adjust to pH 8.0 using HCl. Add 0.17 ml mercapto ethanol only at the time of keeping the buffer in boiling water.
2	Chloroform:Isoamyl Alcohol (24:1)	Chloroform 96 ml Isoamyl alcohol (IAA) 4 ml Store in dark at room temperature.
3	Isopropanol	Keep Isopropanol at –20°C. Use only ice cold Isopropanol.
4	RNase A (10 mg/ml)	Dissolve 100 mg of pancreatic RNase A in 100 ml of 10 mM Tris (pH 7.5) and 15 mM NaCl. Heat in boiling water bath for 15 minutes and allow cooling slowly to room temperature. Dispense into aliquots and store at –20°C. Working stocks may be stored at 4°C.
5	Phenol:Chloroform: Isoamyl Alcohol (25:24:1)	Phenol (equilibrated) 50 ml Chloroform: IAA (24:1) 50 ml, Store at 4°C.
6	Sodium Acetate (3 M, pH 5.2)	Dissolve 40.824 g of sodium acetate in 60 ml distilled water and adjust to pH 5.2 using glacial acetic acid. Make the volume up to 100 ml with distilled water and autoclave.
7	Absolute Ethanol	Store at –20°C
8	70% Ethanol	Absolute ethanol 70 ml Distilled water 30 ml
9	$T_1E_{0.1}$ Buffer	10 mM Tris 121 g 1 mM EDTA 0.0372 g

		Distilled water 100 ml
10	$T_{10}E_1$ Buffer	0.5 M Tris 6.050 g 0.5 M EDTA 9.306 g 2 M NaCl 11.688 g Distilled water 100 ml

Tables

Table 4.1. Analysis of variance for different characters using alpha lattice design in $F_{2:3}$ mapping population of the cross ICGV 06099 × ICGV 93468 in groundnut during rainy season, 2013

Sources of variation	Replication	Replication/Block	Genotypes	Error
df	1	50	259	209
Days to emergence	8.11**	1.19**	0.68	0.71
Days to 75 % flowering	7.23	5.73**	2.84**	2.19
Days to maturity	32.39	33.89**	23.09	18.73
100-kernel weight (g)	1.45	21.54*	20.40*	14.66
Single plant yield (g)	1980.80**	93.28**	73.66**	55.35
Pod yield (g $plot^{-1}$)	443.75*	1042.38**	1673.52**	78.53
Kernel yield (g $plot^{-1}$)	43.12	377.27**	570.75**	85.61
Sound mature kernel percentage (%)	10582.90**	162.10	204.90**	146.10
Shelling Percentage (%)	102.36	56.32*	99.84**	35.09
Oil content (%)	12.90*	4.55*	4.38*	3.27
Protein content (%)	0.40	1.99	0.93	1.03
Kernel iron concentration(mg kg^{-1})	11.4	50.37**	44.81**	20.39
Kernel zinc concentration(mg kg^{-1})	0.79	42.11**	37.42**	17.84
Oleic acid (%)	8.42	14.35	14.29*	10.90
Linoleic acid (%)	7.30	10.26	11.24**	7.84
Palmitic acid (%)	0.12	0.27	0.25	0.21
Stearic acid (%)	0.15	0.05	0.09**	0.06

Where, df – Degrees of freedom

Table 4.3. Descriptive statistics of the parents and $F_{2:3}$ mapping population of the cross ICGV 06099 × ICGV 93468 in groundnut

Character	Mean of the parental lines		$F_{2:3}$ mapping population				
	P_1	P_2	Mean	Range	CV (%)	S.Em	Skewness
Days to emergence	7.00	6.00	7.00	5.00-10.00	12.42	0.04	0.385
Days to 75 % flowering	34.00	33.00	34.00	30.00-39.00	5.06	0.07	-0.110
Days to maturity	114.00	104.00	111.00	103.00 -114.00	4.28	0.22	-1.158
100-kernel weight	33.01	31.36	31.65	19.20 -43.00	13.57	0.19	-0.062
Single plant yield (g)	23.67	11.43	18.24	4.08-78.60	46.91	0.39	1.880
Pod yield per plot (g $plot^{-1}$)	133.58	96.21	104.20	25.60 -244.00	37.44	1.79	0.614
Kernel yield per plot (g $plot^{-1}$)	74.87	51.91	59.67	13.94 -158.50	37.62	1.03	0.802
Sound mature kernel percentage (%)	70.37	62.64	69.47	28.81-95.72	21.66	0.69	-0.270
Shelling percentage (%)	55.55	53.22	58.06	30.49-84.80	5.50	0.39	-0.278
Oil content (%)	49.23	46.09	48.53	43.49-59.61	4.13	0.09	0.714
Protein Content (%)	27.08	27.36	27.22	24.89-29.75	3.84	0.04	-0.221
Kernel iron concentration (mg kg^{-1})	52.50	37.30	45.42	31.77-61.41	13.49	0.28	0.516
Kernel zinc concentration (mg kg^{-1})	79.50	65.00	76.74	59.64-90.40	7.30	0.26	-0.115
Oleic acid (%)	40.04	44.75	41.31	31.66-53.93	8.75	0.17	0.377
Linoleic acid (%)	35.50	30.67	34.08	24.04-42.17	9.24	0.67	-0.495
Palmitic acid (%)	12.63	12.55	12.73	11.01-14.38	3.86	0.02	-0.042
Stearic acid (%)	2.24	2.13	2.22	1.54-3.65	12.93	0.01	0.912

Note: CV–Coefficient of Variation; **S.Em–**Standard Error of mean; **P_1–** ICGV 06099; **P_2–** ICGV 93468

Table 4.4. Estimates of various genetic parameters for different traits and kernel nutrient parameters in $F_{2:3}$ population of the cross ICGV 06099 × ICGV 93468 in groundnut

Character	PCV %	GCV %	h^2_b	GA	GAM
Days to emergence	2.38	1.88	62.36	0.83	3.06
Days to 75 % flowering	4.69	1.68	30.60	0.41	1.24
Days to maturity	3.93	3.15	64.44	5.81	5.22
100-kernel weight (g)	13.22	5.35	37.00	1.41	4.46
Single plant yield (g)	34.45	32.04	86.45	11.19	61.37
Pod yield (g $plot^{-1}$)	28.40	27.10	91.00	55.50	53.26
Kernel yield (g $plot^{-1}$)	30.35	26.10	73.91	27.58	46.22
Sound mature kernel weight percentage (%)	18.23	9.59	27.66	7.21	10.39
Shelling Percentage (%)	14.14	9.80	47.98	8.11	13.98
Oil content (%)	10.72	4.09	44.59	0.58	3.22
Protein content (%)	2.30	1.93	70.40	0.91	3.34
Kernel iron concentration(mg kg^{-1})	12.60	7.71	64.24	4.40	9.72
Kernel zinc concentration(mg kg^{-1})	6.84	4.07	62.21	3.83	4.99
Oleic acid (%)	8.59	3.15	31.81	0.98	2.38
Linoleic acid (%)	9.06	3.82	39.42	1.13	3.32
Palmitic acid (%)	5.05	3.52	48.73	0.64	5.07
Stearic acid (%)	12.91	5.29	37.74	0.09	4.46

Note: PCV – Phenotypic Coefficient of Variation; **GCV –** Genotypic Coefficient of Variation; h^2_b**–**Heritability (broad sense); **GA–** Genetic Advance; **GAM–** Genetic Advance as per cent of Mean.

Table 4.5. Simple correlations among various characters in $F_{2:3}$ mapping population of the cross ICGV 06099 × ICGV 93468 in groundnut

	DE	DF	DMT	HSW	SPYD	PY	KY	SMK %	SH %	OC	PC	Fe	Zn	OAC	LAC	PALM	SAC
DE	-	0.241**	0.132**	-0.060	-0.011	-0.016	-0.018	0.036	0.049	0.095	0.021	0.074	0.060	-0.093	0.092	-0.003	0.069
DF		-	0.167**	-0.084	0.054	0.033	-0.030	-0.019	-0.164**	0.071	0.022	0.093	0.149**	-0.111	0.109*	0.050	0.013
DMT			-	0.202**	0.307**	0.164**	0.158**	0.157**	-0.001	0.286**	0.008	0.057	0.058	-0.175**	0.143**	0.020	0.286**
HSW				-	0.158**	0.340**	0.352**	0.245**	0.014	0.002	0.032	-0.028	0.008	-0.020	0.001	-0.173**	0.152**
SPYD					-	0.323**	0.298**	-0.169**	-0.063	0.263**	0.055	-0.001	-0.067	0.004	-0.009	-0.122*	0.202**
PY						-	0.915**	0.224**	-0.278**	-0.127**	-0.041	-0.060	-0.045	-0.245**	0.239**	-0.044	-0.039
KY							-	0.192**	0.097*	-0.132**	-0.067	-0.068	-0.049	-0.267**	0.266**	-0.014	-0.030
SMK %								-	-0.094	-0.078	-0.082	0.132**	0.093	-0.029	0.041	-0.071	-0.005
SH %									-	0.060	-0.047	-0.001	-0.021	-0.017	0.027	0.050	0.098*
OC										-	0.094	0.082	-0.006	-0.066	0.031	-0.226**	0.765**
PC											-	-0.08	0.074	-0.176**	0.053	-0.245**	0.373**
Fe												-	0.302**	-0.022	0.043	0.049	0.019
Zn													-	-0.049	0.041	0.039	0.038
OAC														-	-0.970**	-0.576**	-0.101*
LAC															-	0.622**	-0.026
PALM																-	-0.384
SAC																	-

Note: DE: Days to Emergence**DF:** Days to Flowering;**DMT:** Days to Maturity; **HSW:** Hundred Seed Weight (g); **SPYD:** Single Plant Yield (g); **PY:** Pod Yield per plot (g); **KY:**KernelYield per plot (g); **SMK %:** Sound Mature Kernel Percentage; **SH %:** Shelling Percentage; **OC:** Oil Content (%); **PC:** Protein Content (%); **Fe:** Kernel iron concentration (mg kg^{-1}); **Zn:** Kernel zinc concentration (mg kg^{-1}); **OAC:** Oleic Acid Content (%); **LAC:** Linoleic Acid Content (%); **PALM:**Palmitic acid content (%); **SAC:** Stearic acid Content (%).

*** –** Significance at 5 % level (0.0962); ******- Significance at 1 % level (0.1262)

Table 4.8. Analysis of variance for different characters of six generations of two crosses in groundnut

sources of variation	df	Days to emergence	Days to flowering	Days to maturity	100-kernel weight (g)	Shelling Percentage (%)	Sound mature kernel percentage (%)	Pod yield per plant (g)	Kernel iron concentration (mg kg^{-1})	Kernel zinc concentration (mg kg^{-1})
Analysis of variance between crosses										
Rep	2	0.10	0.12	1.99	0.53	1.03	1.64	0.40	0.07	1.45
Cross	1	1.372803 ns	0.7744 ns	24.2720*	3.3856*	4.80340 ns	0.5041 ns	2.783336*	6.881878*	14.65614*
Error	2	0.07	0.10	0.68	0.18	0.43	1.76	0.06	0.12	0.54
Analysis of variance between generations within crosses										
ICGV 06040 × ICGV 87141										
Rep	2	6.00	3.84*	6.33	7.99	48.50*	0.30	-27.24	6.23	52.95*
Gen	5	12.06*	1.47 ns	133.75**	57.56**	144.89**	108.82 ns	93.47*	21.17**	72.76**
Error	10	3.03	0.73	5.36	9.99	9.53	61.89	20.69	2.38	8.66
ICGV 06099 × ICGV 93468										
Rep	2	0.34	2.20*	88.98*	11.04	36.16	14.85	-23.42	0.39	18.61
Gen	5	3.25**	1.01 ns	325.11**	42.10*	64.09 ns	43.42 ns	76.35**	16.80*	36.09*
Error	10	0.33	0.37	19.36	10.05	28.67	21.35	9.80	4.22	10.41

Note: Rep – Replication Mean Sum of Squares; Cross – Crosses Mean Sum of Squares; Gen – Genotypic Mean Sum of Squares; Error – Error Mean Sum of Squares.

* – Significance at 5 % level

** –Significance at 1 % level

NS – Non-Significant

Table 4.9. Mean performance of six generations each of two crosses of groundnut for different characters

Character	ICGV 06040 × ICGV 87141													
	P_1		P_2		F_1		F_2		B_1		B_2		S.Em. ±	C.D. (p=0.05)
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range		
DE	11.27	11.0-12.0	15.38	13.0-18.0	12.20	10.00	14.34	11.0-17.0	15.25	12.0-17.0	15.80	11.0-17.0	1.01	3.17
DF	47.94	47.0-51.0	49.61	48.0-53.0	48.80	46.0-51.0	49.83	43.0-56.0	49.46	46.0-55.0	48.37	45.0-56.0	0.49	NS
DM	159.00	159.0	142.00	142.00	159.00	159.00	158.34	142.0-159.0	158.33	157.0-159.0	155.81	142.0-159.0	1.34	4.21
HKW (g)	44.53	31.4-52.3	34.82	30.2-51.5	43.94	31.0-54.5	43.00	22.7-73.9	44.54	22.2-68.8	36.49	23.1-57.1	1.82	5.75
SH (%)	72.54	64.0-85.4	57.74	46.9-65.4	75.40	57.0-87.9	66.07	34.9-86.2	61.05	44.5-76.6	65.51	45.6-85.9	1.78	5.62
SMK (%)	64.70	39.2-82.1	55.24	27.6-70.9	60.85	34.2-77.9	67.50	16.4-95.7	54.38	19.6-86.7	68.88	36.8-84.8	4.54	NS
PY(g)	24.82	15.2-45.7	31.09	16.5-53.8	31.67	14.5-53.0	31.40	7.6-86.3	39.99	15.4-100.3	34.12	11.0-105.7	2.63	8.28
KIC (mg kg^{-1})	33.32	29.2-38.6	25.54	21.7-27.7	28.49	23.7-32.2	28.38	20.1-39.1	29.42	20.4-41.4	31.49	21.0-42.2	0.89	2.81
KZC (mg kg^{-1})	50.91	45.9-57.4	36.05	27.7-41.9	40.27	28.2-55.2	39.80	22.7-57.9	42.46	27.6-54.2	41.98	27.1-60.3	1.70	5.35
Character	**ICGV 06099 × ICGV 93468**													
DE	11.47	11.0-12.0	10.58	10.0-11.0	11.25	11.0-12.0	11.26	10.0-14.0	13.70	12.0-17.0	11.80	11.0-13.0	0.33	1.04
DF	47.42	46.0-48.0	46.91	46.0-49.0	46.95	46.0-49.0	47.74	43.0-53.0	47.17	46.0-51.0	47.44	42.0-52.0	0.35	NS
DM	159.00	159.00	133.00	133.00	147.67	142.0-159.0	156.08	142.0-159.0	155.76	142.0-159.0	136.53	133.0-159.0	2.54	8.00
HKW (g)	46.32	26.0-60.5	44.78	39.5-54.5	46.37	30.0-67.4	36.43	26.0-77.7	45.03	28.6-58.7	44.33	23.2-63.6	1.83	5.77
SH (%)	60.17	45.3-64.3	61.26	47.3-77.5	58.98	41.7-73.3	65.32	38.9-90.1	62.29	35.3-78.7	62.77	28.8-89.7	3.09	NS
SMK(%)	65.18	43.7-75.9	57.86	53.4-65.4	67.88	48.4-76.2	71.79	32.4-90.9	67.34	41.3-86.4	58.28	19.6-91.4	2.67	NS
PY(g)	30.71	20.1-66.9	26.77	16.1-46.7	37.51	18.5-68.6	30.82	4.7-91.3	33.50	9.4-66.7	35.90	9.1-71.7	1.81	5.70
KIC (mg kg^{-1})	25.49	20.9-31.1	20.83	15.8-22.6	21.95	18.7-31.1	25.19	17.3-48.9	26.25	16.2-41.2	24.07	17.6-35.2	1.19	3.74
KZC (mg kg^{-1})	36.58	35.0-54.6	30.39	24.5-35.9	32.01	21.9-41.2	35.08	23.4-60.8	37.27	23.7-50.6	32.91	25.0-42.1	1.86	5.87

Note: DE– Days to Emergence; DF– Days to Flowering; DM– Days to Maturity; HKW –100-Kernel Weight; SH %– Shelling percentage; SMK % – Sound Mature Kernel percentage; PY– Pod yield per plant; KIC– Kernel iron concentration; KZC – Kernel Zinc Concentration; NS – Non-Significant

Table 4.10. Estimates of various genetic parameters for different traits including kernel iron and zinc concentrations for two crosses *viz*., ICGV 06040 × ICGV 87141 and ICGV 06099 × ICGV 93468 of groundnut during post-rainy season, 2013-14

Character	PCV %	GCV %	$h^2_{(b)}$	GA	GAM	$h^2_{(n)}$	Degree of dominance
ICGV 06040 × ICGV 87141							
Days to emergence	52.23	32.52	49.79	2.52	17.86	1.19	2.57
Days to flowering	2.02	1.01	24.94	0.51	1.04	0.29	1.82
Days to maturity	1.39	--	88.88	12.71	8.17	2.87	1.14
100- kernel weight (g)	12.33	9.66	61.36	6.43	15.59	0.15	0.86
Shelling percentage (%)	11.14	10.12	82.56	12.57	18.95	0.59	0.45
Sound mature kernel percentage (%)	14.17	6.36	20.18	3.66	5.89	0.02	1.24
Pod yield per plant (g)	20.7	15.21	53.96	7.45	23.01	1.56	2.11
Kernel iron concentration (mg kg^{-1})	10.01	8.52	72.43	4.39	14.93	0.66	1.88
Kernel zinc concentration (mg kg^{-1})	13.11	11.06	71.15	8.03	19.21	0.51	3.67
ICGV 06099 × ICGV 93468							
Days to emergence	9.69	8.38	74.83	1.76	14.94	7.73	1.8
Days to Flowering	1.86	0.86	36.68	0.58	1.22	2.20	1.4
Days to maturity	7.42	6.8	84.04	19.06	12.84	24.68	1.39
100- kernel weight (g)	10.39	7.42	51.52	4.83	10.91	0.60	0.49
Shelling percentage (%)	11.25	6.04	29.16	3.82	6.05	1.09	5.19
Sound mature kernel percentage (%)	10.88	5.34	25.62	2.83	4.66	0.51	1.8
Pod yield per plant (g)	16.7	13.9	69.36	8.08	23.85	0.32	3.18
Kernel iron concentration (mg kg^{-1})	12.01	8.48	49.85	2.98	12.34	0.23	0.78
Kernel zinc concentration (mg kg^{-1})	12.75	8.57	45.12	4.05	11.86	0.19	0.99

Note: PCV – Phenotypic Coefficient of Variation; **GCV –** Genotypic Coefficient of Variation; $h^2_{(b)}$**–**Heritability (broad sense); **GA–** Genetic Advance; **GAM–** Genetic Advance as per cent of Mean; $h^2_{(n)}$**–**Heritability (narrow sense).

Table 4.11. Estimates of heterosis and inbreeding depression for various traits including kernel iron and zinc concentrations for two crosses *viz*., ICGV 06040 × ICGV 87141 and ICGV 06099 × ICGV 93468 of groundnut

Character	Average Heterosis	RHM	Heterobeltiosis	RHB	ID
ICGV 06040 × ICGV 87141					
Days to emergence	-12.20	4.98	5.88	26.59	-19.56
Days to flowering	-0.049	2.24	1.61	3.94*	-2.29
Days to maturity	5.64**	5.21**	11.97**	11.50**	0.41
100- kernel weight (g)	4.43	2.88	5.43	3.87	1.48
Shelling percentage (%)	15.92**	1.70	32.48	-9.60*	12.26
Sound mature kernel percentage (%)	5.81	13.90	-0.34	7.27	-7.63
Pod yield per plant (g)	44.44**	9.02	27.50*	-3.75	24.51
Kernel iron concentration (mg kg^{-1})	-2.72	-3.65	-14.14**	-14.97**	0.95
Kernel zinc concentration (mg kg^{-1})	-4.43	-7.90	-18.57**	-21.53**	3.63
ICGV 06099 × ICGV 93468					
Days to emergence	1.49	0.93	6.25	5.66	0.55
Days to Flowering	-0.14	1.22	-0.94	0.41	-1.37
Days to maturity	1.14	6.90**	11.02**	17.34**	-5.69
100- kernel weight (g)	1.78	-20.02**	3.53	-18.64**	21.42
Shelling percentage (%)	-9.88	2.49	-6.56	6.27	-13.74
Sound mature kernel percentage (%)	11.28	20.47**	4.67	13.31*	-8.24
Pod yield per plant (g)	32.63**	1.65	14.86	-11.96	23.35
Kernel iron concentration (mg kg^{-1})	-3.66	8.20	-14.14	-3.56	-12.32
Kernel zinc concentration (mg kg^{-1})	-6.33	6.49	-16.75*	-5.35	32.84

Note: RHM – Residual Heterosis over Mid parent; **RHB –** Residual Heterosis over Better parent; **ID –** Inbreeding Depression

Table 4.12. Results of scaling tests and genetic components for various traits including kernel iron and zinc concentrations in the cross ICGV 06040 × ICGV 87141 of groundnut

Scaling test	Days to emergence	Days to maturity	100-Kernel weight (g)	Shelling percentage (%)	Pod yield per plant (g)	Kernel iron concentration (mg kg^{-1})	Kernel zinc concentration (mg kg^{-1})
A	7.034±0.76**	-1.302± 0.28**	4.336±3.90	-25.828±3.81**	23.489±6.96**	-2.976±1.57	-6.271±2.82*
B	4.015±1.08**	-7.650±2.27**	-15.835±3.17**	-2.126±4.05	5.478±7.79	8.949±1.80**	7.636±3.25*
C	6.310±1.15**	-2.628±0.91**	-0.792±4.42	-16.781±5.60**	6.346±8.62	-2.335±1.71	-8.305±4.09*

Genetic component	Days to emergence	Days to maturity	100-Kernel weight (g)	Shelling percentage (%)	Pod yield per plant (g)	Kernel iron concentration (mg kg^{-1})	Kernel zinc concentration (mg kg^{-1})
m	14.343±0.12**	164.022±2.45**	53.599±4.96**	66.077±0.80**	5.336±9.63	21.129±2.47**	33.815±3.84**
d	-0.544±0.50	3.825±1.13**	0.508±1.23	-4.452±2.12*	-3.135±1.93	3.891±0.42**	7.431±0.73**
h	3.608±1.23**	-17.694±7.06*	-30.219±13.71*	-0.918±5.79	77.922±27.64**	21.647±6.97**	17.496±10.88
i	4.740±1.12**	-5.022±2.45*	-10.706±4.81*	-11.173±5.32*	22.621±9.43*	8.308±2.44**	9.671±3.77**
j	1.509±0.59	3.825±1.13	10.085±2.35	-11.851±2.38	9.006±4.682	-5.963±1.15	-6.953±1.80
l	-15.789±2.31**	12.672±4.63**	22.205±9.15*	39.127±10.17**	-51.588±19.11**	-14.281±4.61**	-11.036±7.74

*****– Significant at 5% level of probability

**– Significant at 1% level of probability

m – mean *i* – additive × additive

d – additive *j* – additive × dominance

h – dominance *l* – dominance × dominance

Table 4.13. Results of scaling tests and genetic components for various traits including kernel iron and zinc concentrations in the cross ICGV 06099 × ICGV 93468 of groundnut.

Scaling test	Days to emergence	Days to maturity	100-Kernel weight (g)	Pod yield per plant (g)	Kernel iron concentration (mg kg^{-1})	Kernel zinc concentration (mg kg^{-1})
A	4.107±0.76**	6.401±2.91*	-0.795±3.75	-7.855±5.16	5.051±1.67**	5.943±2.46*
B	1.752±0.53**	-9.417±6.48	-3.40±3.39	5.664±5.53	5.371±1.77**	3.421±2.04
C	0.518±0.41	37.528±4.08**	11.891±2.25*	-19.70±7.32*	10.550±2.30**	12.197±3.16**

Genetic component	Days to emergence	Days to maturity	100-Kernel weight (g)	Pod yield per plant (g)	Kernel iron concentration (mg kg^{-1})	Kernel zinc concentration (mg kg^{-1})
m	11.269±0.07**	186.299±6.84**	59.984±4.67**	13.210±7.60	23.294±0.36**	36.324±2.93**
d	1.624±0.45**	12.006±0.72**	-1.193±1.20	1.972±1.90	2.334±0.703**	3.096±0.88**
h	5.561±0.95**	-82.191±20.17**	-32.293±12.61*	46.154±21.03*	8.957±6.56	2.220±8.03
i	5.341±0.94**	-40.543±6.80**	-16.085±4.51**	15.537±7.36*	-0.128±2.32	-2.834±2.79
j	1.178±0.46	7.909±3.35	1.303±2.11	-4.371±3.58	-0.160±1.15	1.261±1.43
l	-11.199±1.84**	43.559±13.71**	20.280±8.83*	-21.851±14.66	-10.295±4.32*	-6.529±5.49

*****– Significant at 5% level of probability

**– Significant at 1% level of probability

m – mean *i* – additive × additive

d – additive *j* – additive × dominance

h – dominance *l* – dominance × dominance

Table 4.14. Comparison of gene actions for various traits in two crosses of groundnut

	ICGV 06099 × ICGV 93468						
Character	**Genetic component**						**Epistasis**
	ICGV 06040 × ICGV 87141						
	m	***d***	***h***	***i***	***j***	***l***	
Days to emergence	14.343±0.12**	-0.544±0.50	3.608±1.23**	4.740±1.12**	1.509±0.59	-15.789±2.31**	Duplicate
Days to maturity	164.022±2.45**	3.825±1.13**	-17.694±7.06*	-5.022±2.45*	3.825±1.13	12.672±4.63**	Duplicate
100-Kernel weight (g)	53.599±4.96**	0.508±1.23	-30.219±13.71*	-10.706±4.81*	10.085±2.35	22.205±9.15*	Duplicate
Shelling percentage (%)	66.077±0.80**	-4.452±2.12*	-0.918±5.79	-11.173±5.32*	-11.851±2.38	39.127±10.17**	Duplicate
Pod yield per plant (g)	5.336±9.63	-3.135±1.93	77.922±27.64**	22.621±9.43*	9.006±4.682	-51.588±19.11**	Duplicate
Kernel ironconcentration(mg kg^{-1})	21.129±2.47**	3.891±0.42**	21.647±6.97**	8.308±2.44**	-5.963±1.15	-14.281±4.61**	Duplicate
Kernel zinc concentration(mg kg^{-1})	33.815±3.84**	7.431±0.73**	17.496±10.88	9.671±3.77**	-6.953±1.80	-11.036±7.74	Duplicate
Days to emergence	11.269±0.07**	1.624±0.45**	5.561±0.95**	5.341±0.94**	1.178±0.46	-11.199±1.84**	Duplicate
Days to maturity	186.299±6.84**	12.006±0.72**	-82.191±20.17**	-40.543±6.80**	7.909±3.35	43.559±13.71**	Duplicate
100-Kernel weight (g)	59.984±4.67**	-1.193±1.20	-32.293±12.61*	-16.085±4.51**	1.303±2.11	20.280±8.83*	Duplicate
Pod yield per plant (g)	13.210±7.60	1.972±1.90	46.154±21.03*	15.537±7.36*	-4.371±3.58	-21.851±14.66	Duplicate
Kernel iron concentration (mg kg^{-1})	23.294±0.36**	2.334±0.703**	8.957±6.56	-0.128±2.32	-0.160±1.15	-10.295±4.32*	Duplicate
Kernel zincconcentration (mg kg^{-1})	36.324±2.93**	3.096±0.88**	2.220±8.03	-2.834±2.79	1.261±1.43	-6.529±5.49	Duplicate

*****– Significant at 5% level of probability

**– Significant at 1% level of probability

m – mean *i* – additive × additive

d – additive *j* – additive × dominance

h – dominance *l* – dominance × dominance

Table 4.15. Simple correlation among various characters in the cross ICGV 06040 × ICGV 87141 of groundnut

Character	Days to emergence	Days to flowering	Days to maturity	100-kernel weight (g)	Pod yield per plant (g)	Shelling percentage (%)	Sound mature kernel weight percentage (%)	Kerneliron concentration (mg kg^{-1})	Kernelzinc concentration (mg kg^{-1})
Days to emergence	-	0.033	-0.226**	-0.086	0.033	-0.208**	0.172**	0.044	-0.083
Days to flowering		-	0.277**	0.071	-0.048	-0.069	0.017	-0.163**	-0.074
Days to maturity			-	0.158**	-0.006	0.081	-0.176**	-0.26	-0.049
100-Kernel weight (g)				-	0.196**	0.142**	0.197**	-0.225**	0.134*
Pod yield per plant (g)					-	-0.064	-0.062	-0.082	-0.077
Shelling percentage (%)						-	-0.087	-0.180**	-0.023
Sound mature kernel weight percentage (%)							-	0.034	0.018
Kernel iron concentration (mg kg^{-1})								-	0.590**
Kernel zinc concentration (mg kg^{-1})									-

* – Significant at 5% level of probability *i.e.*, r = 0.1062

** – Significant at 1% level of probability *i.e.*, r = 0.1393

Table 4.16. Simple correlation among various characters in the cross ICGV 06099 × ICGV 93468 of groundnut

Character	Days to emergence	Days to flowering	Days to maturity	100-kernel weight (g)	Pod yield per plant (g)	Shelling percentage (%)	Sound mature kernel weight percentage (%)	Kernel iron concentration (mg kg^{-1})	Kernel zinc concentration (mg kg^{-1})
Days to emergence	-	0.002	0.153**	-0.122*	0.003	-0.048	-0.001	0.104*	-0.021
Days to flowering		-	0.151**	-0.010	-0.026	0.118*	-0.053	-0.040	-0.053
Days to maturity			-	0.190**	-0.071	0.112*	0.094	0.158**	0.220**
100-Kernel weight (g)				-	-0.018	0.299**	0.286**	-0.100	0.175**
Pod yield per plant (g)					-	-0.207**	-0.015	-0.103	0.002
Shelling percentage (%)						-	0.025	-0.055	0.024
Sound mature kernel weight percentage (%)							-	-0.061	0.084
Kernel iron concentration (mg kg^{-1})								-	0.549**
Kernel zinc concentration (mg kg^{-1})									-

* – Significant at 5% level of probability *i.e.*, r = 0.1047

** – Significant at 1% level of probability *i.e.*, r = 0.1373

Table 4.7 Kernel iron and zinc concentrations of entries in the cross ICGV 06040 × ICGV 87141 which showed similar scoringas that of entries of genotyping population using three SSR markers each associated with kernel iron and zinc concentrations of the cross ICGV 06099 × ICGV 93468

Sample No.	Iron concentration (mg kg^{-1})	Zinc concentration (mg kg^{-1})
1	25.42	43.29
2	24.16	43.39
3	25.48	41.18
4	21.74	40.38
5	23.69	36.82
6	23.54	38.55
7	21.65	35.38
8	21.94	43.58
9	19.39	34.97
10	22.58	38.87
11	23.59	42.62
12	27.22	43.83
13	24.18	43.40
14	21.12	39.44
15	20.80	36.69
16	19.89	39.65
17	20.22	35.06
18	26.12	39.60
19	25.60	40.44
20	23.04	43.59
21	20.33	41.43
22	19.59	41.08
23	24.31	45.90
24	19.38	36.30
25	21.27	41.32
26	23.92	40.13
27	19.03	34.49
28	20.69	37.55
29	23.00	42.36
30	20.13	40.95
31	21.83	40.17
32	21.96	43.08
33	20.03	43.12
34	12.84	26.51

Illustrations

Fig 4.3a Frequency distribution of the mapping population for days to emergence (DE), days to 75 % flowering (DFL), final plant stand (FPS), days to maturity (DMt), 100-kernel weight (HSW) and single plant yield (SPYD) in the mapping population. (↑ indicates parental lines *viz.*, P_1 - ICGV 06099 and P_2 - ICGV 93468 values for respective traits)

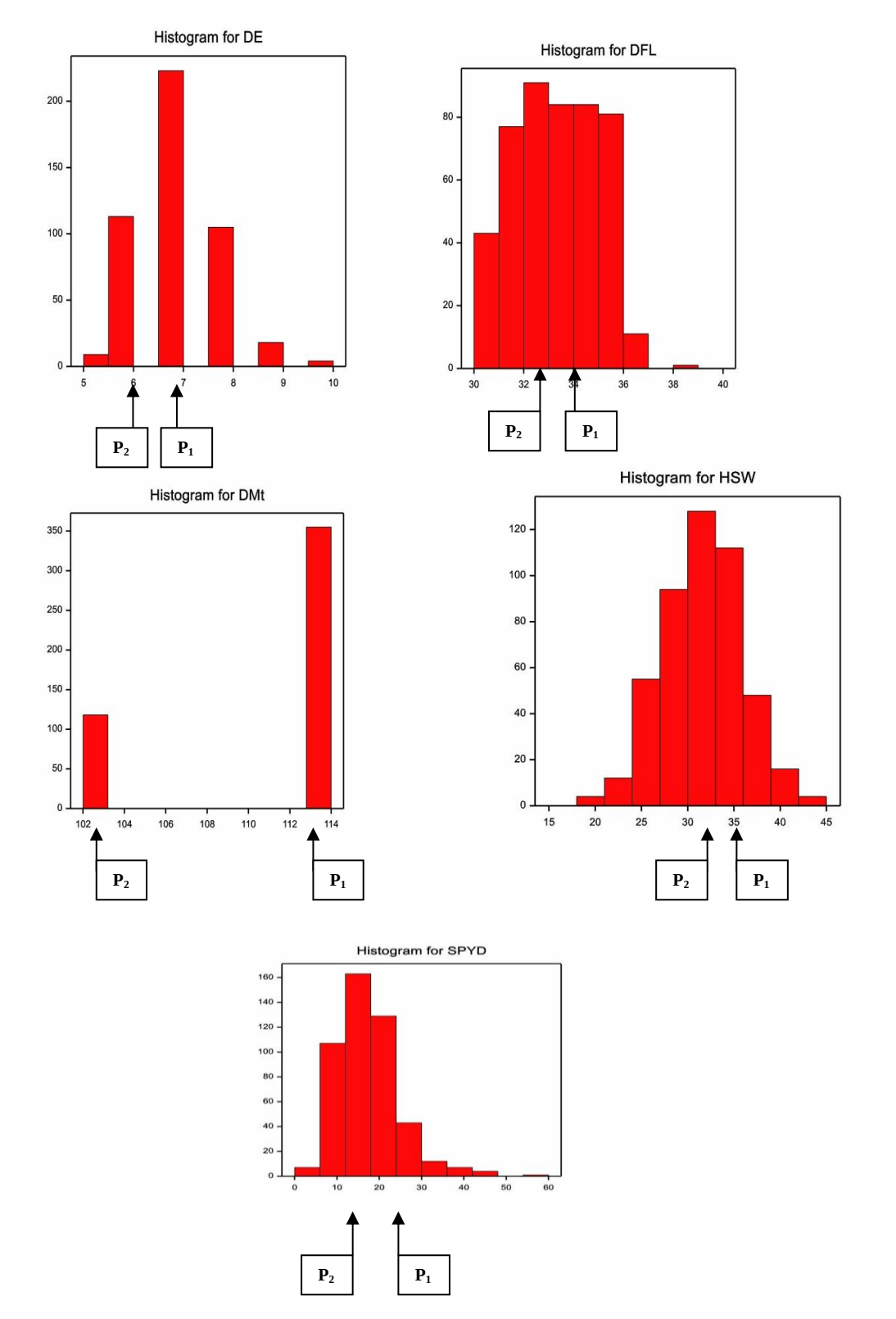


Fig 4.3b Frequency distribution of the mapping population for pod yield per plot (PY), seed yield per plot (SEED_Y), sound mature kernel percentage (SMK%), shelling percentage (SH%), oil content (OIL) and protein content (PRO) in the mapping population. (↑ indicates parental lines *viz.*, P_1 - ICGV 06099 and P_2 - ICGV 93468 values for respective traits)

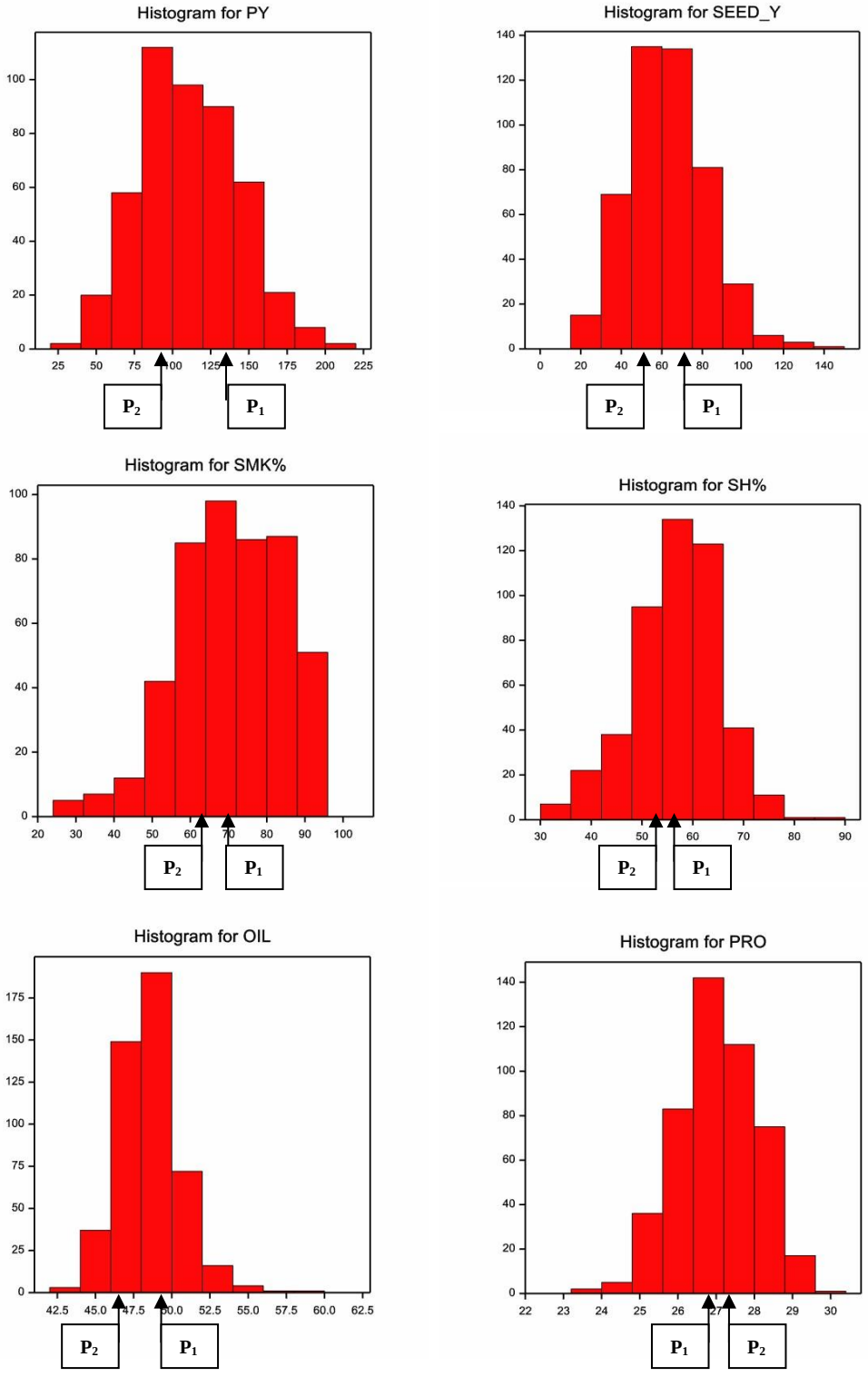


Fig 4.3c Frequency distribution of the mapping population for kernel iron concentration (D_Fe), kernel zinc concentration (D_Zn), oleic acid content (Oleic), linoleic acid content (Linoleic), palmitic acid content (Palmitic) and stearic acid content (Stearic) in the mapping population. (indicates parental lines *viz.*, P_1 - ICGV 06099 and P_2 - ICGV 93468 values for respective traits)

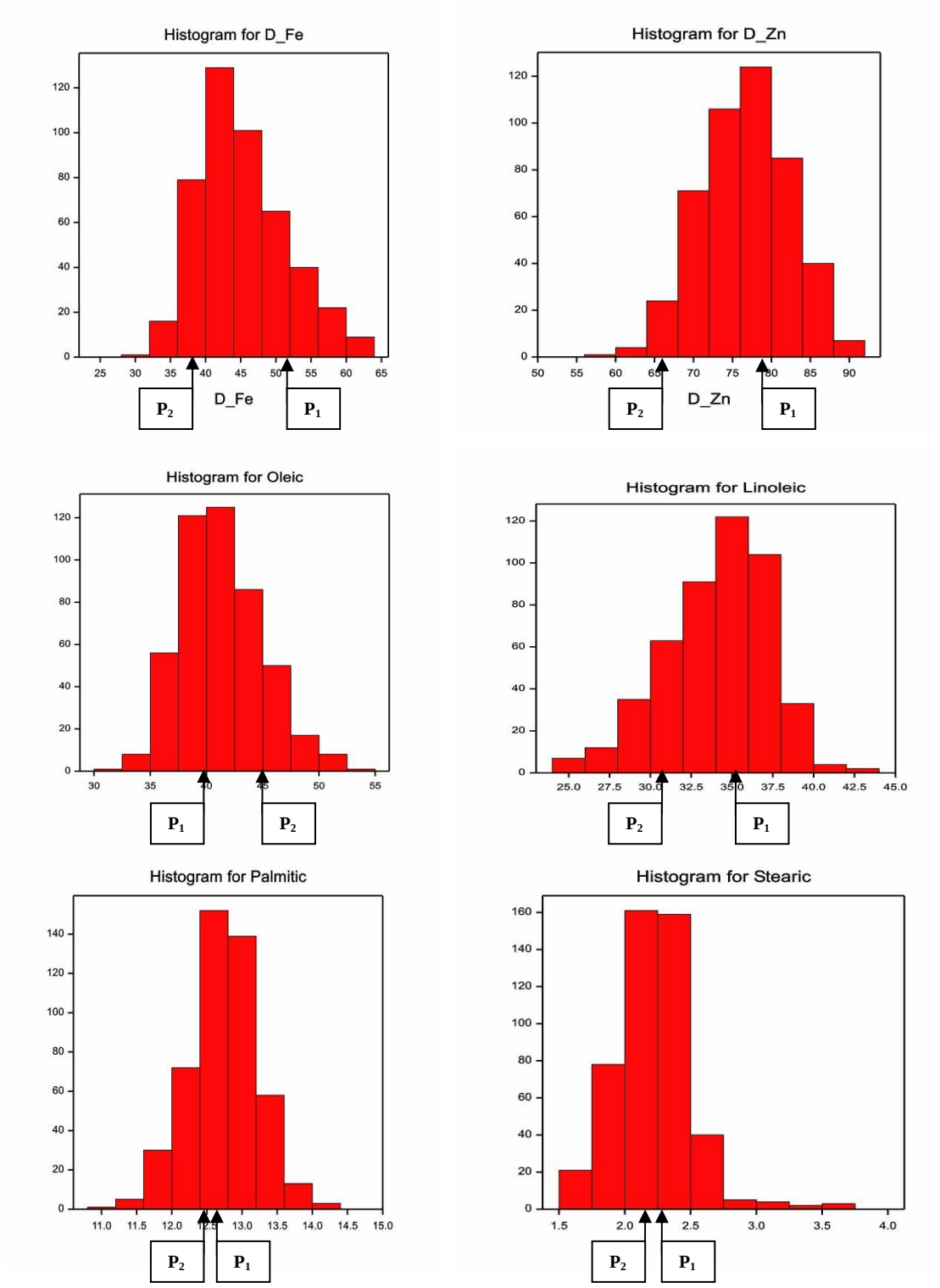


Fig 4.5 Relationship between kernel iron and zinc concentrations in $F_{2:3}$ mapping population of a cross ICGV 06099 × ICGV 93468

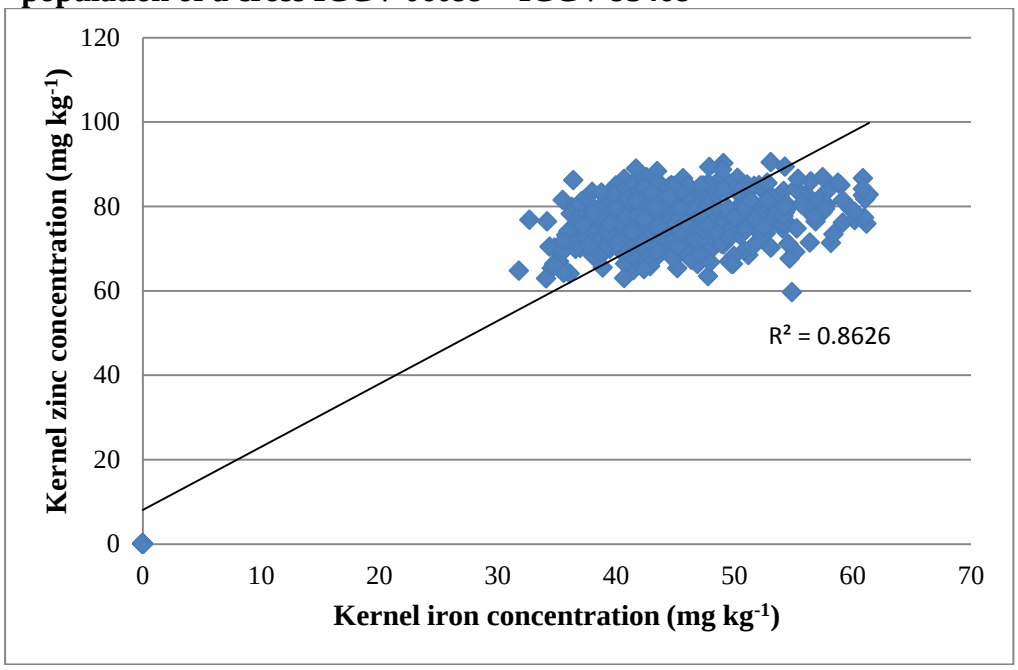
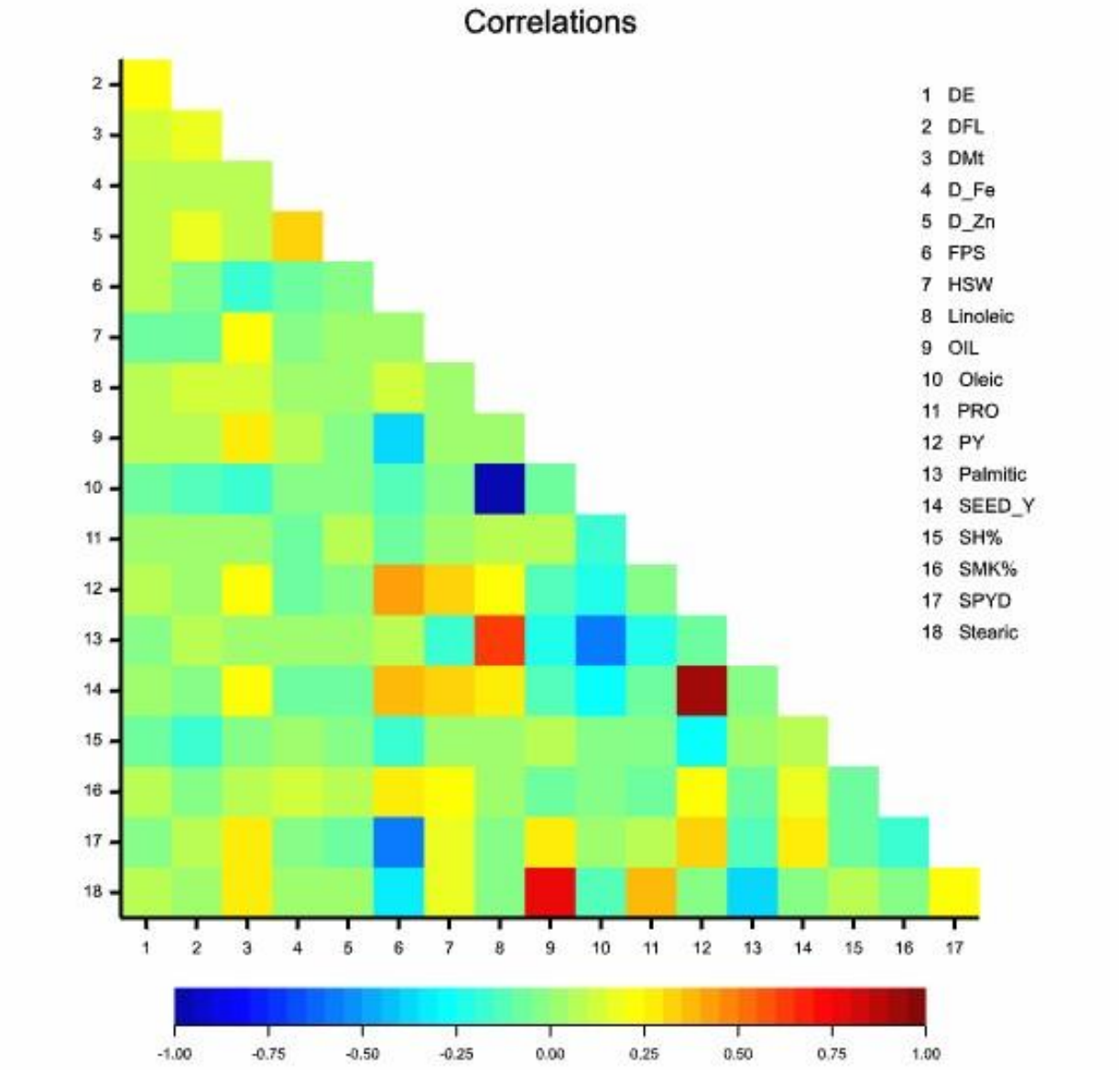
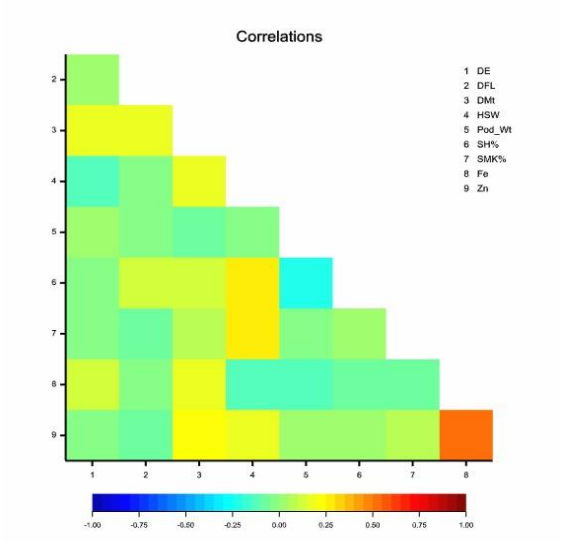


Fig 4.6 Pictorial representation of correlations among various agronomic characters in the mapping population of the cross ICGV 06099 × ICGV 93468

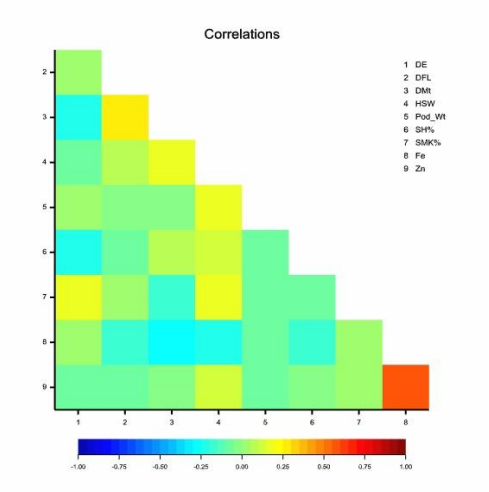


Note: DE - Days to Emergence; **DFL** - Days to Flowering; **DMt** - Days to Maturity; **D_Fe** - Kernel iron concentration; **D_Zn** - Kernel zinc concentration; **FPS** - Final Plant Stand; **HSW** - 100-Kernel weight; **Linoleic** -Linoleic acid content; **OIL** - Oil content; **Oleic** - Oleic acid content; **PRO** - Protein content; **PY** - Pod Yield per plot; **Palmitic** - Palmitic acid content; **SEED_Y** - Seed yield per plot; **SH %** - Shelling percentage; **SMK %** - Sound Mature Kernel percentage; **SPYD** - Single Plant Yield; **Stearic** - Stearic acid content.

Fig 4.10 Pictorial representation of correlations among various agronomic characters in six generations of crosses ICGV 06040 × ICGV 87141 and ICGV 06099 × ICGV 93468



ICGV 06040 × ICGV 87141



ICGV 06099 × ICGV 93468

Note: DE - Days to Emergence; **DFL** - Days to Flowering; **DMt** - Days to Maturity; **HSW** - Hundred Kernel weight; **Pod_wt** - Pod yield per plant; **SH %** - Shelling percentage; **SMK %** - Sound Mature Kernel percentage; **Fe** - Kernel iron concentration; **Zn** - Kernel zinc concentration;

Fig 3.2 Field layout overview of generation mean analysis plot consisting six generations of crosses ICGV 06040 × ICGV 87141 and ICGV 06099 × ICGV 93468

Fig 3.1 Variation in kernel characteristics of four parental lines *viz.*, ICGV 06040, ICGV 87141, ICGV 06099 and ICGV 93468

Fig 4.4 Variation in kernel characteristics of $F_{2:3}$ mapping populations of crosses ICGV 06040 × ICGV 87141 and ICGV 06099 × ICGV 93468

$F_{2:3}$ mapping populations of ICGV 06040 × ICGV 87141

$F_{2:3}$ mapping populations of ICGV 06099 × ICGV 93468

Fig 4.8. Comparison of mean performance of different generations of two crosses of groundnut for days to maturity and 100- kernel weight

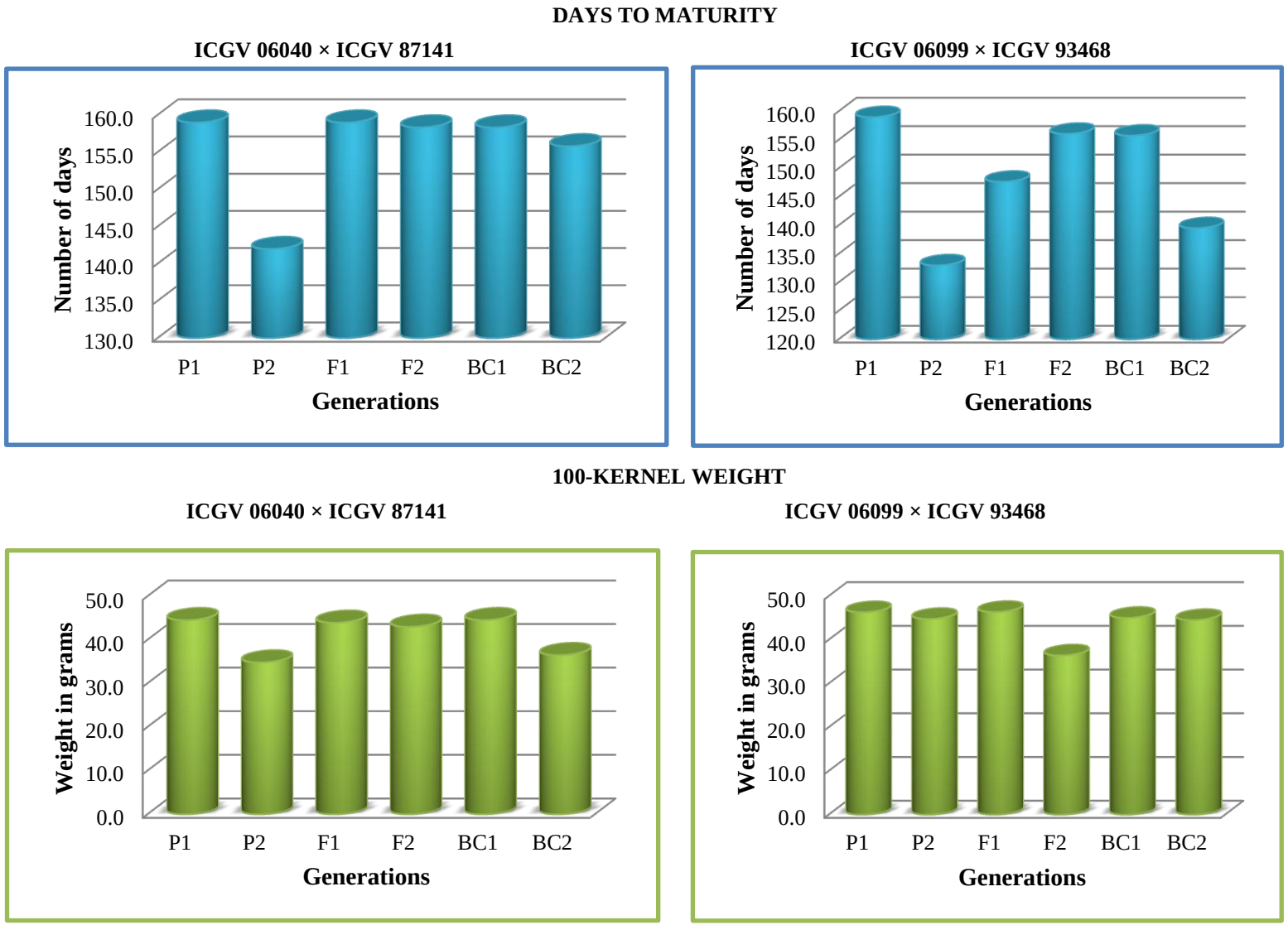


Fig 4.9. Comparison of mean performance of different generations of two crosses of groundnut for pod yield per plant and kernel iron and zinc concentration

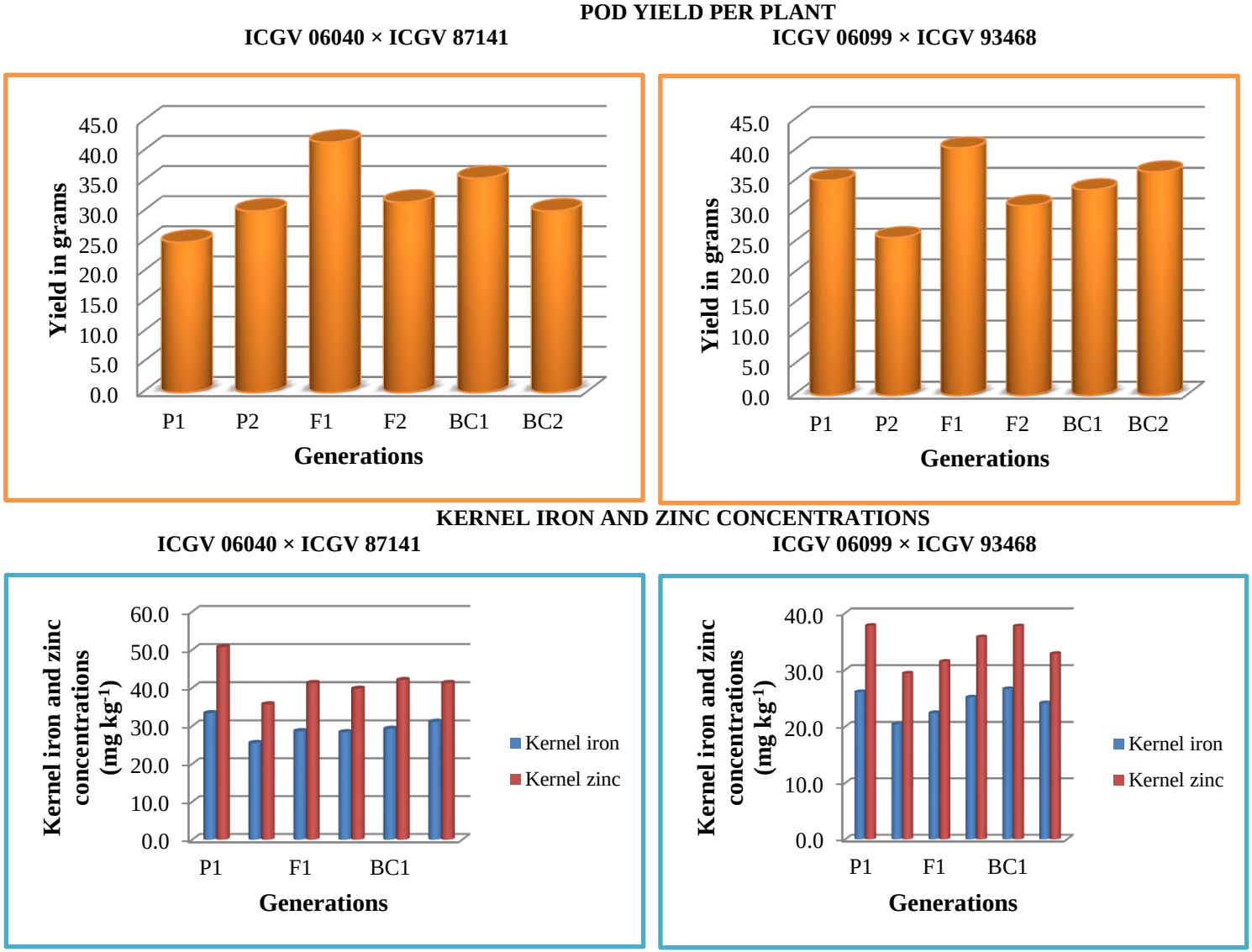
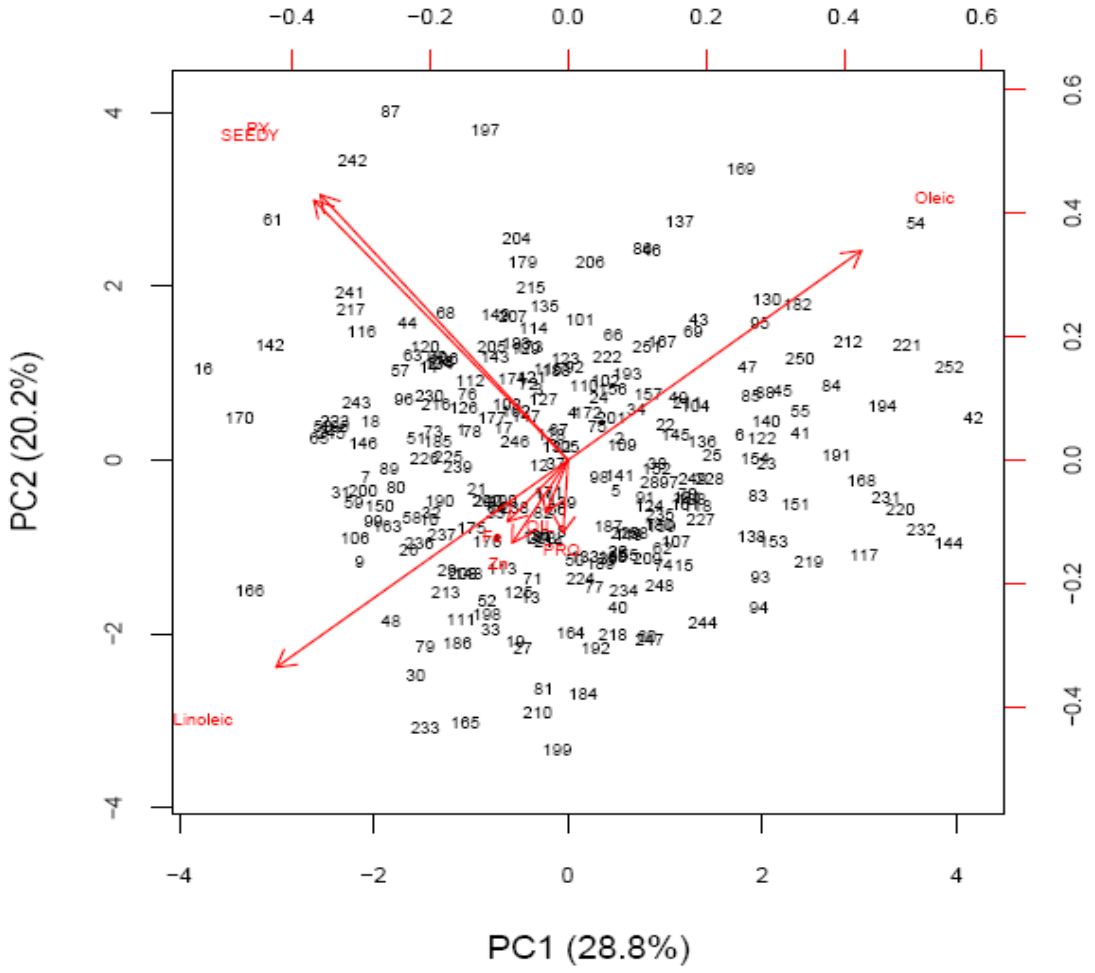


Fig 4.7 Principal Component Analysis (PCA) of various traits including kernel iron and zinc concentrations in $F_{2:3}$ mapping population of the cross ICGV 06099 × ICGV 93468



Where,

PY	: Pod yield per plot
SEED Y	: Seed yield per plot
OIL	: Oil content
PRO	: Protein content
Fe	: Kernel iron concentration
Zn	: Kernel zinc concentration
Oleic	: Oleic acid content
Linoleic	: Linoleic acid content
PC1	: Principal component 1
PC2	: Principal component 2