GENOME-WIDE ASSOCIATION MAPPING FOR Fe, Zn AND YIELD TRAITS IN PIGEONPEA

Dissertation

Submitted to the Punjab Agricultural University
in partial fulfillment of the requirements
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DOCTOR OF PHILOSOPHY
in
PLANT BREEDING & GENETICS
(Minor Subject: Biotechnology)

By

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CERTIFICATE I

This is to certify that the dissertation entitled, **"Genome-wide association mapping for Fe, Zn and yield traits in pigeonpea"** submitted for the degree of **Doctor of Philosophy** in the subject of **Plant Breeding and Genetics** (Minor subject: **Biotechnology**) of the Punjab Agricultural University, Ludhiana, is a bonafide research work carried out by **Harpreet Kaur (L-2017-A-39-D)** under my supervision and that no part of this dissertation has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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CERTIFICATE II

This is to certify that the dissertation entitled **"Genome-wide association mapping for Fe, Zn and yield traits in pigeonpea"** submitted by **Harpreet Kaur (L-2017-A-39-D)** to the Punjab Agricultural University, Ludhiana, in partial fulfillment of the requirements for the degree of **Ph.D.** in the subject of **Plant Breeding and Genetics** (Minor subject: **Biotechnology**) has been approved by the Student's Advisory Committee along with External Examiner after an oral examination on the same.

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ABSTRACT

In the present study, an effort was made to understand the complex nature of yield and quality traits and genome-wide association studies (GWAS) was undertaken to identify QTLs associated with traits. The analysis of variance (ANOVA) revealed significant variation among all genotypes in both the years and pooled data. Measurement of skewness and kurtosis indicated presence of duplicate and complementary gene interaction for different traits. The seed yield exhibited highly positive and significant correlation with number of primary branches per plant, number of secondary branches per plant and number of pods per plant for both the years and pooled data across two years. Path analysis identified pods per plant, number of primary and secondary branches as the major contributing traits to grain yield in both the years and pooled data. Yield per plot and pods per plant exhibited high heritability along with high genetic advance showing additive gene effect and could be considered during selection. In genotypic analysis, a total of 7366 filtered SNPs were used for genome-wide association studies through ddRAD-Seq. A total of 30 highly significant associated SNPs in 2018-19, 23 significant associated SNPs in 2019-20 and 25 significant associated SNPs in pooled data were identified through combination of two or more than two models for different traits. Most of the SNP loci were found to be environment and trait specific but few stable and consistent SNPs were identified. Some of the SNPs associated with days to maturity, yield per plot and grain iron content were observed to be consistent in 2018-19 and pooled conditions. The candidate regions were defined by the average LD decay distance or the LD block. Biological function of the identified candidate genes revealed their role in plant growth and development, organogenesis, pollen development and source-sink relationship. These genes were found to be present on chromosome 02, 03 and 09. Three polymorphic KASP markers for traits *viz.*, grain iron content, number of primary branches and yield per plot were validated and showed that parents have homozygous alleles for the traits and alleles were found to be segregating in the $F_{2:3}$ population. Promising genotypes for grain iron, grain zinc content and yield attributing traits were identified which can be used as donor parents in pigeonpea breeding programme and developed markers can be used in marker assisted selection (MAS) for identifying desirable genotypes having allele of interest.

Keywords: Pigeonpea, GWAS, Fe, Zn, Heritability, Genetic Advance, Correlation

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Signature of Major Advisor **Signature of the Student**

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ਮੁੱਖ ਸਲਾਹਕਾਰ ਦਾ ਨਾਮ ਅਤੇ ਅਹੁਦਾ	:	ਡਾ ਇੰਦਰਜੀਤ ਸਿੰਘ ਪ੍ਰਿੰਸੀਪਲ ਪਲਸ ਬ੍ਰੀਡਰ
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ਮੌਜੂਦਾ ਅਧਿਐਨ ਦੌਰਾਨ, ਝਾੜ ਅਤੇ ਗੁਣਵੱਤਾ ਗੁਣਾਂ ਦੇ ਗੁੰਝਲਦਾਰ ਵਤੀਰੇ ਨੂੰ ਸਮਝਣ ਦੀ ਕੋਸ਼ਿਸ਼ ਕੀਤੀ ਗਈ ਅਤੇ ਗੁਣਾਂ ਨਾਲ ਸਬੰਧਤ QTLs ਦਾ ਪਤਾ ਲਗਾਉਣ ਲਈ ਜੀਨੋਮ-ਵਾਈਡ ਐਸੋਸ਼ੀਏਸ਼ਨ ਅਧਿਐਨ (GWAS) ਕੀਤਾ ਗਿਆ। ਵਿਭਿੰਨਤਾ ਮੁਲਾਂਕਣ (ANOVA) ਨੇ ਦੋਨਾਂ ਸਾਲਾਂ ਦੌਰਾਨ ਅਤੇ ਪੂਲਡ ਅੰਕੜਿਆਂ ਵਿੱਚ ਸਾਰੀਆਂ ਲਾਈਨਾਂ ਵਿੱਚ ਅਰਥਪੂਰਨ ਵਿਭਿੰਨਤਾ ਦਰਸਾਈ। ਸਕਿਉਨੈਸ ਅਤੇ ਕਰਟੋਸਿਸ ਤੋਂ ਵੱਖੋ-ਵੱਖਰੇ ਗੁਣਾਂ ਲਈ ਡੁਪਲੀਕੇਟ ਅਤੇ ਕੰਪਲੀਮੈਂਟਰੀ ਜੀਨ ਇੰਟ੍ਰੈਕਸ਼ਨ ਦੀ ਹੋਂਦ ਦਾ ਪਤਾ ਚੱਲਿਆ। ਦੋਨਾਂ ਸਾਲਾਂ ਦੌਰਾਨ ਪ੍ਰਤੀ ਪੌਦਾ ਮੁੱਢਲੀਆਂ ਸ਼ਾਖਾਵਾਂ, ਪ੍ਰਤੀ ਪੌਦਾ ਸਕੈਂਡਰੀ ਸ਼ਾਖਾਵਾਂ ਅਤੇ ਪ੍ਰਤੀ ਪੌਦਾ ਫ਼ਲੀਆ ਦੀ ਗਿਣਤੀ ਨਾਲ ਬੀਜ ਦੇ ਝਾੜ ਦਾ ਅਰਥਪੂਰਨ ਅਤੇ ਸਾਕਾਰਆਤਮਕ ਸਬੰਧ ਵੇਖਿਆ ਗਿਆ। ਪਾਥ ਮੁਲਾਂਕਣ ਤੋਂ ਪਤਾ ਚੱਲਿਆ ਕਿ ਪ੍ਰਤੀ ਪੌਦਾ ਫ਼ਲੀਆਂ ਦੀ ਗਿਣਤੀ, ਮੁੱਢਲੀਆਂ ਅਤੇ ਸਕੈਂਡਰੀ ਸ਼ਾਖਾਵਾਂ ਦੀ ਗਿਣਤੀ ਦਾ ਬੀਜ ਦੇ ਝਾੜ ਵਿੱਚ ਅਹਿਮ ਯੋਗਦਾਨ ਸੀ। ਪ੍ਰਤੀ ਪਲਾਟ ਝਾੜ ਅਤੇ ਪ੍ਰਤੀ ਪੌਦਾ ਫ਼ਲੀਆਂ ਦੀ ਗਿਣਤੀ ਨੇ ਐਡੀਟਿਵ ਜੀਨ ਪ੍ਰਭਾਵ ਵਾਲੀ ਵਧੇਰੇ ਅਨੁਵਾਂਸ਼ਿਕ ਵਿਕਾਸ ਦੇ ਨਾਲ-ਨਾਲ ਉੱਚਤਮ ਅਨੁਵਾਂਸ਼ਿਕਤਾ ਦਰਸਾਈ ਅਤੇ ਇਸ ਲਈ ਇਸਨੂੰ ਚੋਣ ਲਈ ਵਾਚਿਆ ਜਾ ਸਕਦਾ ਹੈ। ਜੀਨੋਟਿਪਿਕ ਮੁਲਾਂਕਣ ਵਿੱਚ, ਜੀਨੋਮ-ਵਾਈਡ ਸਹਿ-ਸਬੰਧਨ ਅਧਿਐਨ ਲਈ ddRAD-Seq ਜ਼ਰੀਏ ਕੁੱਲ 7366 ਫਿਲਟਰਡ SNPs ਦੀ ਵਰਤੋਂ ਕੀਤੀ ਗਈ। ਵੱਖੋ-ਵੱਖਰੇ ਗੁਣਾਂ ਲਈ ਦੋ ਜਾਂ ਦੋ ਤੋਂ ਜ਼ਿਆਦਾ ਸੰਯੋਜਕਾਂ ਰਾਹੀਂ ਸੰਨ 2018-19 ਵਿੱਚ ਅਰਥਪੂਰਨ ਤੌਰ ਤੇ ਵਧੇਰੇ ਸਬੰਧਤ 30 SNPs, ਸੰਨ 2019-20 ਦੌਰਾਨ ਅਰਥਪੂਰਨ ਤੌਰ ਤੇ ਸਬੰਧਤ 23 SNPs, ਅਤੇ ਪੂਲਡ ਆਂਕੜਿਆਂ ਵਿੱਚ ਅਰਥਪੂਰਨ ਤੌਰ ਤੇ ਸਬੰਧਤ 25 SNPs ਦਾ ਪਤਾ ਚੱਲਿਆ। ਜ਼ਿਆਦਾਤਰ SNP ਵਾਤਾਵਰਣ ਅਤੇ ਗੁਣਾਂ ਨਾਲ ਸਬੰਧਤ ਸਨ ਪਰ ਕੁੱਝ ਸਥਿਰ ਅਤੇ ਇਕਸਾਰ SNPs ਦੀ ਵੀ ਪਹਿਚਾਣ ਹੋਈ। ਪੱਕਣ ਲਈ ਲੱਗੇ ਦਿਨਾ ਦੀ ਗਿਣਤੀ, ਪ੍ਰਤੀ ਪਲਾਟ ਝਾੜ ਅਤੇ ਦਾਣਿਆਂ ਵਿੱਚ ਆਇਰਨ ਦੀ ਮਾਤਰਾ ਨਾਲ ਸਬੰਧਤ ਕੁੱਝ SNPs ਸੰਨ 2018-19 ਅਤੇ ਪੂਲਡ ਹਲਾਤਾਂ ਅਧੀਨ ਇਕਸਾਰ ਪਾਏ ਗਏ। ਔਸਤਨ LD ਡਿਕੇਅ ਦੂਰੀ ਜਾਂ LD ਬਲਾਕ ਦੀ ਵਰਤੋਂ ਨਾਲ ਕੇਂਡੀਡੇਟ ਖੇਤਰ ਨੂੰ ਪ੍ਰਭਾਸ਼ਿਤ ਕੀਤਾ ਗਿਆ। ਪਹਿਚਾਣੇ ਗਏ ਕੇਂਡੀਡੇਟ ਜੀਨਾਂ ਦੇ ਜੈਵਿਕ ਕੰਮ-ਕਾਜ ਤੋਂ ਪਤਾ ਚੱਲਿਆ ਕਿ ਪੌਦੇ ਦੇ ਵਿਕਾਸ ਅਤੇ ਵਾਧੇ, ਓਰਗੈਨੋਜੇਨੇਸਿਸ, ਪਰਾਗਕਣਾਂ ਦੇ ਵਿਕਾਸ ਅਤੇ ਸੋਰਸ-ਸਿੰਕ ਸਬੰਧ ਵਿੱਚ ਉਹਨਾਂ ਦੀ ਭੂਮਿਕਾ ਸੀ। ਇਹ ਜੀਨ ਗੁਣਸੂਤਰ 02, 03 ਅਤੇ 09 ਉੱਪਰ ਮੌਜੂਦ ਸਨ। ਦਾਣਿਆਂ ਵਿੱਚ ਆਇਰਨ ਦੀ ਮਾਤਰਾ, ਮੁੱਢਲੀਆਂ ਸ਼ਾਖਾਵਾਂ ਅਤੇ ਪ੍ਰਤੀ ਪਲਾਟ ਝਾੜ ਲਈ ਤਿੰਨ ਬਹੁਰੂਪਕ KASP ਮਾਰਕਰਾਂ ਦੀ ਪ੍ਰਮਾਣਿਕਤਾ ਸਿੱਧ ਕੀਤੀ ਗਈ ਅਤੇ ਇਸ ਤੋਂ ਪਤਾ ਚੱਲਿਆ ਕਿ ਗੁਣਾਂ ਲਈ ਮਾਪਿਆਂ ਵਿੱਚ ਹੋਮੋਜ਼ਾਈਗੋਸ ਐਲੀਲਸ ਸਨ ਅਤੇ $F_{2:3}$ ਵੰਸ਼ਾਵਲੀ ਦੇ ਪੌਦਿਆਂ ਵਿੱਚ ਐਲੀਲਸ ਵੱਖ-ਵੱਖ ਤੌਰ ਤੇ ਪਾਏ ਗਏ। ਦਾਣਿਆਂ ਵਿੱਚ ਆਇਰਨ ਅਤੇ ਜ਼ਿੰਕ ਦੀ ਮਾਤਰਾ, ਝਾੜ ਨਾਲ ਸਬੰਧਤ ਗੁਣਾਂ ਲਈ ਸੰਭਾਵਿਤ ਜੀਨੋਟਾਈਪਾਂ ਦੀ ਪਹਿਚਾਣ ਕੀਤੀ ਗਈ ਜਿਹਨਾਂ ਨੂੰ ਅਰਹਰ ਦੇ ਬ੍ਰੀਡਿੰਗ ਪ੍ਰੋਗਰਾਮ ਵਿੱਚ ਡੋਨਰ ਮਾਪਿਆਂ ਵਜੋਂ ਵਰਤਿਆ ਜਾ ਸਕਦਾ ਹੈ ਅਤੇ ਲੋੜੀਂਦੇ ਐਲੀਲ ਵਾਲੇ ਜੀਨੋਟਾਈਪਾਂ ਦੀ ਪਹਿਚਾਣ ਲਈ ਵਿਕਸਤ ਕੀਤੇ ਗਏ ਮਾਰਕਰਾਂ ਨੂੰ MAS ਵਿੱਚ ਵਰਤਿਆ ਜਾ ਸਕਦਾ ਹੈ।

ਮੁੱਖ ਸ਼ਬਦ: ਅਰਹਰ, GWAS, ਆਇਰਨ, ਜ਼ਿੰਕ, ਅਨੁਵਾਂਸ਼ਿਕਤਾ, ਅਨੁਵਾਂਸ਼ਿਕੀ ਵਿਕਾਸ, ਸਹਿ-ਸਬੰਧਨ

ਮੁੱਖ ਸਲਾਹਕਾਰ ਦੇ ਹਸਤਾਖਰ **ਵਿਦਿਆਰਥੀ ਦੇ ਹਸਤਾਖਰ**

CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER-I

INTRODUCTION

Pulses are grown all over the world and in many countries these are considered as the major components of human diet. About 50 per cent pulses are produced in Asia. India is the largest producer and consumer of a wide variety of pulses including pigeonpea. The production of pulses in India has increased to 25.58 m tonnes during 2020-21(Gaur 2021). Pigeonpea [*Cajanus cajan* (L.) Millspaugh] is one of the leading, second most important pulse crop of India after chickpea cultivated in semi-arid and arid regions of the world (Singh 1988). It ranks sixth in global grain legume production and worldwide, it is cultivated on about 6.99 m ha area with an annual production of 5.96 m tonnes and a mean productivity of 852 kg/ha. Worldwide, India ranks first in pigeonpea cultivation with an area of 5.58 m ha and production of 4.29 m tonnes (FAOSTAT 2020). Pigeonpea is a major and cheap source of protein as compared to animal protein. It accounts for about 15% of total pulses produced in the country. India is the major pigeonpea producing country, contributing almost 80% of total production and area in the world (Anonymous 2021). Pigeonpea is known by several local and trade names such as red gram, Congo pea, Angola pea, tuar, Oil dhal and Yellow dhal (Saxena *et al* 2008). Among these, pigeonpea is the popular name coined in Barbados, where it was grown for feeding its seeds to pigeons (Saxena *et al* 2010). Being the important source of protein and minerals, this crop has great nutritional as well as economic value distinctively for poor people living in Asia, Africa, South America, Central America and the Carribean region (Mula and Saxena 2010). Pigeonpea seeds have 21-25% protein, supplementing energy rich cereals for a proportional diet (Kumar *et al* 2018). It is a multipurpose crop as it can be used as food, fodder, feed, fuel, fertilizer, for making baskets and fences, and even for pharmaceutical purposes. Because of its deep tap root system and ability to fix atmospheric nitrogen, adding organic matter and micro nutrients and breaking hard soil pan, sometimes referred as biological plough (Saxena *et al* 2010).

Pigeonpea is a drought tolerant, widely adapted and hardy crop with a wide range of temporal variation (97-299 days) for grain maturity. In spite of being the second most important pulse crop and rich source of dietary protein in south Asia, it faces consequential issues in terms of productivity due to insufficient availability of seed of improved varieties seed and various environmental stresses including biotic and abiotic, at an early establishment stage. Long crop duration, tall stature, low harvest index, spreading type and non-synchronous maturity of the pigeonpea varieties make mechanical operations laborious in this crop. Over the decades, crop productivity has not shown much increase in level and remained stagnant at around 700-800 kg ha^{-1} mainly due to the adverse effect of biotic (insect pests and

diseases) and abiotic (drought, salinity and water logging) stresses (Bohra *et al* 2020, Saxena *et al* 2020). The main challenge for pigeonpea improvement is increasing the productivity simultaneously, reducing the yield losses due to various abiotic and biotic stresses. Hence there is an utmost requirement to develop improved varieties to overcome these limitations. An ideal pigeonpea plant is considered as short statured with determinate growth habit, photo-insensitive, early maturing and fast growing with high harvest index (Singh *et al* 1990). On the other hand, some genotypes are intermediate between determinate and indeterminate type called as semi-determinate types which contribute directly to modify grain yield, ease in harvesting and seed production. Determinate type genotypes have many advantages over indeterminate types such as they are early maturing and suitable for mechanical harvesting but they are more prone to insect attack which limits their widespread cultivation. So, there is need to develop and identify genetic resources for trait dissections. Improvement in seed yield depends on the nature and magnitude of genetic variability and heritable associations between yield and its components which will provide useful information on direction, extent and nature of selection of promising genotypes.

The increasing rate of world population is estimated to cross 10 billion over the next 30 years and expected to grow by 25 percent (Hickey *et al* 2019). India is catching up the most populous country China at a very fast rate (Saxena *et al* 2008). To meet the food demands and ensuring nutritional security of the increasing global population with scarce resources is the uttermost challenge of this century. Protein mal-nutrition, more particularly, the micronutrient deficiency is prevalent among poor section of developing and under developed countries. The deficiency of iron (Fe) and zinc (Zn) is one of the top risk factors leading to impaired health (WHO 2017a). According to the Food and Nutrition Board of the Institute of Medicine, National Academy of Sciences, the RDA (Recommended Daily Allowance) of Fe is 8 mg/day for male adults and 18 mg/day for females. The RDA for Zn is 11 mg/day for male adults and 8 mg/day for female adults (https://ods.od.nih.gov/Health_Information/Dietary_Reference_Intakes.aspx). Fe and Zn are essential cofactors of a wide range of metabolic enzymes. Thus, these elements occur in protein-bound forms in plants and animals. In the human body, Fe is required for the synthesis of oxygen transport proteins (haemoglobin and myoglobin) and enzymes involved in electron transfer and oxidation-reductions. Zn is an another important mineral required by human and is involved in many biological functions, such as improving wound healing by its involvement in membrane signaling systems in cell growth and proliferation, protecting cells from oxidative damage by quenching reactive oxygen species, and reducing risk of various types of cancer including prostate and pancreatic.

Iron deficiency is the most common nutritional deficiencies in the world, the impact of which is intensified by malaria, worm infestations and infectious diseases such as HIV and tuberculosis (WHO 2017). Zinc deficiency in human is largely due to inadequate intake or absorption of Zn from the diet. Zinc deficiency leads to physiological disorders affecting the immune, central nervous, skeletal, epidermal gastrointestinal and reproductive systems (Roohani *et al* 2013). Anaemia is the most common human nutritional malaise, resulting from Fe deficiency and affecting 32.9% people worldwide; meanwhile, Zn deficiency affects17% of the world's population, with the highest risk occurring in sub-Saharan Africa and South Asia (Wessells *et al* 2012, Kassebaum *et al* 2014). To mitigate this problem, legumes in the developing world are known to serve food proteins which can be grown under low input level of farming. Among the legumes, pigeonpea or red gram and chickpea holds an important place in developing countries like Asia and Africa due to its high protein content. It is also a valuable source of different types of vitamins (thiamine, folic acid, riboflavin, pyridoxine, niacin), essential minerals (Fe, Zn, Ca, Cu, Mg), dietary fibres and carbohydrates (Jha *et al* 2020). For poor populations of developing countries, grains enriched with micronutrients by biofortification, seem the most appropriate and attractive approach to address the problem (Bohra *et al* 2015). Crop based biofortification is the most efficient, low cost and sustainable means for enriching micronutrient concentration in the edible parts of the crops. The minerals that are essential to human health include a range of macronutrients and micronutrients. Genetic variability revealed the continuous variation for these traits in germplasm and makes these amenable to the molecular analysis using quantitative genetics approach to target the genomic location of gene(s)/QTLs having significant effects on the phenotype. Also, the identification and understanding of genetic variability and genetic factors contributing to the seed Fe and Zn concentrations in the wild and cultivated germplasm will lead to appropriate utilization of resources in breeding programme to enrich the commercial cultivars with micronutrients (Mishra and Acharya 2017).

Genomics-assisted breeding can help the breeders to select suitable parents to recover novel combinations in crossing programme to develop elite breeding lines. The availability of first draft genome sequence of pigeonpea variety Asha has facilitated to fulfil the gap to some extent but there is need to develop high quality reference genome to accelerate more efficient gene discovery and molecular breeding. In recent times, molecular maps and QTLs for important agronomic traits have been reported in pigeonpea using SSR and SNP markers but the level of polymorphism is very low with SSR markers therefore, large no. of SNP markers and high density linkage maps are required (Singh *et al* 2020). Further, the advancement in next- generation sequencing and genotyping technologies has facilitated the generation of

large scale genomic resources such as molecular markers, transcript reads and BAC end sequences in pigeonpea (Varshney *et al* 2013). Genotyping- by- sequencing (GBS) and high-density SNP arrays has emerged as attractive and efficient genotyping tools. Experimental operation and data analysis of huge amount of unknown sequence information generated through GBS are beyond the reach of many of the breeders. On contrary, high throughput genotyping arrays are simple, easy to use and genotype large number of samples within a short time facilitating quick data analysis.

To overcome the shortcomings such as presence or absence (PAV) related to single reference genome sequence, the concept of pangenome is necessary to understand the gene diversity across species. In addition to this integration of next generation sequencing with multi-omics strategies has significantly strengthened the research in pigeonpea. These resources have been used to develop QTL maps, dense genetic maps as well as physical maps for this crop. Hence there is a need of integrated genomics and breeding approach in pigeonpea to boost crop productivity in marginal environments as well as ensuring food security in developing countries. Keeping all these points in view, the present study was planned with the following objectives:

1. Evaluation of diverse pigeonpea germplasm for variability in Fe, Zn content and yield component traits.
2. Genome-wide association studies to identify QTLs governing Fe and Zn content and yield components.
3. Validation of QTLs associated with high Fe, Zn content and yield component traits in bi-parental population

CHAPTER-II

REVIEW OF LITERATURE

Domestication of pigeonpea was reported approximately >3500 years ago from its wild relative, *Cajanus cajanifolius* (Royes 1976). The presence of natural genetic variability in local germplasm and occurrence of several wild relatives concluded that India is the primary center of origin of pigeonpea and it then spread to Africa about 4000 years ago (van der Maesen 1980). The first scientific breeding effort in India with the description of morphological and agronomic traits of 86 elite collections was conducted by Shaw (1933). Large number of germplasm lines have been collected, conserved, characterized and evaluated for various morpho-agronomic traits and are maintained by the National Bureau of Plant Genetic Resources New Delhi, India.

Genetic variability within a species is a fundamental aid required for crop genetic improvement. Development of effective breeding programme mainly depends on the knowledge, especially the magnitude of genetic variability and genetic diversity in the breeding material. Further, yield is a complex character composed of several components some of which affect the yield directly, while, others affect indirectly and it is under polygenic control influenced by environmental factors too. Hence, knowledge of the association between yield and its components helps in formulating selection program. Correlation studies would provide estimates of the degree of association between grain yield and its various components and also among the component traits. Path coefficient analysis further elucidates the intrinsic nature of the association of component traits by determining the direct and indirect contribution of these traits to yield. Genetic parameters like genotypic and phenotypic coefficient of variation, heritability and genetic advance are highly reliable, making effective selection in the breeding material.

Despite of being exposed to various biotic and abiotic stresses, low level of genetic variability and limited genomic resources have been serious bottlenecks in pigeonpea crop improvement through modern breeding techniques (Pazhamala *et al* 2015). Genomics can play a major role in deployment of germplasm resources in breeding programme, by fingerprinting and cataloguing the desirable genes/genomic segments. Recent advances in pigeonpea genomics have helped to identify markers for Fusarium wilt and sterility mosaic diseases, fertility restoration and hybrid testing (Saxena *et al* 2016). Efforts are underway to identify markers for key agronomic traits related to yield and various insect pests like pod borer. The relevant literature is thus reviewed as following headings:

2.1 Genetic analysis for yield and component traits

Determination of genetic diversity of any given crop species is necessary for its genetic improvement because it generates baseline data to guide selection of parental lines and design of breeding scheme. Further, a complete understanding of the association between yield and yield components is must for a plant breeder devising a selection criteria for the improvement of any crop. The optimum combination of yield contributing traits can be accumulated in a particular genotype only by understanding the inter relationships of various traits using correlation and path coefficients (Pandey *et al* 2015). Seed yield is a complex and dependant character and whenever plant breeder goes for selection for yield, it always misleads because it depends on various characters and to some extent on the environmental conditions (Bal Chinmayee 2016). Genetic variability and correlation studies alone are not enough to give an exact picture of relative importance of direct and indirect influence of each of the component traits on grain yield. In such case, path coefficient analysis is an important technique for partitioning the correlation coefficient into direct and indirect effect of independent variables on dependent variable. Therefore genetic variability as well as correlation and path coefficient analysis may be important tools for the breeder for enhancing the production and productivity of pigeonpea. For yield enhancement, selection of superior parents exhibiting better yield contributing traits having high heritability and genetic advance is an essential prerequisite. Heritability, in conjunction with genetic advance, is more useful than heritability alone in the prediction of resultant effect of selecting the best individual.

A set of 29 new pigeonpea genotypes for genetic variability revealed high genotypic and phenotypic coefficient of variability for number of primary branches, number of pods per plant, dry grain yield and dry pod weight (Vange & Moses, 2009). Chetukuri *et al* (2013) evaluated 84 pigeonpea lines for genotypic and phenotypic variation and character association and observed significant variability for all traits. The phenotypic variance was high for all traits compared to genotypic variance. Heritability (broad sense) was generally high for all the traits with an exception of days to pod initiation. All traits were positively correlated with total yield except days to pod initiation. Similarly, the phenotypic correlation of all traits was positive with total yield, except days to pod initiation, 100-seed weight and pod length. Pandey *et al* (2016) investigated 23 pigeonpea genotypes and found that biological yield/plant, 100-seed weight, pods/plant, secondary branches/plant and harvest index had highly significant and positive correlation with seed yield. Ram *et al* (2016) reported that 100-seed weight, pods/plant, primary branches and secondary branches, days to 50% flowering had high direct effect on yield which resulted in significant positive correlation of these traits with grain yield/plant. Pushpavalli *et al* (2018) evaluated 49 pigeonpea genotypes where significant and positive genotypic and phenotypic correlation of number of pods/plant and

number of secondary branches/plant was observed with seed yield. The GCV for the traits under study ranged from 4.55 to 22.07% indicating the existence of sufficient variability present among the pigeonpea genotypes. Path coefficient analysis revealed that days to maturity exhibited maximum direct effect followed by number of pods/plant.

Genotypic coefficient of variation (GCV) which measures the extent of genetic variability of a trait is considered in combination with heritability and genetic advance while assessing the effect of phenotypic selection. The phenotypic and genotypic variances, correlation and path coefficient, heritability and genetic advances were estimated for yield attributing traits in 50 genotypes of pigeonpea by Baldaniya *et al* (2018). High estimates of genotypic and phenotypic variance were observed for days to 50% flowering, days to maturity, plant height, number of pods/plant, grain yield/plant and harvest index. Estimation of high heritability accompanied with high to moderate genetic advance as percentage of mean was observed for number of seeds/pod, harvest index and number of branches/plant which indicated that these characters were governed by additive gene effect. The character grain yield/plant was found to have positive and significant correlation with days to 50% flowering, days to maturity, plant height, number of branches/plant, number of pods/plant, pod length, number of seeds/pod, 100-seed weight, harvest index and protein content at both phenotypic and genotypic level. Hemavathy *et al* (2019) reported highest GCV for number of secondary branches/plant followed by number of pods/plant. High genetic advance was observed for number of secondary branches/plant, number of pods per plant, single plant yield and number of racemes, indicating the prevalence of additive gene action for inheritance of these traits. However, narrow difference between phenotypic and genotypic coefficients of variation was observed for all the traits, indicated little effect of environment on the expression of these traits and variability was mainly due to genetic constitution. Character association studies revealed that number of racemes, number of primary branches, number of secondary branches, pods/plant, clusters/plant, pod length, seeds/pod and 100 seed - weight were strongly correlated with seed yield. Path coefficient analysis revealed that days to 50% flowering and number of pods/plant had high positive direct effect on seed yield. Pod size had moderate direct effect on seed yield. Hence, due emphasis should be given on the number of pods/plant for improvement of seed yield in pigeonpea.

The efficiency of selection not only depends on the magnitude of genetic variability but also on the heritability of the desirable traits. The genotypes under study showed high heritability values for all the characters. Since heritability is also influenced by environment, the information on heritability alone may not help in selecting characters for enforcing selection. The heritability estimates in conjunction with genetic advance will be more reliable. High heritability accompanied with high genetic advance suggests predominance of additive

gene effects and effectiveness of selection for that trait. High heritability along with high genetic advance as per cent of mean were recorded for number of pods per plant, single plant yield, number of racemes, plant height, pod bearing length, number of primary branches and 100-seed weight. Similar observation was reported by Ram (2016) for number of branches/plant, number of pods/plant and seed yield/plant, Mittal *et al* 2010 and Patel and Acharya (2011) for number of pods/plant and number of branches/plant. Thus, selection of these traits is likely to accumulate more additive genes leading to further improvement of their performance and these traits may be used as selection criteria in pigeonpea breeding program.

Saroj *et al* (2013) estimated the genotypic and phenotypic variances, heritability and genetic advances, correlation and path coefficient for grain yield and yield traits in 70 pigeonpea genotypes. Relatively higher value of genotypic variance was found for pods/plant. Genotypic coefficient of variation was the maximum for secondary branches/plant followed by pods/plant and grain yield/plant, the lowest for days to maturity. The value of the genotypic variance for all the yield components were, however, higher than the environmental variance. The low environmental influence observed as compared to genetic factors indicates that the trait may be under pure genetic control without any influence of the environment, hence improvement can be achieved through phenotypic selection. High values of heritability estimates were obtained in characters like days to 50% flowering, days to maturity, 100-seed weight, secondary branches/plant, grain yield/plant, pods/plant, plant height, whereas moderate heritability was recorded for pod length. High heritability tells the effectiveness of selection on the basis of phenotypic performance, it does not show any indication of the amount of genetic progress for selecting the best individuals. High heritability in conjugation with high genetic advance as a percentage of mean in case of grain yield/plant, number of secondary branches/plant, 100-seed weight, pods/plant, indicate that these are simply inherited and the heritability is because of additive gene effects and selection may be effective in early generations for traits. However, pod length, days to maturity and seeds/pod had high heritability accompanied with low genetic advance which indicates non-additive gene effects. Days to 50% flowering had significant and positive association with grain yield/plant, pods/plant, days to maturity, primary branches/ plant, 100- seed weight and plant height at both phenotypic and genotypic levels. Correlation and Path coefficient analysis revealed days to 50% flowering, pods/plant, primary branches and secondary branches and 100-seed weight as main yield components. Rao *et al* (2019) reported moderate to high estimates of PCV and GCV for number of branches / plant and number of pods/plant. High estimates of heritability was observed for days to 50% flowering, days to maturity, 100-seed weight and number of pods/ plant. High heritability coupled with high genetic advance was recorded for number of

pods/ plant and 100-seed weight. Grain yield was positively correlated with plant height and number of pods/plant. Path coefficient analysis indicated that days to 50% flowering, Plant height and number of pods/plant were important component traits the improvement in yield. In another study, Meena *et al* (2017) observed that, PCV estimates in general were higher than the corresponding GCV values for all the characters in all environments. None of the trait showed high GCV and PCV in any of the environments. Over all the environments, high estimates of heritability (>60 %) in conjugation with high genetic advance as percent of mean (>20 %) was observed for number of pods/plant, seed yield/plot, plant height and number of primary branches/plant whereas, remaining traits were having high heritability with low to moderate genetic advance as percent of mean. Seed yield was positively correlated with 100-seed weight and number of pods/plant and these traits are under governance of additive geneaction. Direct selection for number of seeds/plant and 100-seed weight may be rewarding for improvement in yield as these traits have direct effects on yield.

2.2 Breeding for Fe and Zn in pigeonpea

Protein malnutrition is widespread among poor sections of developing and under developed countries. Due to the low protein availability in developing countries with ever increasing human population, nutritional enhancement programmes are facing a challenge to meet the targeted protein requirement as reviewed by Saxena *et al* 2010. Further not only protein malnutrition, nutrient scarcity, more particularly the micronutrient deficiencies or hidden hunger are indeed alarming and need urgent attention (Bohra *et al* 2015). Among legumes, pigeonpea is a valuable source of protein and also rich in calcium, starch, crude fibre, manganese, fat, trace elements (Fe & Zn). Tan *et al* (2017) has reported that Fe deficiency is one of the major problem in both developed and developing countries. The potential options such as supplementation and fortifying processed food have helped to gain a nutritionally- balanced food to alleviate this problem. However, cost associated with these products limits their accessibility and effectiveness, particularly of financially constrained group. One of the potential ways to enhance the micronutrient density is accessing the exploitable natural variation available in the germplasm for the micronutrient-of-interest to mine the genotypes that might act as potential donors in the downstream breeding programmes (Dwivedi *et al* 2012). Further, continuous distribution pattern, the complex quantitative nature of these traits makes them amenable to the molecular analysis using QTL mapping techniques. To identify the genomic location of genes/QTLs, two QTL mapping methods viz. association analysis (LD mapping) and family-based linkage mapping are implemented (Mackay and Powell 2007, Mitchell-Olds 2010, Wurschum 2012, Bohra 2013). By taking into consideration these approaches, a more affordable and sustainable option can

be crop-based biofortification that is more efficient and cost effective means based on enriching micronutrient-density. Significant advances for the biofortification of pulses have been made in chickpea, lentil and pea for high iron accumulating traits by exploiting existing genetic variability. However, the knowledge of the available genetic diversity and to use this variability for the enhancement of iron and zinc in pigeonpea is less. Molecular mechanisms behind these traits are also not clear in leguminous crops.

Recently, Mishra and Acharya (2017) studied the association of Fe and Zn with important nutritional and anti-nutritional factors and tried to identify the genetic factors responsible for enhancing seed Fe and Zn concentration, assisting selection process. Significant differences were observed among one hundred and four diverse genotypes that were scored for seven nutritional traits. Correlation coefficients and path analysis indicated that traits like higher zinc concentration, bolder seeds, lipid content and lesser contents of phytic acid should be used in selection for enhancement of seed Fe concentration. On the other hand, small seeds with high lipid and iron concentrations and lesser contents of phytic acid and proteins should be aimed for improvement of zinc concentration in pigeonpea. Besides micronutrient architecture, the genetic architecture of seed protein content (SPC) and its relationships with agronomic traits is poorly understood in pigeonpea. Fewer studies on genetic control of SPC suggested its quantitative inheritance. Accordingly, five F_2 populations segregating for SPC and four agronomic traits (seed yield, seed weight, days to first flowering and growth habit) were genotyped and phenotyped using GBS approach. Five high-density population-specific genetic maps were constructed and subsequently, integrated into a consensus map. Analysis revealed 192 main effect QTLs with phenotypic variation of 0.7% to 91.3% and 573 epistatic QTLs with PVE ranging from 6.3% to 99.4% across traits and populations. Co-localization of M-QTLs and E-QTLs explained the genetic basis of the significant ($P<0.05$) correlations of SPC with SW, SY, DFF and GH. This relationship suggests the simultaneous improvement of SPC and other important traits.

In lentil a study on genetic variability for Fe & Zn content and effect of genotype × environmental (G×E) interaction in 96 lentil genotypes was carried out in India for 2 years by Kumar et al (2018). Significant variability was observed for iron and zinc content. Iron content varied from 71.3 to 126.2 mg/kg and zinc content varied from 40.1 to 63.6 mg/kg. Presence of broad sense heritability estimates indicated the potential of these genotypes for genetic improvement through hybridization and selection over several generations for increased Fe and Zn content. A significant correlation between seed weight and Fe content, suggesting selection for large seeded lentil was observed. No Correlation was recorded between Zn and Fe content.

A study was conducted by Diapari *et al* (2014) to assess the genetic variability and to identify SNP markers associated with seed Fe and Zn content using 94 diverse accessions of chickpea. Considerable variability was observed in chickpea germplasm for seed Fe and Zn content. They observed negative correlation between grain yield and Zn across all locations and years; whereas significant negative correlation between Fe and grain yield was reported at the Elrose locality. Eight SNP loci associated with iron and zinc concentration were identified. These results open the way for molecular breeding for improvement of seed Zn and Fe concentrations in chickpea as well as in other pulses like pigeonpea where such investigations are yet to be conducted.

Quantitative trait analysis was conducted to decipher variability for seed Fe, Zn, P and phytic acid levels in an $F_{5:7}$ RIL population in common beans (*Phaseolus vulgaris* L.) developed from a cross between AND696 and G19833, of Andean origin. Significant genetic and environmental variability was observed for Fe, Zn and P levels and Fe and Zn were significantly correlated (up to r = 0.53). Three linkage groups were found to have QTLs for seed Fe and Zn, co-localized on these groups. The QTL for Zn (R^2 = 0.39) and Fe (R^2 = 0.36) were found at the same marker interval on B6, both derived from AND696. Similarly, QTL for seed P were identified on six linkage groups and explained 17% to 55% of total phenotypic variation depending on year and environment. Thus, the genetics of seed Zn, Fe and P concentration can be useful in the development of stress-resistant and micronutrient-fortified crops as reported by Cichy *et al* (2009).

Similar study was conducted in lentil to assess the level of genetic variability in the lentil accessions. A preliminary evaluation of 234 germplasm lines from various countries was carried out to understand the macro- and micro-nutrient (Mg, K, P, Ca, Fe, Mn, S, B, Zn and Cu) levels. Significant correlation was observed among macro and micro-nutrients. Positive correlation was observed between Ca and Mg followed by K and S, P and K and Ca and B. Negative correlation of K was observed with B and Ca. Hence, line identified having high nutrient content will be used as parents in developing nutrient rich lentils at ICARDA (Sarker *et al* 2017).

Before starting the breeding programme to enhance the micronutrient level, it is important and foremost step to study the inheritance of trait in breeding material. For this, Blair *et al* 2009 reported the inheritance of seed Fe and Zn accumulation in a recombinant inbred line population of common beans with low and high Fe and Zn content (DOR364 × G19833) using a QTL mapping technique. The population was evaluated over two locations and simultaneously, two analytical methods (Atomic Absorption Spectroscopy and

Inductively Coupled Plasma Spectrometry) were used to determine Fe and Zn concentration in the seeds. Variability for Fe concentration (40.0 – 84.6 ppm) was large than Zn (17.7 – 42.4 ppm) with significant correlation between trials, between methods and between minerals (up to r = 0.715). A total of 26 QTLs were recorded for the mineral × trial × method combinations of which half were for Fe content and half for Zn content. Eleven QTLs for Fe and six QTLs for Zn contributing up to 47.9% phenotypic variance were identified introducing an important locus useful for marker assisted selection (MAS). By taking relevance from these results from common bean, crop improvement for micronutrient fortification can be practised in pigeonpea also.

To improve the cultivars for nutrient levels, identification of required mineral enriched germplasm is important. In lentil, bio-fortification potential of a core collection of 96 wild accessions from 405 global wild annual collections was analysed. Profound variation was observed for different minerals. Hierarchical clustering revealed high intra- and inter-specific variability. Positive significant association among minerals and between minerals and agro-morphological traits was observed. Diversity analysis depicted wide geographical variations across gene pool in core set. Identified trait specific genetic resources could be initial genetic material for broadening the genetic base and bio-fortification of lentil (Kumar *et al* 2018).

However, transfer of favourable wild alleles from unadapted germplasm into elite breeding lines through advanced backcross (AB) method while escaping the epistatic effects of deleterious background genes in the wild is an important method to enhance the micronutrient level in cultivated lines (Tanksley and McCouch 1997). Likewise, Blair *et al* 2012 adopted this logical approach to transfer the seed mineral content trait from wild beans to cultivated beans. They analysed a population of 138 $BC_2F_{3:5}$ introgression lines derived from a wild genotype with very high Fe content, G10022 backcrossed into commercial-type variety ‘Cerinza’ a large-red seeded bush bean cultivar of the Andean gene pool. Alongside, simultaneous testing of adaptation of introgression lines in two replicated yield trials was conducted. Composite interval mapping (CIM) and Single point analysis (SPA) was used to identify associations of markers and mineral traits. It was found that correlations between seed size and mineral content affected the identification of iron and zinc content QTLs on many linkage groups. Thus, the advanced backcross programme produced some introgression lines with high mineral accumulation using a wild donor parent.

Similarly, Diapari *et al* (2015) in 94 diverse accessions of other leguminous crop fieldpea (*Pisum sativum* L.), studied the association analysis of Fe, Zn and Se concentrations. A total of 1,233 EST-based SNPs distributed over the pea genome as Illumina Golden Gate

assay were used for genotyping to estimate the population structure. Eight sub-populations were identified in the population explaining the genetic structure of the panel. Nine SNPs were found to be significantly associated with iron content and two SNPs with zinc content in seeds. These associated SNPs can be used in marker-assisted selection in pea breeding programmes in near future. Inspite of the immense nutritional value, pigeonpea also contain many anti-nutritional factors like trypsin, chymotrypsin inhibitors, polyphenols and galacto oligosaccharides (Singh 1988). Due to the lack of α – galactosidase activity in human small intestines, these sugars can not be hydrolysed and absorbed (Olsen *et al* 1981) and undigested raffinose family of sugars pass into the large intestines, where it gets fermented anaerobically by the microbial flora and leads to flatulence (Liener 1994). Most of the anti-nutritional factors can be easily removed by traditional soaking and cooking methods (Singh 1988). The levels of flatulence-causing sugars are more in mature seed. Germinated pigeonpea produced less flatus than the ungerminated. It has been shown that traditional processing practices do not bring about significant changes in the levels of these sugars except germination. So, there is need to identify and select the genotypes of pigeonpea with low levels of sugars for their utilization in breeding programme to understand the molecular mechanism behind the reduction of these anti-nutritional factors in pigeonpea gene pool.

2.3 Breeding for yield and component traits for increased productivity

Pigeonpea is an important grain legume crop grown predominantly in the tropical and sub-tropical regions of the world. Due to its inherent ability to withstand high temperatures and water scarcity, it is a crop of choice in arid and semi arid tropical (SAT) tropical regions of the world. Despite its importance in the SAT regions and efforts by various research communities, little improvement has been made in increasing pigeonpea production (Mir *et al* 2017). Despite the release of large number of high yielding varieties, productivity in the crop remains stagnant (700 kg ha^{-1}) as compared to its potential yield (2500-3000 kg ha^{-1}). This huge gap in the productivity might be due to several biotic (diseases and insect pests) and abiotic factors (drought, salinity and water logging) etc (Gowda *et al* 2015). Biotic stresses include diseases like Fusarium wilt, sterility mosaic disease (SMD), Phytophthora blight and insect pests like pod borer, which are the major constraints in pigeonpea production. Besides these factors, there are some others reasons of low gain in production and productivity. High genotype × environment interaction on the expression of quantitative traits leading to slow gain in genetic improvement and yield stability in pulses is one of them (Kumar and Ali 2006). Therefore, to strengthen conventional crop improvement programmes, one way is to incorporate genomic tools including molecular marker technology in selection of desirable genotypes or growing of transgenic crops for traits that cannot be improved by using

crossable gene pools due to lack of desirable genes in the gene pools. One alternative way is the use of available high yielding diverse lines as base material for incorporating useful genes from otherwise unadapted cultivars, lines or wild relatives (Kumar *et al* 2011). Some agronomic traits like growth habit is also known to play some role in increasing grain yield. There are two forms of growth habit in pigeonpea known as 'indeterminate' (IDT) and 'determinate' (DT) type. IDT type genotypes grow in height and have vegetative terminal bud and on the other hand DT type genotypes are relatively short in height and have reproductive terminal buds. At the time of domestication, pigeonpea was domesticated as indeterminate and short-day plant. Genotypes that are intermediate between IDT and DT type are called semi-determinate (SDT) types and these contribute directly to grain yield, harvesting and seed production. Despite the advantage of short stature, rapid and early maturity and ease of mechanical harvesting, DT type genotypes are more prone to insect attacks and requires high seed rate (Saxena *et al* 2017). Advances have been made in mapping of growth habit locus *(Dt1*) mapped on the *CcLG03*. Subsequently, QTL analysis highlighted one gene, *CcTFL1*, as a candidate for determinacy, since an Indel marker derived from this gene co-segregated with Dt1 locus. This marker would be useful to differentiate between DT/IDT growth type and was also validated on 262 pigeonpea lines in this experiment. The identification of this user-friendly marker will allow low-cost genotyping without need of automation (Saxena *et al* 2017). In another study, Mir *et al* (2012) reported mapping of determinacy in pigeonpea based on whole-genome scanning. A set of 94 pigeonpea lines including 11 determinate (DT) and 83 indeterminate (IDT) lines were used for genotyping with DArT arrays (with 6144 features) and 768 SNP markers using Golden Gate assay. Significant association ($P \leq 0.01$) of determinacy with 19 SNP and 6 DArT markers explaining 8.05%–8.58% and 7.26%–14.53% phenotypic variation, respectively was observed. Discrimination between DT lines and IDT lines was observed after analysis with associated markers instead of clustering based on entire DArT and SNP markers. Marker–trait associations after validation may prove useful in marker-assisted selection (MAS) involving the development of ideal DT genotypes for environments with moderate growth, tolerance to drought and waterlogging. This is the first report on mapping of determinacy trait as well as the first report on association mapping for any trait in pigeonpea.

Enhancing yield gains in pigeonpea calls for a prolonged focus on reconstructing the ideal plant type using more efficient genomic tools. Plant ideotype breeding intends to deliver suitable genotypes for modern farming practices particularly ideotype breeding seeks accumulating favorable QTLs for various component traits in a given genotype (Wu 1998). Therefore, Patil *et al* (2018) evaluated 133 pigeonpea germplasms including four checks in an augmented design for various traits i.e. initiation of flowering, days to 50% flowering, days to

maturity, plant height, primary branches, seeds/ pod and pod length. The BLUP values were calculated for individual traits, and association analysis was performed in a panel of 95 diverse genotypes having 19 genic SSRs. Out of five flanking SSRs used here for validation, only ASSR295 could show significant association. Additionally, two highly significant SSR markers, ASSR8 and ASSR390 on LG 1 and LG 2,respectively were identified.

Genotype-by-environment (G×E) interaction plays significant role for stability of genotypes across environments with high yield potential. Hence, it is essential to breed stable varieties with high yield potential which perform consistently under different agro-climatic and cropping systems. In this context, a study was conducted by Singh *et al* (2014) on twenty-one short duration genotypes of rainfed pigeonpea during *kharif* season of 2007-08, 2008-09 and 2009-10. Genetic divergence based on mahalanobis D^2 method grouped the genotypes into six different clusters. The genotypes viz., GT 101 showed higher mean performance for grain yield with non-significant deviation from regression, followed by ICPL 87 and PA 134. Besides diversity, the other important thing is character association in pigeonpea which is essential for well planned heterosis breeding programme to validate the characters responsible for increased seed yield of hybrids. Hemavathy *et al* (2019) conducted a study in 57 pigeonpea genotypes to study the variability, path coefficient and correlation in pigeonpea. Number of secondary branches/plant (111.73) followed by number of pods/plant (40.73) recorded high estimates of GCV. Additive gene action was observed for number of pods per plant (83.88), number of secondary branches/plant (230.00), number of racemes (60.41), single plant yield (80.89) and exhibiting high genetic advance for these traits. Days to 50 % flowering and pods/plant showed high positive direct effect on yield. Similarly, Chetukuri *et al* (2013) evaluated 84 pigeonpea lines for genetic and phenotypic variation as well as association of grain yield with component traits using correlation, heritability and variability analysis. Phenotypic variance was high as compared to genotypic variance for all the traits. Heritability in broad sense was high for all traits, with the exception of days to pod initiation (0.0084). Genotypic and phenotypic correlations of yield were positive with all traits except for days of pod initiation, pod length and 100-seed weight. Total plant yield, number of seeds/plant and number of pods/plant have been identified as selection criteria for obtaining good parental lines in a pigeonpea breeding programme. Additionally, Saroj *et al* (2013) estimated phenotypic and genotypic variances, heritability and genetic advances, correlation and path coefficient for grain yield and yield component traits in 70 pigeonpea genotypes. The highest GCV was recorded for number of secondary branches/plant (61.81) followed by pods/plant (38.34). Heritability in broad sense varied from 61.33 (seeds/pod) to 98.26 (days to 50% flowering). High genetic advance was observed for number of secondary branches/plant (154.10), number of primary branches/plant (60.61), pods/plant (92.59), grain yield/plant

(84.65), 100-seed weight (50.82), days to 50% flowering (45.05) and plant height (33.83) suggesting additive gene action for inheritance of these traits. Correlation and path coefficient analysis (genotypic and phenotypic) revealed that pods/plant, 100-seed weight, days to 50% flowering, primary branches and secondary branches had maximum direct effect resulting into the significant positive correlation with grainyield/plant. Hence these traits can be used to improve the grain yield of pigeonpea.

Kaoneka *et al (2017)* have identified genomic regions associated with yield and related traits in three newly developed F_2 population of pigeonpea. GBS was performed for genetic analysis and linkage analysis was done using JoinMap version 4. QTL analysis of eight yield and yield-related traits was performed using single marker analysis (SMA) and composite interval mapping (CIM) using stepwise regression linear model. A total of 42 QTLs were detected that were co-localized within the same genomic regions indicating the close genetic linkage. This study will help in further fine mapping and validation of QTLs and transfer in several independent mapping populations. Likewise Kumawat *et al* (2012) constructed a high density intra-specific linkage map using 296 genic-SNP and SSR markers s covering a total adjusted map length of 1520.22 cM for mapping of major quantitative trait loci (QTLs) for key agronomic traits. A population of 186 $F_{2:3}$ lines derived from an intra-specific cross between inbred lines 'Pusa Dwarf' and 'HDM04-1' was used. This is the first dense intra-specific linkage map of pigeonpea with the highest genome length coverage. Thirteen QTLs for the six agronomic traits were identified with phenotypic variance ranging from 3.18% to 51.4%. by the individual QTLs. Ten of these QTLs were clustered in just two genomic regions, indicating pleiotropic effects or close genetic linkage. The QTLs identified in this study provide a strong foundation for further validation and fine mapping for utilization in the pigeonpea improvement.

2.4 **NGS technologies accompanied with Genome-wide association studies**

Advances in molecular biology techniques and next-generation sequencing technologies have encouraged generation of genomic tools such as transcript reads, molecular markers BAC-end sequences in pigeonpea. This has lead to development of dense genetic maps, QTL maps and physical maps. These above mentioned advances in genomics have hastened the crop improvement in the form of marker-assisted selection for hybrid purity assessment, marker-assisted backcrossing (MABC) for introgression of QTL region. In addition, the use of advanced-backcross (AB-backcross) breeding and special populations (MAGIC population) will help in creating new variations to develop superior lines (Varshney *et al* 2013). To meet the challenges of feeding this ever growing population, scientists are focusing on development of new and more efficient breeding strategies for the efficient

exploitation of genomic tools with precise phenotyping to utilize natural and induced variation in a better way. Over the last decade, rapid developments in next generation sequencing technologies (NGS) have made it possible to explore the relationship between genotype and phenotype with greater resolution than ever before. Due to the decreased cost of sequencing now a days, it's a routine practice for breeders to sequence large populations to discover QTLs with high resolution and setting the basis for modeling complex genotype-phenotype relationships at the whole genome level. Many NGS technologies are now available like whole genome sequencing (WGS), whole genome re-sequencing (WGRS), genotyping by sequencing (GBS), transcriptome and epigenetic analysis. For gene and QTL discovery with increased mapping resolution, various approaches like genome-wide association studies (GWAS) utilize association mapping or linkage disequilibrium (LD) mapping, map QTLs by taking advantage of historic LD to identify significant phenotype-genotype associations (Varshney *et al* 2014).

There are two types of genomic-assisted breeding: Marker assisted selection (MAS) and genomic selection (GS) (Varshney *et al* 2005). MAS is the most widely used form of genomics-assisted breeding. It is useful for introgressing particularly recessive alleles, pyramiding genes with overlapping phenotypic effects, for traits that are difficult to phenotype (Collard and Mackill 2008). For traits with complex inheritance associated with major effect QTLs, MAS can be used to target the traits like 'grain yield' and 'yield under drought' in rice (Imai *et al* 2013), and drought tolerance in chickpea (Varshney *et al* 2014). Bulked segregant analysis and modifications in combination with NGS based mapping speed up the identification of candidate genes (James *et al* 2013). Pooled DNA from two phenotypic extremes are used to identify the genomic regions underlying the trait (Michelmore *et al* 1991). An approach known as QTL-Seq, involves WGRS on bulked DNA samples from two phenotypic extremes of a population of RILs or F_2 individuals to find the causal SNPs for target trait. NGS simultaneously performs SNP discovery, SNP validation and SNP genotyping in a population, therefore, it is very efficient for map-based gene discovery. Single nucleotide polymorphisms (SNPs) are markers of choice due to their higher abundance. Saxena *et al* (2012) used RNA-seq and allele-specific sequencing approaches for discovering single-nucleotide polymorphisms (SNPs, >2000) in pigeonpea (*Cajanus cajan*). Competitive allele-specific polymerase chain reaction (KASPar) assays were developed for 1616 SNPs and referred to as PKAMs (pigeonpea KASPar assay markers). Out of 1154 polymorphic markers, 1094 PKAMs showed polymorphisms between parental lines of the reference mapping population (*C. cajan* ICP 28 × *C. scarabaeoides* ICPW 94). A comprehensive genetic map comprising 875 PKAMs with an average intermarker distance of 1.11 cM on 167 F_2 lines from the population was developed. Higher degree of synteny with the genome of

Glycine max followed by *Medicago truncatula* and *Lotus japonicus* and least with *Vigna unguiculata* was observed. These PKAMs will be useful for genetics research and breeding applications in pigeonpea and for utilizing genome information from other legume species. On the other hand, despite being the availability of several types of genotyping platforms for assaying large number of SNPs, high throughput SNP genotyping platforms (e.g., iScan or Infinium) may not be cost effective. In this view, Golden Gate assays based on VeraCode technology using Illumina BeadXpress seems to be the most cost-effective platform. The study was conducted with the objective to develop cost-effective SNP genotyping platforms in chickpea (*Cicer arietinum* L.) and pigeonpea (*Cajanus cajan* L.). Two sets of SNPs, one each for chickpea (96 SNPs) and pigeonpea (48 SNPs), were developed and tested by genotyping 288 diverse genotypes from respective reference sets. Two major groups were observed in the case of pigeonpea whereas no unique pattern was observed in chickpea reference set. Assays developed for chickpea and pigeonpea in this study are highly informative and cost effective for undertaking genetic studies in these legume species (Roorkiwal *et al* 2013).

With the completion of draft genome sequence, pigeonpea has been considered among sequenced legumes such as *Lotus*, *Medicago* and Soybean. Re-sequencing of landraces, wild relatives and cultivars would ensure recovery of novel haplotypes associated with domestication and other important traits (Bohra *et al* 2013). However, in recent times, it has been shown that a single reference sequence cannot capture entire gene content of species because of significant gene presence or absence variation(PAV) (Golicz *et al*, 2015a, Hurgobin *et al*, 2018a). Therefore, to understand gene diversity across the species and apply this for crop improvement, a pangenome is necessary. The pangenome concept was first introduced to represent the full complement of genes within a bacterial species and it comprises the core complement of genes common to all members of a species (Tettelin *et al* 2005). The first pangenome in pigeonpea based on 89 accessions mainly from India and the Philippines, indicated the genetic diversity in Philippine individuals not present in Indian individuals as reported by Zhao *et al* (2020). Annotation of variable genes revealed their association with self-fertilization and response to disease and identification of 225SNPs associated with nine agronomically important traits over three locations and two different time points, with SNPs associated with genes for transcription factors and kinases. The results will pave the way to an improved pigeonpea breeding programme. On the other hand, integration of next -generation sequencing techniques with 'omics' technologies is an effective strategy to find critical regulatory regions governing various seed traits. A collaborative and extensive germplasm evaluation is required to utilize its full potential. There is need to find the potential genes/alleles in genomic selection for quality trait improvement

such as complex traits of seed quality and nutritional content. Therefore, the understanding of complex genetic architecture of qualitative and quantitative traits is very important for the development of nutrient rich varieties for value addition (Singh *et al* 2020).

In view of the above mentioned strategies, a study was conducted by taking the advantage of GBS along with multilocation evaluation of two interspecific backcross populations to discover the novel alleles from wild species. Single-nucleotide polymorphism markers associated with yield related traits were identified. A total of 86 QTLs explaining 12%–21% phenotypic variation and 107 QTLs explaining 11%–29% phenotypic variation were detected in BP-1 and BP-2 respectively. Interestingly, 11 QTLs in BP-2 were associated with more than one trait. One SNP marker in BP-2 population has been found associated with four traits, days to 50% flowering, days to 75% maturity, primary branches/ plant and secondary branches/ plant with positive additive effect. Although most QTLs were environment and trait specific, few stable and consistent QTLs were also detected which can be used for improvement of traits through genomics-assistedbreeding (Saxena *et al* 2020).

Once markers associated with QTLs governing high Fe, Zn and high yield are available; these QTLs can be pyramided in one genotype in different combinations using simultaneous marker-assisted selection for multiple traits (van Berloo and Stam 2001). Population size and trait heritability are the main factors influencing MAS results (Moreau *et al* 1998, Van Berloo and Stam 1998). It is the transfer of several useful genes in different germplasm lines into one genome by using conventional, backcross and compound hybrid technique and then selection of single plant with target genes in different combinations using molecular markers among segregating popualtions (Lan and Chao 2011).

High-density SNP arrays and genotyping-by-sequencing (GBS) have come as attractive and efficient genotyping tools. Simultaneous SNP discovery and genotyping using NGS platforms facilitated the development of many more SNP markers and construction of dense linkage maps for the identification of QTLs for various agronomic characters (Tayeh *et al* 2015b, Huang *et al* 2017, Ma *et al* 2017, and Gali *et al* 2018). But at the same time, there is a need to identify more number of trait-associated markers in a large gene pool such as diverse set of germplasm lines than the bi-parental mapping populations (Gali *et al* 2019). To dissect the genetic nature of complex traits in genetically diverse set of germplasm lines, genome-Wide Association Study proves to be an efficient approach providing higher mapping resolution in genomics era (Korte and Farlow 2013). GWAS is a powerful tool effectively used for investigation of natural variation occurring in various traits for identification of causal loci/genes. Historical recombination events leading to high-resolution mapping and incorporation of large number of alleles in GWAS are being exploited. Due to advancements

in NGS technologies, the cost of sequencing has markedly reduced which enabled the access to whole genome sequences and genome-wide SNPs across many accessions at a time. Hence, using the large number of genome-wide SNPs across large number of accessions, GWAS has emerged as an alternative approach for bi-parental QTL mapping. In GWAS, development of mapping population is not required as compared to genetic bi-parental mapping population. Furthermore, accessions genotyped once, can be used for GWAS analysis for number of traits without genotyping again, leading to dissection of genetic architecture of various traits at a quick instance (Nakano *et al* 2020).

CHAPTER-III

MATERIAL AND METHODS

The present study was carried out at the experimental field area of pulses section, Department of Plant Breeding and Genetics, Punjab Agricultural University (PAU), Ludhiana and School of Agricultural Biotechnology, PAU, Ludhiana. Major objectives were phenotypic evaluation of germplasm lines for Fe, Zn and yield component traits, genome-wide association studies to identify the QTLs governing Fe, Zn content and component traits related to yield and further validation of identified QTLs for these traits in bi-parental population. Material and methodology used for conducting different experiments is discussed in this chapter.

3.1 Experiment No. 1: Evaluation of diverse germplasm lines for yield and quality component traits.

3.1.1 Plant material

A set of 178 diverse germplasm lines including released varieties, advanced breeding lines and indigenous collections was used. The germplasm lines were planted in a randomized complete block design consisting of two replications in a single row plot of length 4 m with 75 cm of row spacing during *kharif* 2018-19 and 2019-20. Standard cultural practices were adopted for raising a healthy crop.

3.1.2 Phenotypic evaluation for yield component traits

Phenotypic data for yield component traits were recorded on three randomly taken plants of each genotype in both the replications. The selected three plants were harvested separately and all other plants of the test rows were bulk harvested. Observations were recorded for yield attributing traits *viz.,* plant height (PH), number of primary branches (PB), number of secondary branches (SB), number of pods/plant (PD), number of seed/pod (SP), 100-seed weight (SW), yield/plot and for earliness traits *viz.,* days to 50% flowering (DF) and days to maturity (DM).

3.1.2.1 Days to 50% flowering: Days to 50% flowering was scored as number of days from the date of sowing to which flowers are open in 50% of plants in a row.

3.1.2.2 Days to maturity: Days to maturity was scored as number of days from the date of sowing to when 80% of the pods turned brown in plants in a row.

3.1.2.3 Plant height (cm): Plant height was measured from the base of the plant to the tip of the main axis.

3.1.2.4 Number of primary branches: Number of primary branches originating from the main shoot bearing at least one pod were counted.

Table 3.1: List of genotypes used for investigation

S. No.	Genotypes	Origin	S. No.	Genotypes	Origin	S. No.	Genotypes	Origin
1	AL 292	PAU	35	AL 1721	PAU	69	IC 245533	NBPGR
2	AL 303	PAU	36	AL 1725	PAU	70	IC 245560	NBPGR
3	AL 304	PAU	37	AL 1730	PAU	71	AH 06-1	HAU
4	AL 307	PAU	38	AL 1740	PAU	72	AH 06-7	HAU
5	AL 311	PAU	39	AL 1747	PAU	73	AH 06-12	HAU
6	AL 986	PAU	40	AL 1759	PAU	74	AH 09-1	HAU
7	AL 997	PAU	41	AL 1790	PAU	75	AH 09-3	HAU
8	AL 1313	PAU	42	AL 1801	PAU	76	AH 09-11	HAU
9	AL 1396	PAU	43	AL 1813	PAU	77	AH 15-01	HAU
10	AL 1430	PAU	44	AL 1820	PAU	78	AH 15-1	HAU
11	AL 1449	PAU	45	AL 1823	PAU	79	AH 15-02	HAU
12	AL 1452	PAU	46	AL 1835	PAU	80	AH 15-06	HAU
13	AL 1455	PAU	47	AL 1847	PAU	81	AH 15-07	HAU
14	AL 1459	PAU	48	AL 1848	PAU	82	AH 15-08	HAU
15	AL 1465	PAU	49	AL 1849	PAU	83	AH 15-16	HAU
16	AL 1466	PAU	50	AL 1853	PAU	84	AH 15-19	HAU
17	AL 1471	PAU	51	AL 1856	PAU	85	AH 15-25	HAU
18	AL 1474	PAU	52	IC 3977	NBPGR	86	H 005	HAU
19	AL 1476	PAU	53	IC 245139	NBPGR	87	H 01-3	HAU
20	AL 1477	PAU	54	IC 245176	NBPGR	88	H 01-18	HAU
21	AL 1486	PAU	55	IC 245183	NBPGR	89	H 01-37	HAU
22	AL 1487	PAU	56	IC 245186	NBPGR	90	H 02-65	HAU
23	AL 1490	PAU	57	IC 245219	NBPGR	91	H 03-28	HAU
24	AL 1491	PAU	58	IC 245245	NBPGR	92	H 03-29	HAU
25	AL 1508	PAU	59	IC 245268	NBPGR	93	H 04-20	HAU
26	AL 1533	PAU	60	IC 245273	NBPGR	94	H 05-7	HAU
27	AL 1538	PAU	61	IC 245294	NBPGR	95	H 05-12	HAU
28	AL 1543	PAU	62	IC 245314	NBPGR	96	H 05-35	HAU
29	AL 1554	PAU	63	IC 245315	NBPGR	97	H 05-71	HAU
30	AL 1584	PAU	64	IC 245497	NBPGR	98	H 49-3	HAU
31	AL 1592	PAU	65	IC 245507	NBPGR	99	H 61-21	HAU
32	AL 1594	PAU	66	IC 245523	NBPGR	100	H 93-32	HAU
33	AL 1627	PAU	67	IC 245524	NBPGR	101	ICP 8947	ICRISAT
34	AL 1676	PAU	68	IC 245525	NBPGR	102	ICPL 2037	ICRISAT

S. No.	Genotypes	Origin
103	ICP 9202	ICRISAT
104	ICP 9900	ICRISAT
105	ICP 11250	ICRISAT
106	ICPL 9308	ICRISAT
107	ICPL 98015	ICRISAT
108	ICPL 2033	ICRISAT
109	ICPL 920	ICRISAT
110	Paras	HAU
111	Pant A 37	GBPUAT
112	Pant A-51	GBPUAT
113	Pant A-163	GBPUAT
114	Pant A-174	GBPUAT
115	Pant A 234	GBPUAT
116	Pant A 251	GBPUAT
117	Pant A 252	GBPUAT
118	Pant A 855	GBPUAT
119	Pusa 84	IARI
120	Pusa 991	IARI
121	Pusa 992	IARI
122	P 994	Unknown
123	P 212-1	Unknown
124	P 226	Unknown
125	P 2001-6	Unknown
126	P 2002	Unknown
127	P 2003	Unknown
128	P 2007	Unknown
129	CORG 105	TNAU
130	CORG 111	TNAU
131	CORG9704	TNAU
132	CORG6012	TNAU
133	MTH 103	Unknown
134	MN-1	USA
135	MN -5	USA
136	CIPB 5120	Unknown
137	V 10099	Unknown
138	T -21	IIPR
139	TAT 108	BARC
140	AP 1371	Unknown
141	AF 370	Unknown
142	Manak	HAU
143	AL 1922	PAU
144	AL 2087	PAU
145	AL 2091	PAU
146	AL 2025	PAU
147	AL 2207	PAU
148	IC 245350	NBPGR
149	AL 1781	PAU
150	H 94-8	HAU
151	H 2000-14	HAU
152	AL 1789	PAU
153	AH 06-9	HAU
154	AH 06-3	HAU
155	PUSA2001	IARI
156	IC 245219	NBPGR
157	AH 09-41	HAU
158	AL 1846	PAU
159	AL 1782	PAU
160	AL 2170	PAU
161	AL 2133	PAU
162	VLA-1	Unknown
163	PAU 881	PAU
164	PADT -16	Unknown
165	AL 2132	PAU
166	AL 2128	PAU
167	Sarita	Unknown
168	AL 2131	PAU
169	AL 882	PAU
170	AL 2062	PAU
171	PBR-2	Unknown
172	PANTA250	GBPUAT
173	IC 245294	NBPGR
174	Pant -37	GBPUAT
175	AH 09-2	HAU
176	H-61-21	HAU
177	Pant -234	GBPUAT
178	AL 2204	PAU

3.1.2.5 Number of secondary branches: Number of branches originating from the primary branches were counted for individual plant at maturity

3.1.2.6 Number of pods/ plant: The total number of pods borne on the primary and secondary fruiting branches was counted at maturity from each plant.

3.1.2.7 Number of seeds/ pod: Mean number of seeds that were counted from all the effective pods for each plant at maturity.

3.1.2.8 100-seed weight: Weight of the randomly sampled 100 seeds was taken.

3.1.2.9 Yield/plot: Plot yield was recorded by weighing the seeds from all the plants in a row.

3.1.2.10 Grain Fe content: Grain Fe content was estimated using micro digestion (Inductively coupled plasma spectrometry) method.

3.1.2.11 Grain Zn content: Grain Zn content was estimated using micro digestion (Inductively coupled plasma spectrometry) method

3.1.3 Estimation of Fe and Zn content in grains

For estimation of Fe and Zn content, 5 g of seed was ground in mortar and pestle (sterilized using 70% ethanol to avoid contamination). 0.25 gm of the ground seed was added to the test tubes along with 10 ml of di-acid mixture (1:4 perchloric acid and nitric acid). This mixture was kept overnight and digested in a microwave. After microwave digestion, the final volume was made to 30 ml, using double distilled water in a fume hood to prevent contamination. This sample was further processed in ICP-OES (Perkin Elmer USA) for the estimation of Fe and Zn.

3.1.4 Analysis of variance (ANOVA)

Statistical analysis of variance for every trait was performed using SAS 9.4 version (SAS institute 2002). It helps to divide the total variance into three components *viz*., genotypic, phenotypic, and environmental variance. it also helps to check the statistical significance of the difference among genotypes for a given trait.

3.1.5 Estimation of parameters of genetic variability

The parameters of genetic variability i.e., the genotypic coefficient of variance (GCV), phenotypic coefficient of variance (PCV), heritability in broad sense and genetic advance for different traits under study were calculated as per the procedures described by Burton and Devane (1953) and Johnson *et al* (1955) as given below:

3.1.5.1 Genotypic and phenotypic variances – The variability was measured by simple statistical measures such as range, mean, genotypic and phenotypic variances and coefficient of variation. Formula suggested by Singh and Chaudhary (1985) was used to measure variances and coefficient of variations

$$\sigma^2 p = \sigma^2 g + \sigma^2 e$$

where; $\sigma^2 p$ = Phenotypic variance,

$\sigma^2 g$ = Genotypic variance,

$\sigma^2 e$ = Environmental variance = Error mean square

$$\sigma^2 g = (MSg - MSe)/r$$

where; MSg = Mean square of genotypes,

MSe = Mean square of error,

r = Number of replication

3.1.5.2 Genotypic and Phenotypic Coefficients of Variance

Genotypic Coefficient of variation (GCV) = $(\sigma^2 g)$ ½/ $\mu \times 100$

Phenotypic Coefficient of variation (PCV) = $(\sigma^2 p)$ ½/$\mu \times 100$

Where,

σ^2_g = Genotypic variance

σ^2_p = Phenotypic variance

μ = Mean of the population for a trait under investigation

Measure of GCV and PCV:

> 20 % = High

10 - 20 % = Moderate

< 10 % = Low

3.1.5.3 Heritability in broad sense [h^2 (bs)]

Heritability (per cent) in broad sense was calculated as per the formula described by Allard (1960).

$$h^2(bs) = \frac{\sigma^2_g}{\sigma^2_p} \times 100$$

Where, σ^2_g = Genotypic variance

σ^2_p = Phenotypic variance

Measure of heritability:

> 80 % = High

50 - 80 % = Moderate

< 50 % = Low

3.1.5.4 Genetic Advance (GA)

Genetic advance was measured as per the formula described by Miller *et al* (1958).

$$\text{Genetic Advance} = h^2.k.\sigma_p$$

Where,

h^2 = Heritability

k = Selection Differential (At 5 % selection intensity, k = 2.06)

σ_p = Phenotypic Standard Deviation

Genetic advance as % of mean was measured as under:

$$\text{GA (\% of mean)} = \frac{GA}{\bar{x}} \times 100$$

3.1.6 Correlation coefficient – Minitab 16 for windows was used to measure a matrix of simple correlation coefficients between traits determine the relationship between Fe and Zn content and different component traits with seed yield.

3.1.7 Path coefficient analysis – In order to measure the relations among a set of variables, path coefficient analysis was done as per Singh and Chaundhary (1985) to determine direct and indirect effects of component characters on seed yield.

The path coefficients were analyzed according to the method described by Dewey and Lu (1959) by using matrix method. The matrix inverse of correlation coefficients was done and R package variability was used to calculate direct and indirect effects of various component traits.

3.2 Experiment No.2: Genotyping of germplasm lines

3.2.1 Plant material

Genomic DNA from diverse germplasm lines including released varieties, advanced breeding lines and indigenous collections was extracted by pooling young leaves of five different plants from each germplasm line using CTAB (Cetyl Trimethyl Ammonium Bromide) extraction method (Murray and Thompson 1980), with the chloroform-isoamyl alcohol purification step repeated twice to obtain good quality DNA.

3.2.2 Extraction of DNA

Following steps were used for extraction of DNA:

3.2.2.1 Sample preparation

- Leaf samples were collected from seedlings of 15 days after germination.
- White butter paper was used to preserve the leaf tissues (70-100 mg) marked with appropriate plant number on it and stored in -80ºC deep freezer till further use.
- Pestle and mortar was used for crushing the leaves by adding liquid nitrogen which left them fragile and reduce DNase activity.

3.2.2.2 CTAB extraction

- **A**fter grinding, 2X CTAB extraction buffer in volume of 15 ml of was poured to the powdered samples Table 3.2.
- Buffer was added and mixed to crushed leaves by tilting the tubes carefully. Then tubes containing leaves and buffer were incubated for 45-60 min at 65ºC with periodical shaking at intervals in a water bath.

3.2.2.3 Solvent extraction

- 450 µl of C:I (24:1) mixture was added to each tube and were shaked on a rotary shaker for 45 min for the uniform mixing of the contents, untill it turns into a dark green emulsion Tubes were centrifuged at 5, 500 rpm for 10 min.
- Aqueous layer was transferred to fresh tubes Using 300 µl tips.

3.2.2.4 Precipitation of DNA

- 0.7 µl Isopropanol (stored at -20°C) of was added to aqueous layer (210) of each tube and mixed by tilting.
- Tubes were centrifuged at 5000 rpm for 15min.
- After discarding the supernatant DNA pellet was dried.

3.2.2.5RNase treatment

- RNase (3 µl) was added to each test tube containing DNA and kept in water bath for 30 minutes to get rid of RNA in DNA.

3.2.2.6 Solvent extraction

- Phenol-chloroform-isoamyl alcohol (200µl) in ratio of 25:24:1 was poured to every tube and inverted two times for mixing and were centrifuged for 5 minutes at 5000 rpm.

3.2.2.7 DNA Purification

- Ethanol-acetate (315 µl) was added to each tube and kept for 5minutes at 20ºC. and centrifuged.
- DNA pellet obtained after removing supernatant was washed using70%ethanol and then centrifuged.
- Pallet was dried for1hour after removing supernatant
- Pellet was dissolved in TE buffer and storedat4ºC.

3.2.2.8 Assessment of quality and quantity of DNA

Agarose gel electrophoresis was used to assess the quantity and quality of DNA. 0.8 g of agarose was dissolved in 100 ml of 1X TBE electrophoresis buffer(45 mM Tris base, 45 mM boric acid and 1mM EDTA).The mixture was heated untill completely dissolved and solution became transparent. Then cooled down to 55-60ºC with constant stirring and 5 µl of ethidium bromide (10 mg/ml) was added to a final concentration of 0.5 µg/µl buffer. The agarose solution was poured into a gel mould with combs and kept for 30-40 min for polymerization. Meanwhile, DNA samples were prepared for loading by adding loading dye, Bromophenol blue (3X loading dye consists of 0.4% Bromophenol blue, 0.4% Xylene cyanol, 50% glycerol in sterile water) (Table 3.4) to DNA such that the final concentration of loading dye was 1X.Upongel solidification, it was transferred to the electrophoresis tank and the DNA samples were loaded into wells with the help of a micropipette. Alongwith the DNA samples, DNA of known concentration of variable ranges (100 ng/µl, 200 ng/µl, 400 ng/µl, 500 ng/µl and 1000 ng/µl) 35 was also loaded. After loading, the gel was run for about 3-4 hr at a

constant voltage of 5 V/cm. The gel was then visualized under a UV transilluminator. Using photo gel documentation system, the DNA samples were photographed. The intensity of fluorescence of each sample was compared with that of the known DNA concentration and then DNA concentration of each sample was ascertained. The quality of DNA sample was judged based on whether the DNA formed a single high molecular weight band (good quality) or a smear (degraded or poor quality).

3.2.3 Stocks and solutions

3.2.3.1 Sodium acetate solution (3M) – 204.12 g of sodium acetate was dissolved in 350 ml of distilled water and pH was adjusted to 5.2 and final volume was made up to 500 ml and autoclaved.

3.2.3.2 RNase A (10 mg/ml) – RNase A of 100mg was dissolved in 10 ml of 10 mM Tris pH 7.5 and 15 mM NaCl, heated in boiling water for 15 min and was cooled slowly to room temperature. Thereafter, it was dispensed into aliquots and stored at-20°C.

3.2.3.3 10 X TBE (Tris Borate EDTA) buffer – Tris base, boric acid and 0.5 M EDTA (pH 8.0) were dissolved in 800 ml distilled water and the solution was made up to a volume to 1000 ml. The buffer was autoclaved and stored at 4°C.The 1X working solution was prepared from stock solution by diluting it 10 times(Table 3.5).

Table 3.2: Composition of CTAB extraction buffer

S. No.	Component	Quantity/Litre	Final concentration
1	CTAB	20 g	2.0%
2	1 M Tris HCl	100 ml	100 mM
3	NaCl	81.8 g	1.4 M
4	0.5 M EDTA	40 ml	20 mM
5	β- mercaptoethanol (added just before use)	10ml	0.2%

Table 3.3: Composition of low salt TE buffer

S. No.	Component	Final concentration
1	10 mM Tris	1.21 g
2	1 mM EDTA	0.372 g

Table 3.4: Composition of 3X loading dye

S. No.	Component	Final concentration
1	"5 M NaOH	0.2 ml
2	95% Formamide	95 ml
3	Bromophenol blue	50 mg
4	Xylenecynol	50 mg

The final volume was adjusted to 100 ml with double distilled water.

Table 3.5: Composition of 10 X TBE buffer

S. No.	Component	Final concentration
1	Tris base	108 g
2	Boric acid	55 g
3	0.5 M EDTA	’40 ml

3.2.4 ddRAD library construction

Double digest RAD (ddRAD) libraries were prepared following a modified protocol by Peterson *et al* (2012).

3.2.4.1 Restriction enzyme digestion

Double digestion of genomic DNA (1 microgram) was carried out using Sphl and Mluc1 Restriction enzymes. Used a digestion buffer appropriate for both enzymes. Digestion was run as appropriate for the chosen REs. To ensure complete digestion, double digests were run for 3 hours at 37°C, holding at 4°C.Do not heat kill the enzymes, as this may skew base composition in the resulting fragment library. Reaction was cooled to room temperature. Alternatively, reactions were stored at 4°C overnight. Double digest was cleaned with AMPure XP beads following the provided protocol, using a magnetic plate (SPRI Plate Super Magnet Plate) or rare earth magnets and a tube rack to separate beads from the solution. The concentrations of cleaned digests were quantified. Fluorometric (Spectramax plate reader or Qubit [Invitrogen]) measurement of DNA concentration is highly recommended. These measurements were used for preparing ligations.

3.2.4.2 Adapter Ligation

Double-stranded adapters in paired combinations were used to uniquely tag samples which were quantified and pooled in equimolar amounts.P1 (Barcoded) and P2 adapters were ligated to the restriction digested DNA fragments using T4 DNA ligase.

3.2.4.3 Pooling

After the ligation step, individual barcoded samples with a unique P1 adapter were pooled and cleaned. The end goal was to pool every uniquely barcoded individual into a final 30µl sample such that the entire pool of ligation products could be loaded onto a Pippin Prep for size selection. The number of individuals that could be pooled into the final 30µl for size selection depended on the number of unique P1 adapters used.

3.2.4.4 Size Selection

Size selection of the product was done after 2% agarose gel electrophoresis. Although gel extractions can recover fragment sizes appropriate for Illumina sequencing, automated DNA size selection provides far superior results. Fragments ligated to both P1 and P2 adapters were clustered during sequencing and fragments with P1 Adapters on both 5‘ and 3‘ ends were still amplified in the subsequent PCR step. If the final sequencing library contains many fragments with two adapter P1 ends, then it becomes difficult to quantify how much sample to load on the sequencer for optimal cluster generation. Fragments with only adapter P1 ends were removed from libraries using Streptavidin-coated Dynabeads and biotin-labeled P2.2 oligos. After the size-selection step, standard Invitrogen protocol was followed for both bead preparation and hybridization of beads with biotin-labeled DNA.

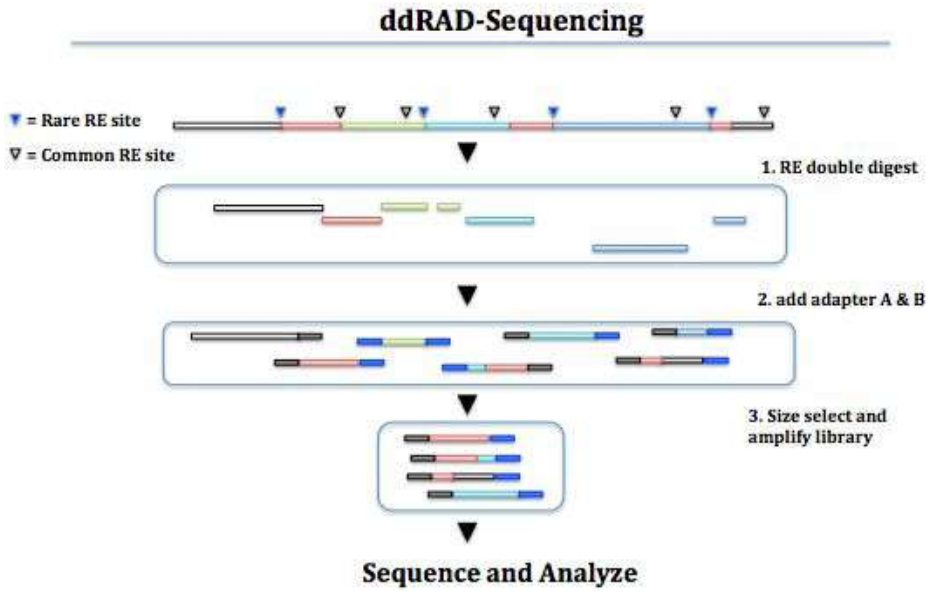


Figure 3.1: Library Preparation Workflow

3.2.4.5 Generation of Illumina Sequencing Libraries thro ugh PCR Amplification

PCR amplification with a Phusion™ Polymerase kit to add Illumina flow cell annealing sequences was performed. For large-scale combinatorial multiplexing of samples, a

set of 12 uniquely indexed PCR primer 2 sequences was included. Each of these primers were added a unique index sequence to all fragments in the PCR. Therefore, it was possible to uniquely label 576 individuals [48 (adapter P1 barcodes) × 12 (PCR2 indices)] to combine in a single lane of sequencing. For each Pippin Prep elution or gel extraction, 4-8 PCR reactions were set up in 20µl total volume:

- For each PCR, combined~20ng of size-selected sample, PCR primers 1 and 2 at final concentration 2µM each, and the recommended amount of 5X-HF buffer, dNTPs, water and Phusion polymerase in a standard 200µl PCR tube.
- Performed 8-12 cycles of PCR. Increasing cycle number beyond this could introduce substantial (>1%) base mis-incorporation and exacerbate size and composition bias in final libraries.
- Combined the completed reactions and cleaned with AMPure XP beads, eluting in 30-40µl.
- To quantify molarity and library fragment size distribution, cleaned PCR samples were run on Bioanalyzer.
- At this point, samples with distinct multiplexing indices introduced in the PCR could be combined in equimolar ratios to compose a final library for each sequencing lane.

3.2.4.6 Sequencing

ddRAD libraries were high throughput sequenced using IlluminaTrueSeq chemistry on IlluminaHiSeq 2000 platform.

3.2.4.7 Bioinformatics Analysis

Steps for reference-based ddRAD sequencing were as followed:

1. Pre-processing through Fastq:
   - De-multiplexing: Demultiplexing was done to produce reads for every genotype
   - Regions that were showing low quality bases were deleted from start or end
   - The trimming of adapters at 5’ and 3’ ends was performed
2. Alignment: Processed reads were then aligned to the reference genome using Bowtie 2 programme.
3. Variant calling: Samtools program for variant calling of aligned genotypes with reference genome was used.
4. Variant annotation: Annotation for Indels and SNPs was done using pigeonpea reference genome

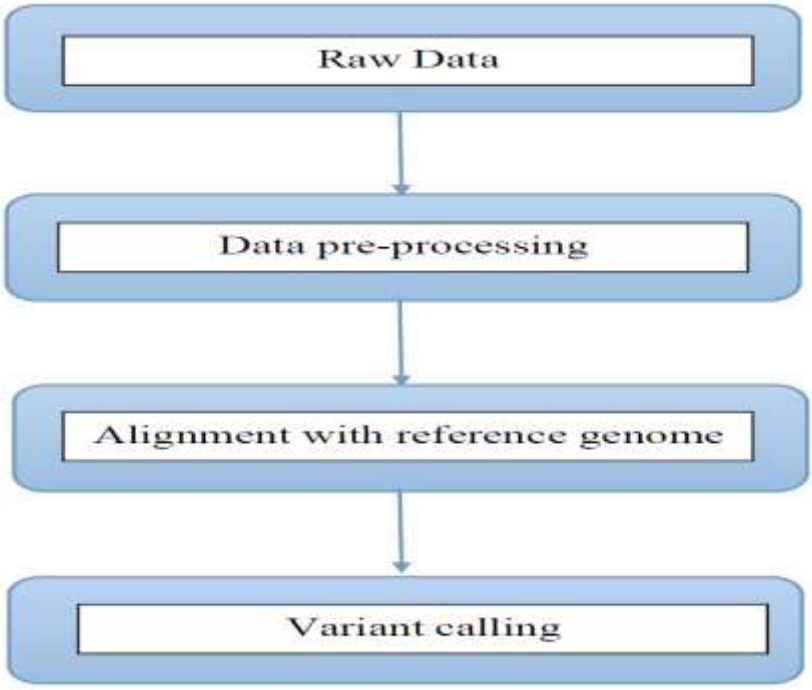


Figure 3.2: dd-RAD Reference-based analysis pipeline

3.3 Experiment No. 3: Validation of QTLs identified through genome-wide association studies in bi-parental population

3.3.1 Plant material

Bi-parental population ($F_{2:3}$) developed from crossing two contrasting genotypes for yield attributing traits were used for validation of QTLs identified through GWAS analysis. The population comprising of 157 $F_{2:3}$ lines was planted in a randomized complete block design during *Kharif* 2020. Standard cultural practices were followed for raising the crop.

3.3.2 Methodology

Genotypes showing contrasting characters were selected from the germplasm and used for phenotypic evaluation. Genotype AL1449 with indeterminate growth habit was used as male parent to cross with MN-1 having determinate growth habit as female parent in main season 2018. Seeds obtained from the cross were planted as F_1 in off season during 2018-19 (Jan-Feb) in polyhouse facility of Department of Plant Breeding and Genetics, PAU. Seeds obtained from F1 plants were planted as individual F_2 plants in main season during 2019-20. Further during the main season of 2020-21, the seeds obtained from individual F_2 plants were sown as plant to progeny rows and a total of 158 F_2 plants were planted during this season. Genomic DNA from the 158 lines was extracted for further analysis with KASP markers.

3.3.3 Genomic DNA isolation

Genomic DNA from 158 $F_{2:3}$ lines was extracted using CTAB (cetyltrimethyl ammonium bromide) method as modified by Saghai-Maroof *et al* (1984).

3.3.3.1 Genomic DNA quantity assessment

Quantification of genomic DNA was done using Nanodrop™. In this equipment, a 2µl DNA sample was loaded at the top of fibre optic pedestals(the receiving fibre).The source fibre optic cables were then brought into contact with the receiving fibres with the sample in between the two fibre optic cables. The light source was provided by Xenon flash lamp and the light passing through the sample was analysed by spectrophotometer. The sample quantity and quality was indicated by spectral data and purity ratio.

3.3.3.2 Normalization of genomic DNA

The quantified DNA was normalized by preparing a intermediate dilution with concentration of 25ng/µl in 200µl final volume with molecular biology (MB) grade water. For KASP assay, the dilutions were prepared from the intermediate dilution by keeping final concentration of 5ng/µl in 50µl of final volume with Molecular biology grade water.

3.3.3.3 Designing of KASP markers

Kompetettive allele specific PCR (KASP) markers were designed by the Primer3 software given by Rozen *et al* (2000).The significantly associated SNP loci were used to design KASP markers. For each associated SNP loci, three primer set was generated. The 200 bp upstream and downstream sequences of the associated SNP loci were fetched from the reference genome (Varshney *et al* 2012) assembly. A total of 400 bp fragment carrying the associated SNP loci was used for designing the primers. For KASP marker designing, oligolength of 25-35 bp, melting temperature (Tm) of 45^0 C – 80^0 C, without any palindromic sequences, hairpin and primer dimer were taken into consideration. Unlabelled tail sequences FAM and HEX were added to the two oligos alternate alleles

3.3.3.4 KASP assay

The KASP genotyping assay included primer mix, KASP master mix and sample DNA. The primer mix included three oligos i.e. two allele specific oligos and one common primer. The 2X KASP master mix carried FAM™ and HEX™ FRET cassette, Taq polymerase specially modified for allele-specific PCR and optimized buffer. The sample DNA was loaded first of all in 384 well microtitre plate. A reaction mixture (Table 3.6) carrying KASP marker and primers was prepared and loaded into each well containing DNA sample.

Table 3.6: KASP PCR reaction profile

Constituent	Volume (1X) (µl)
DNA	2
Primer mix	0.054
KASP mix	1.944

Table 3.7: Thermal cycling conditions for KASP chemistry

Temperature/Duration	Cycle
95^0C for 15 minutes	Hot-start activation
95^0C for 20 seconds 64^0C for 1 minute (dropping 0.6^0C per cycle)	10 cycles
95^0C for 20 seconds 57^0C for 1 minute	30 cycles

3.3.4.5 Visualization of KASP PCR

High throughput TECAN infinite F200 PRO plate reader was used for scoring and genotyping of samples. Fluorescent dyes specific filters measured the amount of fluorescence in each well by placing the384 well plate inside the plate reader. To view the FAM and HEX data in an x-y plot, Klustercaller software was used. The homozygous alleles signal were plotted near the x and y axis for FAM and HEX dyes, respectively whereas heterozygotes (FAM/HEX) were plotted in between the two axis. ROX dye was used for normalization.

CHAPTER IV

RESULTS AND DISCUSSION

The present study was carried out at the experimental area, Department of Plant Breeding and Genetics, Punjab Agricultural University (PAU), Ludhiana and School of Agricultural Biotechnology, PAU, Ludhiana with the objectives to conduct genome-wide association studies for identification of QTLs associated with Fe, Zn and yield related traits in pigeonpea germplasm and validation of QTLs in biparental population developed by crossing contrasting genotypes for Fe, Zn and yield attributing traits. For conducting the present study, 178 germplasm lines available with the pulses section of Department of Plant Breeding and Genetics were planted in randomized complete block design (RCBD). Observations were recorded for all yield related traits along with grain Fe and Zn content over two years.

Over two-third of the world population diets lack one or more essential mineral elements and consequently, suffer from mineral malnutrition, as they are dependent on plant-based foods that often have a low mineral density (Waters & Grusak, 2008). Among the mineral elements, Fe and Zn play a major role in many metabolic functions vital for human growth and development (Bourre 2006, Ozturk *et al* 2018). Ensuring a sufficient intake of micronutrients is one of the components of food security (Meenakshi *et al* 2010). Biofortification is an approach targeted at the enhancement of quantity and quality of nutrients in agricultural crops, reducing micronutrient deficiencies in human diet. Being the major source of protein and essential mineral elements in Asia and Africa, there is a need to develop high yielding biofortified varieties in pigeonpea. Crop yield is one of the complex characters controlled directly and indirectly by several component characters. To enhance the crop productivity, the availability of variability for yield and component traits in population is crucial (Hemavathy *et al* 2019). The desired combination of yield contributing traits can be accumulated in a particular genotype only by understanding the inter -relationships of various traits by studying the correlation component between them. (Pandey *et al* 2015). Seed yield is a character dependent on various characters and to some extent, on the environmental conditions. Therefore, selection for yield always misleads the breeder (Bal Chinmayee 2016). A complete understanding of the relationship between yield and yield components is a must for a plant breeder towards the improvement of any crop. Genetic parameters like phenotypic and genotypic coefficient of variation, heritability and genetic advance are highly reliable for making effective selection in the breeding material (Pushpavalli *et al* 2018). Various studies have been reported for character association in pigeonpea.

A diverse set of 178 germplasm lines was planted in a randomized complete block design consisting of two replications. The phenotypic data were collected for two essential mineral elements, Zn and Fe along with yield and component traits *viz.*, days to 50 %

flowering, days to maturity, plant height, number of primary branches, number of secondary branches, pods/plant, seeds/pod, 100-seed weight and yield/plot. The analysis of variance (ANOVA) was calculated to estimate the significant difference between genotypes and genotype × year (G×Y) interactions. Least square means (LSMEANS) were estimated using the linear model. Least square means are the linear combination (sum) of the estimated effects (means etc) from a linear model. LSMEANS statement produces means which are adjusted for the average value of the specific covariate. In case of missing values also, LSMEANS are preferred because they reflect the model that is being fit to the data. Pearson correlation coefficient was estimated to assess the association between yield and component traits and between mineral elements. Descriptive statistics were calculated for each trait depicting wide variation available in pigeonpea germplasm. Principal Component Analysis (PCA), which is used to transform a number of possibly correlated variables into a smaller number of uncorrelated variables called principal components, was also conducted. Path coefficient analysis was estimated to measure the direct and indirect contribution of various independent characters on a dependent character. Genotyping of mapping population including parents was conducted using double digestion restriction-site associated DNA sequencing (ddRAD-Seq). Double digestion restriction-site associated DNA sequencing facilitates high-throughput genome-wide genotyping with next-generation sequencing technology.

4.1 Genetic variability and character association for grain Zn and Fe content, seed yield and its components in pigeonpea germplasm

4.1.1 Analysis of variance

The data on various phenological and biochemical traits *viz.,* days to 50% flowering, days to maturity, plant height, primary branches, secondary branches, pods/plant, seeds/pod, 100-seed weight, yield/plot, grain Fe content and grain Zn content were recorded over two crop seasons (2018-19 & 2019-20) for a diverse set of germplasm lines. The results of analysis of variance indicated that significant variability was present among genotypes for all the traits studied in both the years. For traits like plant height, number of primary branches, number of secondary branches and 100-seed weight, the variation due to year was observed to be non-significant which indicated that there was no environmental effect on the expression of these traits. Significant genotype-by-year interaction was observed for traits *viz.,* days to 50% flowering, days to maturity, number of pods/plant and number of seeds/pod, indicating the effect of environmental factors on trait manifestation. For traits like plant height, number of primary branches, number of secondary branches, 100-seed weight, yield/plot, grain Fe content and grain Zn content the G×E interaction was found to be non-significant. This is an indication of existence of sufficient variability for the traits under study.

Table 4.1: Analysis of variance for biochemical characters, seed yield and its components for two years and pooled data

Variable	Year	Mean Square (MS)		
		Year (Y)	Genotype (G)	GxY
DTF	2018		124.55***	
	2019		323.92***	
	Pooled	6205.62***	331.06***	117.42***
DTM	2018		235.13***	
	2019		136.15***	
	Pooled	1965.57***	308.53***	62.75***
PH	2018		932.68***	
	2019		867.67***	
	Pooled	686.24	1729.27***	71.08
PB	2018		22.48***	
	2019		16.10***	
	Pooled	33.74	33.83***	4.76
SB	2018		40.74***	
	2019		32.81***	
	Pooled	55.62	65.02***	8.52
PD	2018		6410.75***	
	2019		5811.65***	
	Pooled	3343.89***	11848.66***	373.73***
SP	2018		17.80***	
	2019		14.69***	
	Pooled	142.03***	18.72	13.77***
SW	2018		1.99	
	2019		1.30	
	Pooled	64.38	19.67	18.28
YLD	2018		18409.03	
	2019		16769.35	
	Pooled	11145.35***	34496.18	682.20
Fe	2018		57.89	
	2019		51.14	
	Pooled	267.41***	97.53	11.50
Zn	2018		61.78	
	2019		56.20	
	Pooled	530.59***	110.16	7.82

DTF =days to 50% flowering, DTM = days to maturity, PH = plant height, PB = number of primary branches, SB = number of secondary branches, PD = number of pods/ plant, SP = number of seeds /pod, SW = 100-seed weight, YLD = yield/ plot, , Zn = grain zinc content, Fe = grain iron content.

Saroj *et al* (2013) and Pushpavalli *et al* (2018) also revealed significant difference between the genotypes, indicating sufficient variability for various traits studied. Mishra *et al* (2017) used one hundred and four diverse pigeonpea genotypes including wild and cultivated accessions to estimate the variance analysis. They observed significant differences among wild and cultivated accessions for grain Fe and grain Zn concentration in pigeonpea germplasm. These findings were in accordance with the results of the present study.

4.1.2 Genetic variability

Various parameters of genetic variation for seed yield and its components and biochemical characters in pigeonpea are presented in Table 4.2.

4.1.2.1 Character wise range and mean performance

4.1.2.1.1 Days to 50 % flowering

The days to 50 % flowering varied from 75.50 to 117.50 days with mean value of 102.08 days in the year 2018-19 and from 74.50 to 116.50 days with mean of 96.18 days in the year 2019-20. For pooled data of two years it ranged from 76.75 to 117.00 days with mean of 99.13days.

4.1.2.1.2 Days to maturity

Days to maturity varied from 101.50 to 147.50 days with mean of 123.44 days in 2018-19 and in the year 2019-20, it varied from 103.50 to 139.50 days with mean of 120.12 days. For the pooled data of two years, it varied from 103.00 to 141.75 days with mean of 121.78days.

4.1.2.1.3 Plant height (cm)

Plant height ranged from 99.50 cm to 266.50 cm with mean value of 208.19 cm in the year 2018-19, 105.50 cm to 261.00 cm with mean value of 206.23 cm in 2019-20 and 102.50 cm to 263.75 cm with mean value of 207.21 cm for pooled data of two years, respectively.

4.1.2.1.4 Number of primary branches

Number of primary branches varied from 7.50 to 23.50 with mean number of 13.13 for the year 2018-19. For the year 2019-18, it varied from 7.00 to 24.50 with mean of 12.69 and 7.80 to 24.00 with mean of 12.91 for the pooled data of two years.

4.1.2.1.5 Number of secondary branches

Number of secondary branches per plant varied from 10.00 to 31.50 with mean of 18.43 for the year 2018-19. For the year 2019-18, it varied from 10.00 to 29.50 with mean of 17.87 and 10.50 to 30.50 with mean of 18.15 for the pooled data from two years.

4.1.2.1.6 Number of pods/plant

Number of pods/plant in the year 2018-19 ranged from 69.50 to 342.50 with mean of 171.81. In the year 2019-20, it ranged from 55.00 to 332.50 with mean of 167.48 and for pooled data of two years, it ranged from 70.50 to 329.00 with mean value of169.64.

4.1.2.1.7 Number of seeds/pod

For the first season of year 2018-19, number of seeds/pod ranged from 1.8 to 4.25 with mean value of 3.63. For the second year 2019-20, it ranged from 2.75 to 4.40 with mean value of 3.54 and for pooled data of two years, it ranged from 2.48 to 4.20 with mean of 3.58.

4.1.2.1.8 100-seed weight (g)

The mean value of 100-seed weight was 7.92 g and it ranged from 5.75 g to 9.55 g during 2018-19. On the other hand, mean value was 8.30 g and range was 6.25 to 10.00 during 2019-20 and mean value for pooled data of two years was 8.11 g and it ranged from 6.05g to 9.65g.

4.1.2.1.9 Yield/plot (g)

Average yield/plot was 262.67 g with range of 85.00 g to 545.00 g for the year 2018-19. Average yield for the year 2019-20 was 254.76 g and range was 82.50 g to 507.50 g and for pooled data of two years, it ranged from 86.25 g to 522.50 g with average yield of 258.71 g.

4.1.2.1.10 Grain iron (Fe) content (ppm)

Grain Fe content in pigeonpea germplasm varied from 9.09 to 42.15 ppm with mean iron content of 24.42 ppm for the year 2018-19. For the year 2019-20, it varied from 10.50 ppm to 40.00 ppm with mean of 25.64 ppm. For the pooled data of two years, its range was 9.79 ppm to 39.05 ppm with mean of 25.03 ppm.

4.1.2.1.11 Grain zinc (Zn) content (ppm)

Grain Zn content in pigeonpea germplasm varied from 8.96 to 51.48 ppm with mean zinc content of 23.99 ppm in the year 2018-19. For the year 2019-20, it varied from 9.00 to 43.00 ppm with mean of 25.71 ppm. It varied from 9.48 to 47.24 ppm with mean of 24.85 ppm for pooled data of two years.

The measurement of available variability in genetic material has been the basic requirement of any breeding programme. In the present investigation, a wide range of variability was observed for all the traits *viz*. days to 50% flowering, days to maturity, plant height, number of primary branches, number of secondary branches, pods/plant, seeds/pod, yield/plot, grain Fe content and grain Zn content. Whereas, low variability was observed for 100-seed weight in the germplasm lines. These findings are in general agreement with the findings of Hamid *et al* (2011), Patel and Acharya (2011), Yerimani *et al* (2013) and Chetukuri *et al* (2013).

Table 4.2: Statistical parameter estimation of various characters in pigeonpea for two years and pooled data

Trait	Year	Minimum	Maximum	Mean	SE Mean	Std. Dev.	CV (%)	Skewness	Kurtosis
DTF	**2018**	75.50	117.50	102.08	0.59	7.89	7.73	-0.87	0.56
	2019	74.50	116.50	96.18	0.95	12.73	13.23	-0.22	-1.44
	Pooled	76.75	117.00	99.13	0.68	9.09	9.18	-0.13	-1.04
DTM	**2018**	101.50	147.50	123.44	0.81	10.84	8.78	0.29	-0.66
	2019	103.50	139.50	120.12	0.62	8.25	6.87	0.11	-0.87
	pooled	103.00	141.75	121.78	0.66	8.78	7.21	0.08	-0.89
PH (cm)	**2018**	99.50	266.50	208.19	1.62	21.59	10.37	-1.17	4.24
	2019	105.50	261.00	206.23	1.56	20.83	10.10	-1.47	5.14
	pooled	102.50	263.75	207.21	1.56	20.79	10.03	-1.20	4.28
PB	**2018**	7.50	23.50	13.13	0.25	3.35	25.54	0.70	0.31
	2019	7.00	24.50	12.69	0.21	2.84	22.36	0.91	1.42
	pooled	7.75	24.00	12.91	0.22	2.91	22.53	0.88	1.03
SB	**2018**	10.00	31.50	18.43	0.34	4.51	24.49	0.23	-0.53
	2019	10.00	29.50	17.87	0.30	4.05	22.67	0.18	-0.51
	pooled	10.50	30.50	18.15	0.30	4.03	22.22	0.23	-0.49
PD	**2018**	69.50	342.50	171.81	4.24	56.62	32.95	0.24	-0.69
	2019	55.00	332.50	167.48	4.04	53.91	32.19	0.18	-0.61
	pooled	70.50	329.00	169.64	4.08	54.43	32.08	0.20	-0.68

Trait	Year	Minimum	Maximum	Mean	SE Mean	Std. Dev.	CV (%)	Skewness	Kurtosis
SP	**2018**	1.80	4.25	3.63	0.22	2.98	8.23	-1.58	7.34
	2019	2.75	4.40	3.54	0.20	2.71	7.66	-0.04	1.00
	pooled	2.47	4.20	3.58	0.16	2.16	6.04	-0.59	3.58
SW (g)	**2018**	5.75	9.55	7.92	0.06	0.78	9.87	-0.49	-0.27
	2019	6.25	10.00	8.30	0.06	0.81	9.71	-0.48	-0.00
	pooled	6.05	9.65	8.11	0.05	0.72	8.94	-0.61	0.14
YLD (g)	**2018**	85.00	545.00	262.67	7.19	95.94	36.53	0.67	-0.16
	2019	82.50	507.50	254.76	6.86	91.57	35.94	0.69	-0.08
	pooled	86.25	522.50	258.71	6.96	92.87	35.90	0.71	-0.11
Fe (ppm)	**2018**	9.08	42.15	24.42	0.40	5.38	22.04	0.22	1.09
	2019	10.50	40.00	25.64	0.38	5.06	19.72	-0.30	0.54
	pooled	9.79	39.05	25.03	0.37	4.94	19.73	-0.12	0.91
Zn (ppm)	**2018**	8.96	51.48	23.99	0.42	5.56	23.17	0.63	2.98
	2019	9.00	43.00	25.71	0.40	5.30	20.61	-0.25	0.78
	pooled	9.48	47.24	24.85	0.39	5.25	21.12	0.15	1.74

DTF =days to 50% flowering, DTM = days to maturity, PH = plant height, PB = number of primary branches, SB = number of secondary branches, PD = number of pods/plant, SP = number of seeds/ pod, SW = 100-seed weight, YLD = yield/ plot, Zn = grain zinc content, Fe = grain iron content.

4.1.2.2 Skewness and kurtosis

For the year 2018-19,days to maturity, number of primary branches, number of secondary branches, pods/ plant, yield/plot, grain Fe content and grain Zn content showed positive skewness whereas, days to 50% flowering, plant height, seeds/pod and 100-seed weight showed negative skewness. For the traits like days to maturity, number of secondary branches, pods/plant, 100seed weight and grain Fe content, the value of skewness was between -0.5 to +0.5 indicating that the data was fairly distributed and symmetrical. On the other hand, in the year 2019-20, days to maturity, number of primary branches, number of secondary branches, pods/plant and yield/plot showed positive skewness. Whereas, days to 50% flowering, plant height, seeds/pod, 100-seed weight, grain Fe content and grain Zn content showed negative skewness. For the traits like days to 50% flowering, days to maturity, plant height, number of secondary branches, number of pods/plant, number of seeds/pod, 100-seed weight, grain Fe content and grain Zn content, the value of skewness lied between -0.5 to +0.5 showing fair and symmetrical distribution of the data. For the pooled data of two years, days to maturity, number of primary branches, number of secondary branches, pods/plant, yield/plot and grain Zn content showed positive skewness and days to 50% flowering, plant height, seeds/pod, 100-seed weight and grain Fe content showed negative skewness. The value of skewness for days to 50% flowering, days to maturity, number of secondary branches, pods/plant, grain Fe content and grain Zn content were between -0.5 to +0.5 indicating symmetrical data distribution of these traits. Kurtosis is about the distribution of tails peakness or flatness. In other terms, it is a measure of outliers present in the distribution. Low kurtosis values indicate light tails or lack of outliers. If the kurtosis is close to zero, a normal distribution is assumed and the distribution is said to be mesokurtic. If the kurtosis is less than zero and greater than zero, the distribution is light tailed and heavy tailed and said to be platykurtic and leptokurtic, respectively. Positive kurtosis indicates a relatively peaked distribution. Negative kurtosis indicates a relatively flat distribution. In the present study, during 2018-19, days to maturity, secondary branches, pods/ plant, 100-seed weight and yield/ plot exhibited lesser than zero values for kurtosis and the distribution was observed to be platykurtic with no outliers in the data set. For the year 2019-20, days to 50% flowering, days to maturity, number of secondary branches, pods/plant, 100-seed weight and yield/plot showed value of kurtosis less than zero and the distribution was observed to be platykurtic with no outliers in the data set. Whereas, for the pooled data across two years, days to 50% flowering, days to maturity, number of secondary branches, pods/plant and yield/plot exhibited values less than zero for kurtosis showing light tailed distribution with no outliers in the data set. For the traits viz., days to maturity, number of secondary branches, pods/ plant and yield/plot, the distribution was observed to be positively skewed and platykurtic in both the years and pooled across the years. Mariyammal *et al* (2019) has reported positive

skewness and platykurtic distribution for the traits; days to 50% flowering, plant height, number of clusters/plant and number of pods/cluster in green gram. Sumathi *et al* (2018) has reported positive skewness for days to 50% tasseling, days to 50% silking, days to maturity, cob diameter, 100-seed weight and grain yield per plant in Maize. The information on distribution using skewness and kurtosis give insights into the nature of gene action (Fisher 1932) and number of genes controlling the traits (Robson 1956). Positive skewness is associated with complementary gene action while negative skewness is associated with duplicate (additive × additive) gene interactions (Venkatesha *et al* 2016). In the present investigation, days to 50% flowering, plant height, seeds/pod and 100 seed weight was observed to possess negative skewness which indicated duplicate (additive × additive) gene action for these traits. For other traits like days to maturity, primary and secondary branches, pods/plant, yield/plot, it showed positive skewness thus indicating complementary gene interaction for these traits. For grain Fe content, negative skewness was observed for 2019-20 and pooled data and positive skewness was observed for 2018-19 indicating complementary and duplicate gene interaction. For grain Zn content, negative skewness was observed for 2019-20 and positive skewness was observed for 2018-19 and pooled data indicating complementary and duplicate gene interaction.

4.1.2.3 Coefficient of variation (CV)

Coefficient of variation for plant height, primary branches, secondary branches, pods/plant, yield/plot, grain Fe content and grain Zn content was more than 10% indicating more phenotypic variation as compared to other traits studied. Whereas, traits like days to 50% flowering, days to maturity, seeds/pod and 100 seed weight had coefficient of variation less than 10 % indicating limited variability for these traits in the year 2018-19. For the year 2019-20, coefficient of variation for days to 50% flowering, plant height, primary branches, secondary branches, pods/plant, yield/plot, grain Fe content and grain Zn content was more than 10%, indicating more phenotypic variation as compared to other traits studied whereas remaining traits had coefficient of variation less than 10 % indicating limited variability for these traits. For the pooled data across two years, coefficient of variation for plant height, primary branches, secondary branches, pods/plant, yield/plot, grain Fe content and grain Zn content was more than 10%, indicating more phenotypic variation as compared to other traits studied. Whereas, remaining traits had coefficient of variation less than 10 % indicating limited variability for these traits (Table 4.2).

4.1.3 Genotypic and phenotypic coefficient of variation, heritability & genetic advance as % of mean

The value of genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability and genetic advance as % of mean (GAM) was computed for all the

traits for all the traits for the season 2018-19, 2019-20 and pooled for both the years (Table 4.3). The genotypic coefficient of variation varied from 7.41% to 36.13% and phenotypic coefficient of variation ranged from 7.86% to 36.91% in 2018-19. The values of genotypic coefficient of variation and phenotypic coefficient of variation were observed to be highest for yield/ plot followed by pods/plant and number of primary and secondary branches for the season 2018-19. The values of genotypic coefficient of variation and phenotypic coefficient of variation were moderate for number of primary branches, number of secondary branches, grain Fe content and grain Zn content, while low for days to 50% flowering, days to maturity, plant height, seeds/pod and 100-seed weight. During 2019-20, the range of genotypic coefficient of variation and phenotypic coefficient of variation was observed to be 6.86% to 35.28% and 6.88% to 36.08%, respectively. The highest value of genotypic coefficient of variation and phenotypic coefficient of variation was recorded for yield/plot followed by pods/plant in this season. GCV and PCV estimates were moderate for number of primary branches, secondary branches, grain Fe content and grain Zn content and low for days to 50% flowering, days to maturity, plant height, seeds/pod and 100-seed weight. For the pooled data, the genotypic coefficient of variation ranged from 6.44% to 35.54% and phenotypic coefficient of variation ranged from 7.91% to 36.25%. The yield/ plot showed highest value of genotypic coefficient of variation and phenotypic coefficient of variation. Observations in pooled data indicated the high value of genotypic coefficient of variation and phenotypic coefficient of variation for yield/plot and pods/plant, moderate estimates for number of primary branches and number of secondary branches, grain Fe content and grain Zn content while low GCV and PCV was recorded for days to 50% flowering, days to maturity, plant height, seeds/pod and 100-seed weight. It is noteworthy that GCV and PCV values for the traits like yield/plot and pods/plant were consistently high and traits like number of primary and secondary branches, grain Fe and grain Zn content consistently showed moderate values, while for traits like days to 50% flowering, days to maturity, plant height, seeds/pod and 100-seed weight, consistently low estimates were observed in both the years as well as in pooled data across two years.

The value of phenotypic coefficient of variation and genotypic coefficient of variation components facilitates to estimate the extent of genetic variation present in a population for the trait *per se*. Moderate to high value of genotypic coefficient of variation and phenotypic coefficient of variation were observed for pods/plant, yield/plot, number of primary branches, number of secondary branches while low value for days to 50% flowering, days to maturity and 100-seed weight was observed by Chetukuri *et al* (2013), Saroj *et al* (2013), Hemavathy *et al* (2019) which was in agreement with our results.

Table 4.3: Genotypic and Phenotypic Coefficient of variation, heritability and genetic advance for all the traits in pigeonpea germplasm lines

Variables		DTF	DTM	PH(cm)	PB	SB	PD	SP	SW(g)	YLD(g)	Fe(ppm)	Zn(ppm)
2018	**GCV (%)**	7.60	8.74	10.39	23.65	22.85	32.36	7.41	9.62	36.13	18.73	20.92
2019		13.13	6.86	9.25	19.55	19.77	31.72	7.29	9.35	35.28	19.36	20.38
Pooled		7.37	6.44	9.83	20.85	20.71	31.57	7.37	7.99	35.54	18.52	20.32
2018	**PCV (%)**	7.86	8.82	10.39	27.41	26.01	33.54	8.95	10.11	36.91	24.91	25.22
2019		13.33	6.88	10.90	24.75	25.26	32.64	7.91	10.06	36.08	20.08	20.74
Pooled		10.68	7.91	10.25	24.06	23.63	32.58	7.93	9.79	36.25	20.86	21.83
2018	**h^2 (%)broad sense**	93.54	98.12	72.03	74.45	77.23	93.07	68.61	90.46	95.81	56.56	68.80
2019		96.95	99.59	71.93	62.41	61.29	94.44	84.97	86.39	96.65	92.97	96.54
Pooled		92.64	96.15	77.11	75.11	76.78	93.89	78.22	84.67	96.12	78.83	86.63
2018	**Genetic advance as % of mean**	15.14	17.84	21.40	42.03	41.37	64.30	12.65	18.84	72.86	29.02	35.75
2019		26.63	14.11	16.16	31.82	31.89	63.51	13.85	17.91	71.08	38.45	41.25
Pooled		10.48	10.79	19.46	37.23	37.38	63.02	10.48	13.45	71.78	33.87	38.96

DTF =days to 50% flowering, DTM = days to maturity PH = plant height, PB = number of primary branches, SB = number of secondary branches, PD = number of pods/plant, SP = number of seeds/ pod, SW = 100-seed weight, YLD = yield/plot, Zn = grain zinc content, Fe = grain iron content.

High heritability was recorded for the traits like days to 50% flowering, days to maturity, pods/plant, 100-seed weight and yield/plot, moderate heritability for plant height, number of primary branches, number of secondary branches, seeds/pod, grain Fe content and grain Zn content in the year 2018-19. Whereas, in the year 2019-20, traits like days to 50% flowering, days to maturity, pods/plant, seeds/pod, 100-seed weight, yield/plot and grain Fe and Zn content showed high values of heritability while other traits like plant height, number of primary and number of secondary branches showed moderate values of heritability. For the pooled data of two years, high heritability was observed for days to 50% flowering, days to maturity, number of pods/plant, 100-seed weight, yield/plot and grain Zn content while moderate heritability was observed for plant height, number of primary branches, number of secondary branches, seeds/pod and grain Fe content. High heritability estimates for traits like days to 50% flowering, days to maturity, pods/plant, 100-seed weight and yield/plot were consistent over both the years as well as across two years data (Table 4.3). High or moderate values of heritability for traits like plant height, number of primary branches, number of secondary branches, and yield/plot reported by Saroj *et al* (2013) were in agreement with our results. High heritability indicates the scope of genetic improvement of traits through selection. However, selection based on the high heritability values does not represent the amount of genetic progress for selecting individuals. According to Johnson *et al* (1955), heritability estimates along with genetic advance are usually more useful.

The value of genetic advance as % of mean were high (>60%) for the traits like yield/plot followed by pods/plant and moderate (30-40%) for number of primary branches, number of secondary branches, grain Fe content and grain Zn content while low (< 30%) genetic advance was observed for 50% flowering, days to maturity, plant height, seeds/pod and 100-seed weight in the year 2018-19. For the year 2019-20, genetic advance as % of mean was high for yield/plot followed by number of pods/plant, moderate for days to 50% flowering, days to maturity, plant height, number of primary branches and number of secondary branches, seeds/pod and 100-seed weight, grain Fe content and grain Zn content. For the pooled data, yield/plot showed high genetic advance as per cent of mean followed by number of pods/ plant while days to 50% flowering, days to maturity, plant height, number of primary branches, number of secondary branches, seeds/pod and 100-seed weight, grain Fe content and grain Zn content showed moderate genetic advance as percent of mean. Yield/plot and pods/ plant exhibited high heritability along with high genetic advance showing additive gene effect, therefore, it can be considered as effective for selection in breeding programme. Other traits like days to 50% flowering, days to maturity, plant height, number of primary branches, number of secondary branches, seeds/pod, 100 -seed weight and grain Fe content and grain Zn content had moderate to high heritability along with moderate genetic advance

and indicated that selection could be effective for these traits. High heritability linked with high genetic advance indicates predominance of additive gene effects and effectiveness of selection for that trait. High heritability along with high genetic advance as percent mean was recorded for pods/plant and yield/plot. Similar observation was reported for pods/plant and seed yield/plant Ram (2016), pods per plant Mittal *et al* (2010) seed yield Padi (2003). Thus, these observations indicated that heritability was due to additive gene effects and selection might be effective in early generations and likely to accumulate more additive genes or these traits may be used as selection criteria in pigeonpea breeding programme.

4.1.4 Frequency distribution

The graphical representation of the variation observed among genotypes for various morphological traits *viz.,* days to 50% flowering, days to maturity, plant height, number of primary branches, number of secondary branches, pods/plant, seeds/pod, 100-seed weight, yield/plot, grain Fe content and grain Zn content for year 2018-19, 2019-20 and pooled data were shown in Fig 4.1, Fig 4.2 and Fig 4.3,respectively. The X-axis represents traits which were divided into equal class intervals and Y-axis represents the frequency of genotypes for the respective traits. However, the mean and range of values for the characters under study varied and hence, the peaks were seen at distinct points for each character, indicating high influence of the environment.

4.1.5 Identification of promising genotypes and their performance against check varieties

On the basis of pooled data**,** the promising genotypes with high mean performance were identified (Table 4.4) for all the traits studied in comparison to the check variety AL 882. The comparative performance of identified genotypes along with the checks is given as in Table 4.4.

The genotype ICPL 920 was the earliest to flower (71.5 days) followed by MN 5 (70.3), PADT 16 (72.3), AL 2091 (74.3), AL 2025 (74.3), AL 2204 (76.0), H 0535 (77.8) and these were numerically superior to the best check AL 882 (79 days). Likewise ICPL 920 was showing early maturity (119.5 days) and was significantly superior to the best check AL 882 (133.3 days). The other genotypes AL 2204 (122.5), AL 2091 (123.5), PADT 16 (124.3), AL 2025 (129.5), MN 5 (129.8) and H 0535 (132.0) were numerically superior to the best check AL 882 (133.3 days). For plant height few genotypes with short stature viz., AL 2204 (102.5 cm) followed by AL 2091 (132.8 cm), PADT 16 (137.5 cm) were identified and these were found to be significantly superior and short in height to best check AL 882 (181.8 cm). IC 245350 (175.8) was numerically superior to best check AL882 (181.8 cm). For number of primary branches, genotypes H 0535 (24.0), followed by H 0512 (22.3), AL 997 (21.8), IC

3977 (20.5), AL1487 (19.0), H948 (19.0), IC 245315 (18.8) and MN 5 (19.0) were identified with highest number of primary branches. These were significantly superior to best check AL 882 (11.8). For number of secondary branches, genotypes H 0535 (28.0), H 0512 (30.5), AL 997 (28.8), IC 3977 (23.5), AL 1487 (25.5), H 948 (25.0), IC 245315 (24.3) and MN 5 (25.3) were identified having highest number of secondary branches. These were significantly superior to best check AL 882 (14.5).

Table 4.4: List of promising genotypes for important yield component traits of pigeonpea

Trait	Check AL 882 Promising Genotypes
Days to 50% flowering	**AL 882**©(79.0), **ICPL 920** (71.5), **PADT 16** (72.3), **AL 2091** (74.3), **AL 2204** (76.0), **AL 2025** (74.3), **MN 5** (70.3), **H 0535** (77.8)
Days to maturity	**AL 882**©(133.3), **ICPL 920** (119.5), **PADT 16** (124.3), **AL 2091** (123.5), **AL 2204** (122.5), **AL 2025** (129.5), **MN 5** (129.8), **H 0535** (132.0)
Plant height (cm)	**AL 882** © (181.8), **AL 2204** (102.5), **AL 2091** (132.8), **PADT 16** (137.5), **IC 245350** (175.8),
Primary branches	**AL 882**©(11.8)**, H 0535** (24.0), **H 0512** (22.3), **AL 997** (21.8), **IC 3977** (20.5), **AL 1487** (19.0), **H 948** (19.0), **IC 245315** (18.8), **MN 5** (19.0)
Secondary branches	**AL 882©(**14.5), **H 0535** (28.0), **H 0512** (30.5), **AL 997** (28.8), **IC 3977** (23.5), **AL 1487** (25.5), **H 948** (25.0), **IC 245315** (24.3), **MN 5** (25.3)
Pods/plant	**AL 882**©(161.0), **H 0512** (329.0), **H 0535**(327.3), **H 005** (286.5), **IC 3977** (272.8), **AL 997** (243.8), **IC 245176** (256.8), **AL 1856** (245.0)
Seeds/pod	**AL 882**©(3.45), **H 0512** (3.90), **AL 1730** (4.20), **AL 1676** (4.13), **H 493** (4.08), **Pant A163** (4.05), **Pant A51** (4.00), **MN 1** (3.95), **AL 1487** (3.85)
100 seed weight (g)	**AL 882©(**8.3), **AL 1740** (9.7), **AH 0930** (9.7), **MTH 103 (**9.5), **AL 1430** (9.5), **MN 5** (9.3)
Seed yield/ plot (g)	**AL 882**©(360.0), **H 0512** (513.8), **H 0535**(496.3), **H 005** (522.5), **IC 3977** (427.5), **AL 997** (478.8), **IC 245176** (417.5), **AL 1856** (375.0)
Grain Fe content (ppm)	**AL 882©(**27.8**), AL 1477** (39.1), **AL 1721** (38.6), **AL 1490** (37.1), **AL 1396** (36.0), **AL 1449** (35.1), **AL 986** (33.7), **AL 1313 (**33.4)
Grain Zn content (ppm)	**AL 882©(**28.7**), AL 1449** (47.2), **IC 245176** (36.7), **ICPL 9308** (36.3), **AL 1313** (35.9), **AL 1430** (34.6), **AL 986 (**34.3)

The genotype H 0512 was showing profuse podding (329) followed by H 0535 (327.3), H 005 (286.5) and IC 3977 (272.8), AL 997 (243.8), IC 245176 (256.8) and AL 1856 (245.0). These were significantly superior to best check AL 882 (161.0). For seeds/pod genotype AL 1730 (4.20) with highest number of seeds/pod followed by AL 1676 (4.13), H 493 (4.08), Pant A163 (4.05), Pant A51 (4.0), MN 1 (3.95), AL 1487 (3.85) and these were significantly superior to best check AL 882 (3.45). Genotypes AL 1740 (9.7g) and AH 0930

(9.7g) were having highest 100 seed weight followed by MTH 103 (9.5g), AL1430 (9.5g), MN 5 (9.3g) and these were significantly superior to best check AL 882 (8.3g). The genotype H 005 (522.5g) was showing highest yield/plot followed by H 0512 (513.8g), H 0535 (496.3g), AL 997 (478.8g), IC 3977 (427.5), IC 245176 (417.5), AL 1856 (375.0) and these were significantly superior to best check AL 882 (360.0g). The genotype AL 1477 (39.1ppm) with high grain Fe content followed by AL 1721 (38.6ppm), AL 1490 (37.1ppm), AL 1396 (36.0ppm), AL 1449 (35.1ppm), AL 986 (33.7ppm), AL 1313 (33.4) and these were significantly superior to best check AL 882 (27.8ppm). For grain Zn content genotype AL 1449 (47.2ppm) followed by IC 245176 (36.7ppm), ICPL 9308 (36.3ppm), AL 1313 (35.9ppm), AL 1430 (34.6), AL 986 (34.3) with highest grain Zn content and these were significantly superior to best check AL 882 (28.7ppm).

Based on evaluation, few promising genotypes compared to check varieties were identified for different traits. These were ICPL 920, PADT 16, AL 2091, AL 2204, AL 2025, MN 5, H 0535 for early flowering and early maturity; PADT 16, AL 2204, AL 2091, IC 245350 for short stature; H 0512, H 0535, H 005, IC 3977, AL 997, IC 245176, AL 1856 for profuse podding and seed yield; H 0535, H 0512, AL 997, IC 3977, AL 1487, H 948, IC 245315, MN 5 for more primary and secondary branching habit; H 0512, AL 1730, AL 1676, H 493, Pant A163, Pant A51, MN 1 for more number of seeds per pod; AL 1740, AH 0930, MTH 103, AL 1430, MN 5 for higher 100-seed weight; AL 1449, AL 1313, AL 986 for high grain Fe and Zn content.

4.1.6 Correlation coefficient analysis

Correlation coefficient is a statistical measure used to find out the degree and direction of relationship between two or more than two variables. Mutual relationship between various characters and component characters can be measured with correlation coefficient analysis on which selection can be based for genetic improvement. Knowledge about inter-relationship between yield and yield component characters facilitates the choice of appropriate breeding method to be adopted. To estimate the association between two characters, correlation coefficients were worked out in all possible combinations among zinc, iron content and yield components (Table 4.5).

The yield/plot exhibited positive and significantly high correlation with number of primary branches (0.83, 0.74, 0.81), number of secondary branches (0.83, 0.71, 0.80) and pods/plant (0.87, 0.85, 0.87) for the year 2018-19, 2019-20 and pooled data across two years, respectively. Yield/plot showed positive and significant correlation with seeds/pod (0.22, 0.23) for 2018-19 and pooled data across years but the value of correlation was observed to be low.

Pods/plant had positive and significantly high correlation with number of primary branches (0.76, 0.66, 0.74) and number of secondary branches (0.80, 0.66, 0.76) for the year 2018-19, 2019-20 and pooled data across two years. Another highly significant and positive correlation of number of primary branches with number of secondary branches (0.86, 0.76, 0.84) was also recorded for 2018-19, 2019-20 and pooled across both years. The high values of significant and positive correlation among these traits indicate that these traits contribute considerably towards seed yield in pigeonpea.

Among other traits, days to 50% flowering showed positive and significant correlation with plant height (0.28, 0.23) and pods/plant (0.17, 0.16) for the year 2018-19 and pooled data, though the values of correlation were observed to be low. Days to 50% flowering and days to maturity showed negative significant correlation with grain Fe content (-0.23, -0.12) and (-0.22, -0.21) for 2018-19 and pooled data across two years, respectively indicating that late maturing cultivars will have less grain Fe content .

For grain Fe and grain Zn, content moderate value of positive and significant correlation (0.55, 0.52, 0.57) for 2018-19, 2019-20 and pooled data across two years, respectively was observed between these traits which shows that with the increase in the grain Fe content there would be simultaneously increase in the grain Zn content, therefore, selection for one trait will be effective in selecting the other and vice-versa.

An overall perusal of correlation analysis revealed that number of primary branches, number of secondary branches, pods/plant and seeds/pod exhibited significant and positive correlation with yield/plot across the years. On the other hand, grain Zn content and grain Fe content were found to be highly associated with each other and were found to show consistent association across the years. However, there has not been any correlation found between Zn, Fe content and yield components in the present study. Hence, it depicts that direct selection for these traits may lead to the development of high yielding pigeonpea genotypes. From nutritional point of view, grain Fe and Zn content can also be improved simultaneously. The experimental findings on correlation coefficient analysis are in general agreement with the results reported earlier by Hamid *et al* (2011), Pahwa *et al* (2013), Singh *et al* (2016), Pushpavalli *et al* (2018), Patel *et al* (2018) and Rao *et al* (2019).

4.1.7 Path coefficient analysis

Path coefficient analysis measures the direct and indirect contribution of various independent characters on a dependent character. Path coefficient analysis given by Dewey and Lu (1959) was used to estimate the magnitude and direction of direct and indirect effects

of various yield contributing characters on yield. Correlation coefficients along with path coefficients together provide more reliable information which can be effectively predicted in crop improvement programme. If the correlation between yield and a character is due to direct effect of that character, it reveals true relationship between them and direct selection for that trait will be rewarding for yield improvement. However, if the correlation coefficient is mainly due to indirect effects of the character through another component trait, indirect selection through such trait will be effective for yield improvement. Path coefficients of various yield attributing characters having direct and indirect effects on seed yield are presented in Table 4.6.

The highest positive direct effect contributing to yield/plot was observed due to pods/plant (0.494) followed by number of primary branches (0.356) and number of secondary branches (0.113). Pods/ plant also showed positive indirect effect on seed yield via number of primary branches (0.230) in the year 2018-19. During the year 2019-20, similar results were obtained and the highest positive direct effect contributing to seed yield/plot was exerted by pods/plant (0.574) followed by number of primary branches (0.299) and number of secondary branches (0.128). Pods/ plant also had indirect positive effect on seed yield via number of primary branches (0.218). For the pooled data, the highest positive direct effect contributing to seed yield/plot was observed due to number of primary branches (0.445) followed by seed/pod (0.455) and number of secondary branches (0.358). Pods/plant exerted positive indirect effect on seed yield through primary branches, secondary branches and seeds/pod. Number of primary branches had positive indirect effect on seed yield via number of secondary branches (0.311). As we have seen earlier, pods/plant, number of primary branches, number of secondary branches and seeds/pod exhibited positive and significant correlation with yield/plot. These characters are principal components of seed yield and can be considered as selection criteria for increasing the yield of pigeonpea. Similar results were earlier reported by Mahajan *et al* (2007), Devi *et al* (2012), Saroj *et al* (2013), Patel *et al* (2018), Pushpavalli *et al* (2018) and Hemavathy *et al* (2019).

Table 4.5: Pearson's correlation coefficients for grain zinc, iron content and yield component traits

		DTF	DTM	PH	PB	SB	PD	SP	SW	YLD	Fe
DTM	**2018**	0.069									
	2019	0.106									
	Pooled	-0.124									
PH	**2018**	0.280*	-0.020								
	2019	0.139	0.047								
	Pooled	0.229*	0.012								
PB	**2018**	0.023	-0.062	0.086							
	2019	0.069	0.042	0.178*							
	Pooled	0.050	-0.023	0.140							
SB	**2018**	0.049	-0.088	0.094	0.859*						
	2019	0.063	0.051	0.189	0.760						
	Pooled	0.076	-0.027	0.148*	0.837*						
PD	**2018**	0.167*	-0.078	0.089	0.757*	0.797*					
	2019	0.110	0.062	0.095	0.663*	0.663*					
	Pooled	0.158*	-0.017	0.096	0.736*	0.760*					
SP	**2018**	0.097	-0.085	-0.080	0.102	0.205*	0.258*				
	2019	0.056	0.126	0.094	0.040	0.052	0.114				
	Pooled	0.128	0.042	0.008	0.083	0.127	0.267*				
SW	**2018**	-0.106	0.029	0.108	0.051	0.113	0.072	-0.055			
	2019	-0.113	0.141	0.014	0.222*	0.132	0.152*	-0.040			
	Pooled	-0.116	0.121	0.070	0.138	0.131	0.122	-0.099			
YLD	**2018**	0.050	-0.100	0.128	0.829*	0.831*	0.874*	0.220*	0.084		
	2019	0.077	0.027	0.125	0.735*	0.708*	0.857*	0.123	0.098		
	Pooled	0.077	-0.048	0.132	0.814*	0.798*	0.871*	0.225*	0.101		
Fe	**2018**	-0.226*	-0.223*	0.077	0.083	0.103	0.001	-0.098	0.012	0.104	
	2019	-0.058	-0.128	-0.007	0.091	0.112	-0.018	-0.040	0.058	0.084	
	Pooled	-0.148*	-0.207*	0.029	0.104	0.125	-0.010	-0.087	-0.001	0.101	
Zn	**2018**	-0.073	-0.075	0.059	0.035	0.043	-0.029	-0.137	0.016	-0.013	0.553*
	2019	-0.032	-0.134	0.137	-0.035	0.028	-0.091	0.002	-0.014	-0.035	0.523*
	Pooled	-0.042	-0.116	0.096	0.010	0.050	-0.055	-0.101	0.006	-0.022	0.568*

DTF =days to 50% flowering, DTM = days to maturity PH = plant height, PB = number of primary branches, SB = number of secondary branches, PD = number of pods/plant, SP = number of seeds/pod, SW = 100-seed weight, YLD = yield/plot, Zn = grain zinc content, Fe = grain iron content.

Table 4.6: Path coefficients for yield and its components in pigeonpea germplasm

		DTF	DTM	PH	PB	SB	PD	SP	SW	Fe	Zn
DTF	**2018**	**-0.0407**	0.0002	0.0136	0.0057	0.0051	0.0842	0.0051	-0.0032	-0.0282	0.0069
	2019	**-0.0254**	-0.0018	-0.0014	0.0263	0.0101	0.0654	0.0023	0.0074	-0.0031	0.0003
	Pooled	**-0.2820**	0.1346	0.0224	0.0250	0.0396	0.0094	0.1528	-0.0148	0.0115	-0.0007
DTM	**2018**	-0.0029	**0.0030**	-0.0010	-0.0257	-0.0113	-0.0397	-0.0040	0.0008	-0.0279	0.0063
	2019	-0.0027	**-0.0169**	-0.0005	0.0143	0.0078	0.0369	0.0044	-0.0094	-0.0072	0.0013
	Pooled	0.1397	**-0.2717**	0.0012	-0.0125	-0.0074	-0.0006	0.0682	0.0208	0.0142	-0.0028
PH	**2018**	-0.0115	-0.0001	**0.0480**	0.0333	0.0114	0.0448	-0.0041	0.0031	0.0097	-0.0053
	2019	-0.0040	-0.0009	**-0.0088**	0.0682	0.0264	0.0596	0.0040	-0.0014	-0.0005	-0.0014
	Pooled	-0.0842	-0.0042	**0.0752**	0.0985	0.0571	0.0045	0.0095	0.0093	-0.0012	0.0020
PB	**2018**	-0.0007	-0.0002	0.0045	**0.3558**	0.1000	0.3878	0.0055	-0.0002	0.0122	-0.0036
	2019	-0.0022	-0.0008	-0.0020	**0.2993**	0.0987	0.4021	0.0027	-0.0112	0.0058	0.0004
	Pooled	-0.0159	0.0076	0.0116	**0.4447**	0.3106	0.0346	0.0475	0.0169	-0.0075	0.0004
SB	**2018**	-0.0018	-0.0003	0.0048	0.3138	**0.1133**	0.4065	0.0099	0.0019	0.0143	-0.0051
	2019	-0.0020	-0.0010	-0.0018	0.2307	**0.1280**	0.4045	0.0026	-0.0042	0.0072	-0.0004
	Pooled	-0.0313	0.0056	0.0120	0.3862	**0.3577**	0.0359	0.0548	0.0165	-0.0008	0.0014
PD	**2018**	-0.0069	-0.0002	0.0044	0.2796	0.0934	**0.4935**	0.0132	0.0014	0.0008	0.0019
	2019	-0.0029	-0.0011	-0.0009	0.2098	0.0902	**0.5736**	0.0045	-0.0086	-0.0010	0.0009
	Pooled	-0.0585	0.0037	0.0075	0.3403	0.2836	**0.0453**	0.2394	0.0154	0.0006	-0.0011
SP	**2018**	-0.0045	-0.0003	-0.0043	0.0426	0.0242	0.1408	**0.0463**	-0.0018	-0.0133	0.0125
	2019	-0.0017	-0.0022	-0.0010	0.0232	0.0098	0.0744	**0.0343**	0.0019	-0.0021	0.0000
	Pooled	-0.0947	-0.0407	0.0016	0.0464	0.0431	0.0238	**0.4552**	-0.0298	0.0091	-0.0040
SW	**2018**	0.0047	0.0001	0.0054	-0.0027	0.0077	0.0256	-0.0031	**0.0276**	0.0027	-0.0019
	2019	0.0029	-0.0025	-0.0002	0.0525	0.0085	0.0776	-0.0010	**-0.0639**	0.0035	0.0001
	Pooled	0.0370	-0.0502	0.0062	0.0668	0.0522	0.0062	-0.1203	**0.1126**	0.0030	0.0003
Fe	**2018**	0.0108	-0.0008	0.0044	0.0408	0.0153	0.0035	-0.0058	0.0007	**0.1062**	-0.0465
	2019	0.0015	0.0022	0.0001	0.0320	0.0170	-0.0105	-0.0013	-0.0041	**0.0544**	-0.0051
	Pooled	0.0554	0.0660	0.0015	0.0571	0.0539	-0.0004	-0.0709	-0.0057	**-0.0586**	0.0123
Zn	**2018**	0.0034	-0.0002	0.0031	0.0157	0.0071	-0.0113	-0.0071	0.0006	0.0605	**-0.0815**
	2019	0.0008	0.0023	-0.0013	-0.0110	0.0049	-0.0539	0.0001	0.0009	0.0293	**-0.0094**
	Pooled	0.0100	0.0380	0.0074	0.0076	0.0245	-0.0024	-0.0906	0.0014	-0.0355	**0.0203**

Path coefficients residual effects = 0.130, 0.168, 0.096 for 2018-19, 2019-20 and pooled conditions respectively; direct effects on main diagonal **(bold figures)**

DTF =days to 50% flowering, DTM = days to maturity PH = plant height, PB = number of primary branches, SB = number of secondary branches, PD = number of pods/plant, SP = number of seeds/pod, SW = 100-seed weight, YLD = yield/plot, Zn = grain zinc content, Fe = grain iron content.

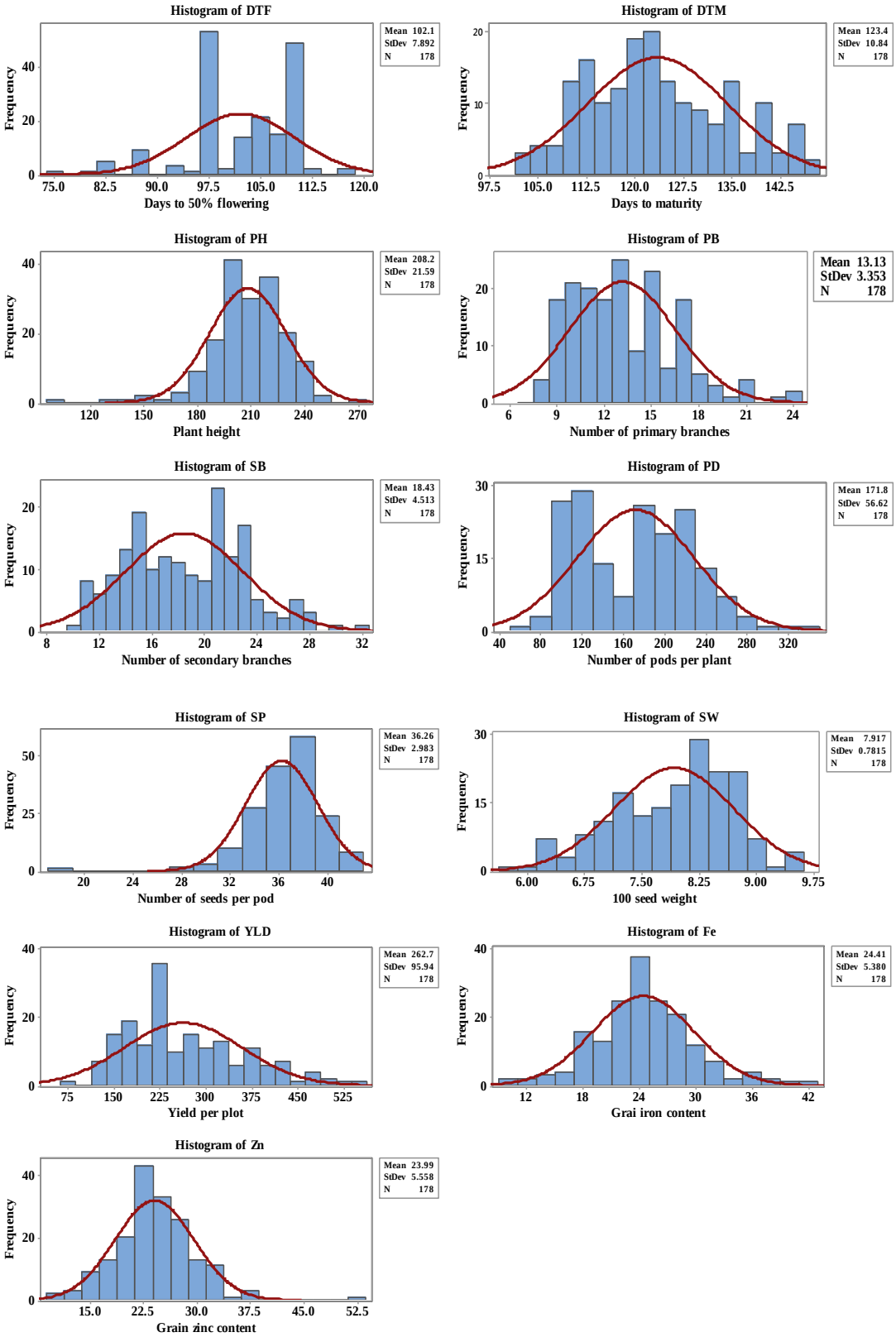


Fig. 4.1: Graphical representations of genotypes for the various traits in pigeonpea for 2018-19. DTF= days to 50% flowering, DTM = days to maturity, PH = plant height, PB = number of primary branches, SB = number of secondary branches, PD = number of pods/plant, SP = number of seeds/pod, SW = 100-seed weight, YLD = yield/ plot, Zn = grain zinc content, Fe = grain iron content.

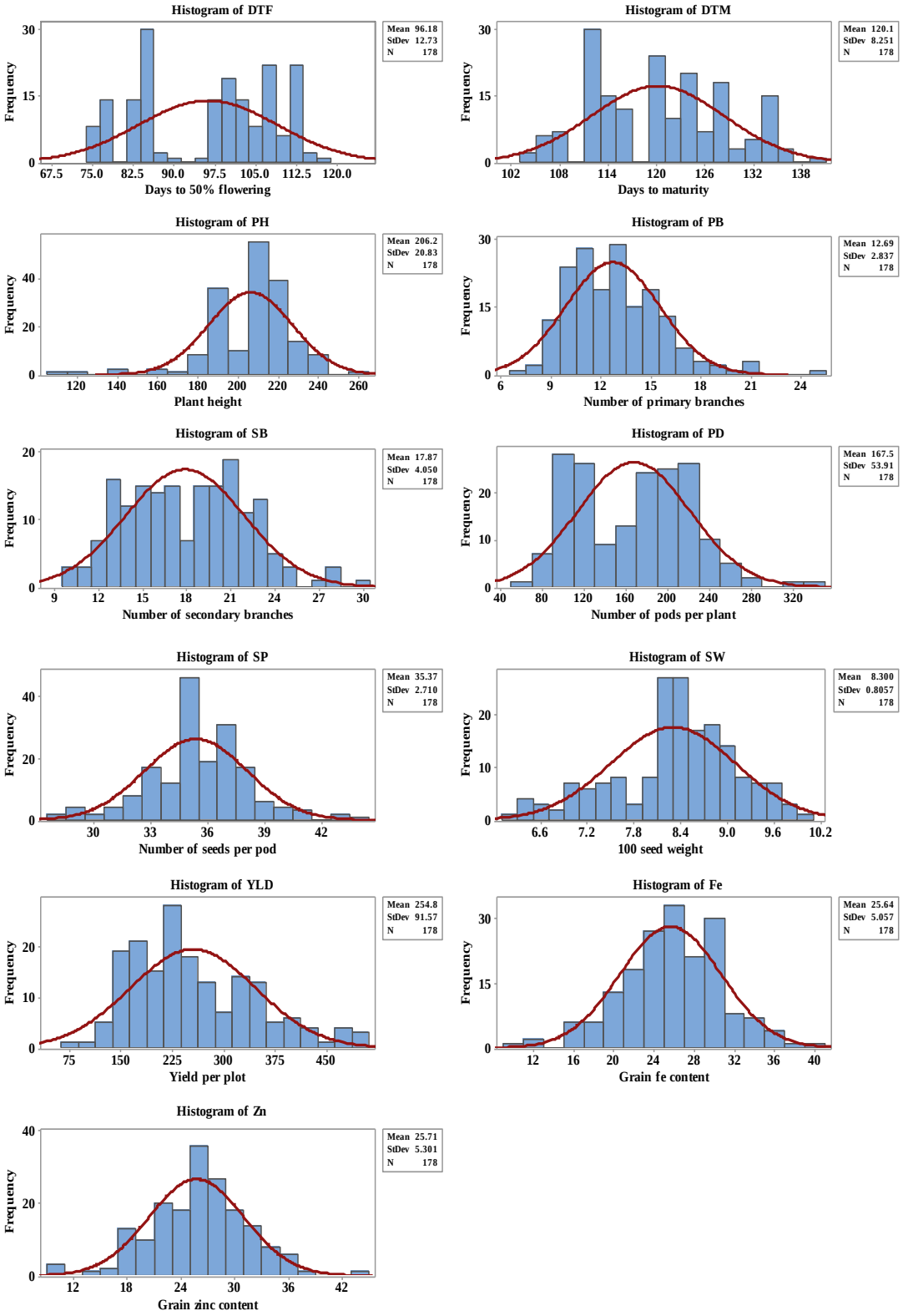


Fig. 4.2: Graphical representations of genotypes for the various traits in pigeonpea for 2019-20. DTF= days to 50% flowering, DTM = days to maturity, PH = plant height, PB = number of primary branches, SB = number of secondary branches, PD = number of pods/plant, SP = number of seeds/pod, SW = 100-seed weight, YLD = yield/plot, Zn = grain zinc content, Fe = grain iron content.

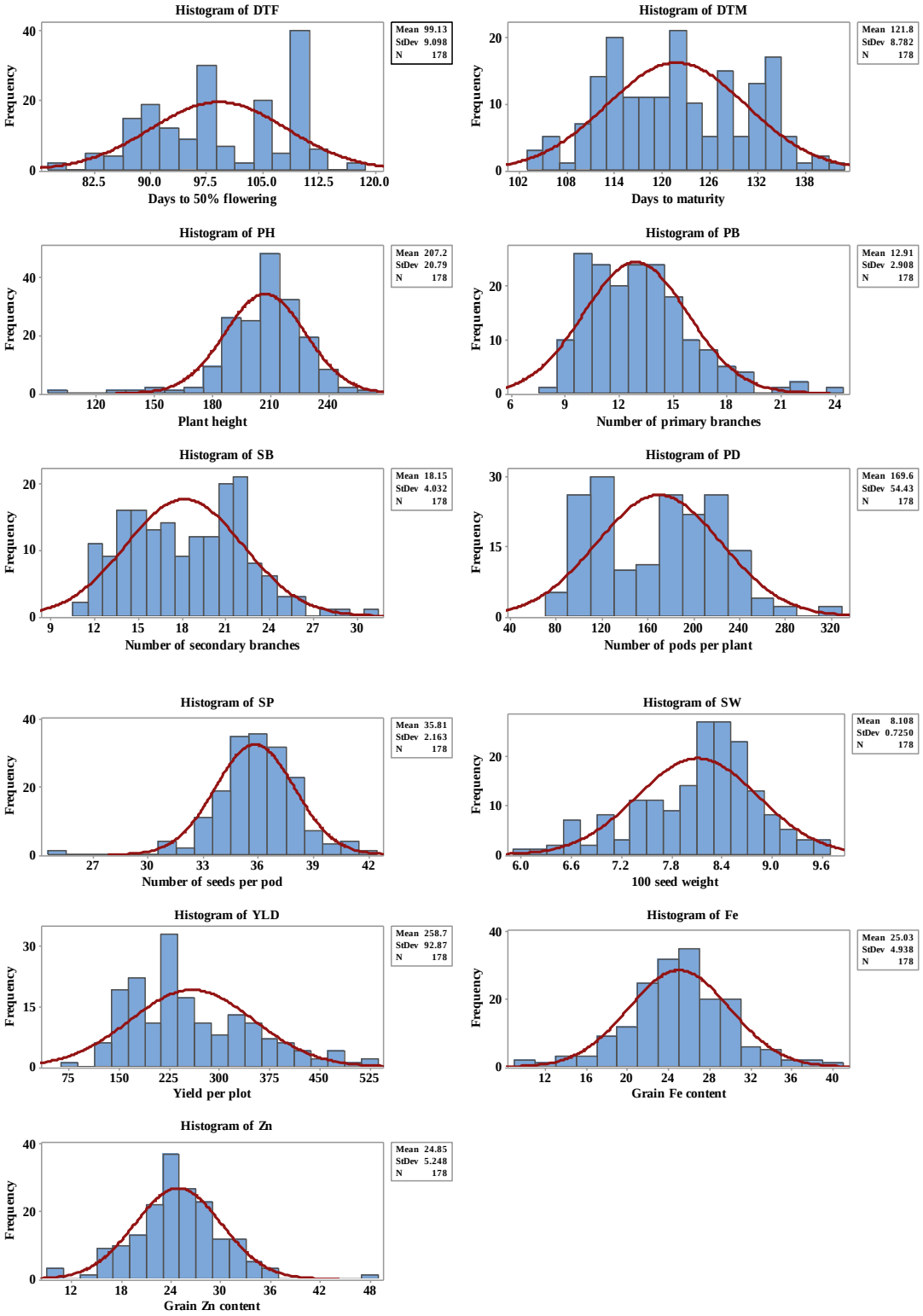


Fig. 4.3: Graphical representations of genotypes for the various traits in pigeonpea for pooled data. DTF= days to 50% flowering, DTM = days to maturity, PH = plant height, PB = number of primary branches, SB = number of secondary branches, PD = number of pods/plant, SP = number of seeds/pod, SW = 100 seed weight, YLD = yield/plot, Zn = grain zinc content, Fe = grain iron content.

4.2 Genotypic analysis

4.2.1 Genotyping by ddRAD sequencing, data analysis and SNP discovery

The diverse set of germplasm lines were genotyped using ddRAD sequencing which was outsourced and data were received in the form of raw reads. A total of 318 million reads were generated with an average of 1.7 million reads per germplasm line for the diverse set of 178 germplasm lines. The number of reads varied from 1.58 million to 1470.14 million. The raw reads were filtered for the presence of RAD TAGs [*Mluc1* and *Sph1*]. After this, the Illumina 5‘ and 3‘ adapter sequences and the bases from 5‘ and 3‘ were removed from the reads for final analysis. The processed reads were aligned to the pigeonpea reference genome (http://www.ncbi.nlm.nih.gov/bioproject?term=PRJNA72815) using Bowtie 2 (version 2-2.2.9) program with default parameters. The alignment file along with the reference genome sequence were used for variant calling using SAM tools program (SAM tools version 0.1.18). Variant calling by using these aligned samples for read depth 5 are given in Annexure V. SAM tools identified a total of 912459 SNPs in 178 germplasm lines. After filtering for minimum read depth, minimum allele frequency of 0.1 and missing data rate 0.8, 9369 SNPs were identified. After removing the indels, finally 7366 high quality SNPs were retained for GWAS analysis.

4.2.2 Phenotype - Genotype Association analysis

To identify significant SNPs, multi–locus methods which capture small effect loci in complex quantitative traits has recently become an attainable approach. The main advantage to apply multiple methods is to get benefitted from the algorithms of different models and support one by the other (Zhang *et al* 2019). Multi-locus GWAS methods included in the R package multi-locus random-SNP-effect mixed linear model (mrMLM) were applied (Wen *et al* 2017). The different algorithms included in these methods are such as FASTmulti-locus random-SNP-effect EMMA (FASTmrEMMA) (Wen *et al* 2016), FASTmulti-locus random-SNP-effect (FASTmrMLM) (Tamba and Zhang 2018), the Iterative modified-Sure Independence Screening EM-Bayesian LASSO (ISIS EM-BLASSO) (Tamba *et al* 2017), mrMLM (Wang *et al* 2016), the integration of Kruskal–Wallis test with Empirical Bayes under polygenic background control (pKWmEB) (Ren *et al* 2018), and the polygene-background-control-based Least Angle Regression plus Empirical Bayes (pLARmEB) (Zhang *et al* 2017). False discovery rate (FDR) correction at $\alpha = 0.05$ was applied for all models to identify significant QTNs and to control type I error in multiple comparison. A log of odds (LOD) score of 3 was used to detect robust association signals for these six methods. Genome–wide association studies were carried out using phenotypic data for all the traits in this study in mrMLM package of R. A total of 7, 366 high quality SNPs, the PCA variable

(the first three PCs) and phenotypic data of all the traits for each year were imported to the mrMLM package for GWAS. Figure results in GWAS comes in the form of Manhattan plots and quantile-quantile (Q-Q) plots. The manhattan plot is a scatter plot that summarizes GWAS results. The X-axis is the genomic position of each SNP, and the Y-axis is the negative logarithm of the P-value on left side and LOD value on the right side obtained from the GWAS model. Manhattan plots show Significant SNP loci associated with all the traits in 2018-19, 2019-20 and pooled across were shown in Figs 4.4, 4.5 and 4.6 respectively. The quantile-quantile (Q-Q)- plot is an important tool for assessing how well the model used in GWAS accounts for the population structure and familial relatedness. The negative logarithms of the P-values from the models fitted in GWAS were plotted against their expected value under the null hypothesis of no association with the trait. Most of the SNPs tested were probably not associated with the trait and lied on the diagonal line in the Q-Q plot. The presence of spurious associations was shown by deviation from the diagonal line due to population structure and familial relatedness while the SNPs on the upper section of the graph deviating from the diagonal were most likely associated with the trait studied. The Quantile-quantile (Q-Q)- plot produced by mrMLM for all the traits in 2018-19, 2019-20 and pooled across were shown in Figs 4.4, 4.5 and 4.6 respectively.

A total of 69 highly significant SNPs associated with all the traits studied were identified, except days to flowering. Out of these identified SNPs, 30 were identified with atleast two or more than two models indicating multiple evidence for the marker-trait association for the year 2018-19. The phenotypic variance explained by these 30 SNPs for Fe, Zn and eight yield traits ranged from 2.42% to 37.29%. For plant height, six SNPs on three chromosomes 06, 09 and 11 were identified through combination of five different models. primary branches had two associated SNPs on chromosomes 06 and 09 that were identified through combination of four different models. For secondary branches, five SNPs on four chromosomes 01, 04, 07 and 11 were identified by combination of five different models. For number of pods/plant, two SNPs were identified through combination of four different models on chromosomes 06 and 07. Chromosome 04 had one SNP for number of seeds/pod identified through combination of two models. For 100- seed weight, five SNPs were identified by combination of three models on four different chromosomes 01, 02, 03 and 08. For plot yield, three SNPs on two chromosomes 03 and 08 were identified through combination of five different models. For days to maturity, two SNPs were identified on chromosome 11 with combination of four models. Chromosome 06 had single SNP for grain zinc content that was observed to be identified through combination of five models. For grain iron content two SNPs on chromosome 02 through combination of five models were identified. The SNP position with ID and combination of models associated was given in Table 4.15.

Table 4.7: Summary of SNPs associated with Fe, Zn and yield component traits in pigeonpea for year 2018-19

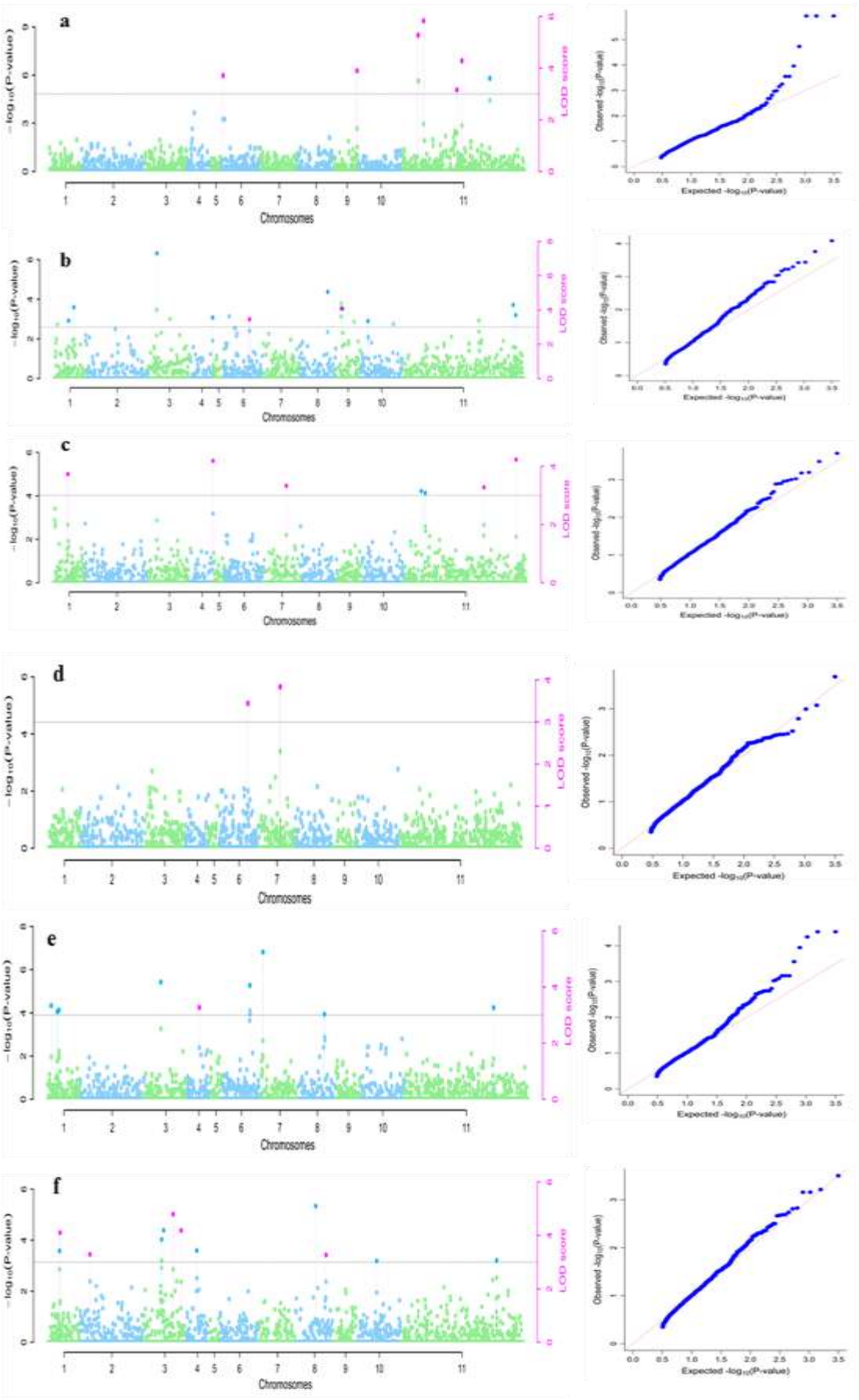
Trait[$]	Chromosome	Marker position (bp)	LOD score	r2 (%)	MAF	allele	Method
DM	S011_43054490	43054490	3.25 – 4.00	9.39 – 13.27	0.1032	G/T	mrMLM pLARmEB ISIS EM-BLASSO
	S011_5131233	5131233	3.26 – 5.91	20.24 - 32.94	0.3254	G/A	mrMLM pLARmEB pKWmEB ISIS EM-BLASSO
PH	S011_9288497	9288497	3.34 – 6.46	10.86 – 20.51	0.2579	G/T	mrMLM, FASTmrMLM, FASTmrEMMA, pLARmEB ISIS EM-BLASSO
	S011_23950335	23950335	3.19 – 4.28	6.38 – 10.55	0.3373	T/C	mrMLM FASTmrEMMA pLARmEB ISIS EM-BLASSO
	S009_10223064	10223064	3.38 – 4.41	4.61 – 12.89	0.3711	C/G	FASTmrMLM FASTmrEMMA pLARmEB ISIS EM-BLASSO
	S011_7167265	7167265	5.27 – 6.16	19.68 – 31.99	0.496	C/G	mrMLM pLARmEB ISIS EM-BLASSO

Trait[$]	Chromosome	Marker position (bp)	LOD score	r2 (%)	MAF	allele	Method
	S006_1627856	1627856	3.7135	15.24 - 15.75	0.4883	A/G	pLARmEB ISIS EM-BLASSO
	S011_23068266	23068266	3.1586	4.39 - 4.54	0.293	G/C	pLARmEB ISIS EM-BLASSO
PB	S009_1724822	1724822	3.60 – 4.70	13.09 – 19.06	0.4841	C/T	mrMLM FASTmrMLM pLARmEB ISIS EM-BLASSO
	S006_15697379	15697379	3.4516	15.48	0.4922	A/G	FASTmrMLM ISIS EM-BLASSO
SB	S004_12460240	12460240	3.07 – 7.47	5.29 – 20.80	0.4841	G/A	mrMLM FASTmrMLM FASTmrEMMA pLARmEB ISIS EM-BLASSO
	S011_30827589	30827589	3.13 – 3.41	8.88 – 17.67	0.1032	T/C	mrMLM FASTmrMLM
	S011_42153301	42153301	3.94 – 5.05	12.91 – 15.20	0.4802	G/A	mrMLM FASTmrMLM pLARmEB
	S001_6980165	6980165	3.46 - 3.99	13.20 – 20.47	0.2063	G/A	mrMLM FASTmrMLM
	S007_11519993	11519993	3.09 – 3.73	4.53 - 6.31	0.4766	G/A	FASTmrMLM pLARmEB ISIS EM-BLASSO

Trait[$]	Chromosome	Marker position (bp)	LOD score	r2 (%)	MAF	allele	Method
PD	S006_17287558	17287558	3.44	7.51 – 15.31	0.2897	C/T	mrMLM FASTmrMLM pLARmEB
	S007_11400172	11400172	3.83 - 3.18	15.53 – 29.04	0.1071	A/G	mrMLM FASTmrMLM pLARmEB ISIS EM-BLASSO
SP	S004_10172349	10172349	3.22 – 3.32	9.64 – 23.31	0.3571	A/C	mrMLM FASTmrMLM
SW	S008_16298680	16298680	3.13 – 3.82	12.41 – 15.66	0.0595	A/G	mrMLM FASTmrMLM ISIS EM-BLASSO
	S003_25410650	25410650	3.36 - 5.00	14.24 - 20.44	0.457	C/T	FASTmrMLM ISIS EM-BLASSO
	S001_6405903	6405903	4.06 - 4.14	5.27 - 6.02	0.3047	G/A	pLARmEB ISIS EM-BLASSO
	S002_6316980	6316980	3.04 - 3.54	2.42 - 5.45	0.4336	T/G	pLARmEB ISIS EM-BLASSO
	S003_20834869	20834869	4.33 – 5.25	6.03 – 7.60	0.1992	A/G	pLARmEB ISIS EM-BLASSO
YLD	S003_11913973	11913973	3.15 – 4.05	2.73 – 5.55	0.2619	C/G	mrMLM FASTmrMLM ISIS EM-BLASSO

Trait[$]	Chromosome	Marker position (bp)	LOD score	r2 (%)	MAF	allele	Method
	S003_12357630	12357630	4.50 - 4.56	21.24 - 22.80	0.4531	G/A	pLARmEB ISIS EM-BLASSO
	S008_2935407	2935407	3.76 - 3.20	15.99 - 32.02	0.2227	C/T	pLARmEB pKWmEB
Zn	S006_17877070	17877070	3.15 – 4.36	21.83 – 37.29	0.1071	A/G	mrMLM FASTmrMLM pLARmEB pKWmEB ISIS EM-BLASSO
Fe	S002_16766436	16766436	3.13 – 4.37	4.93 – 18.18	0.254	A/G	mrMLM FASTmrMLM FASTmrEMMA pLARmEB ISIS EM-BLASSO
	S002_10735749	10735749	4.05 – 4.16	18.50 – 22.17	0.3945	T/G	FASTmrMLM pLARmEB

DTF= days to 50% flowering, DTM = days to maturity, PH = plant height, PB = number of primary branches, SB = number of secondary branches, PD = number of pods/plant, SP = number of seeds/pod, SW = 100 seed weight, YLD = yield/plot, Zn = grain zinc content, Fe = grain iron content.


a
b
c
d
e
f
−log10(P-value)
LOD score
Chromosomes
Observed -log10(P-value)
Expected -log10(P-value)

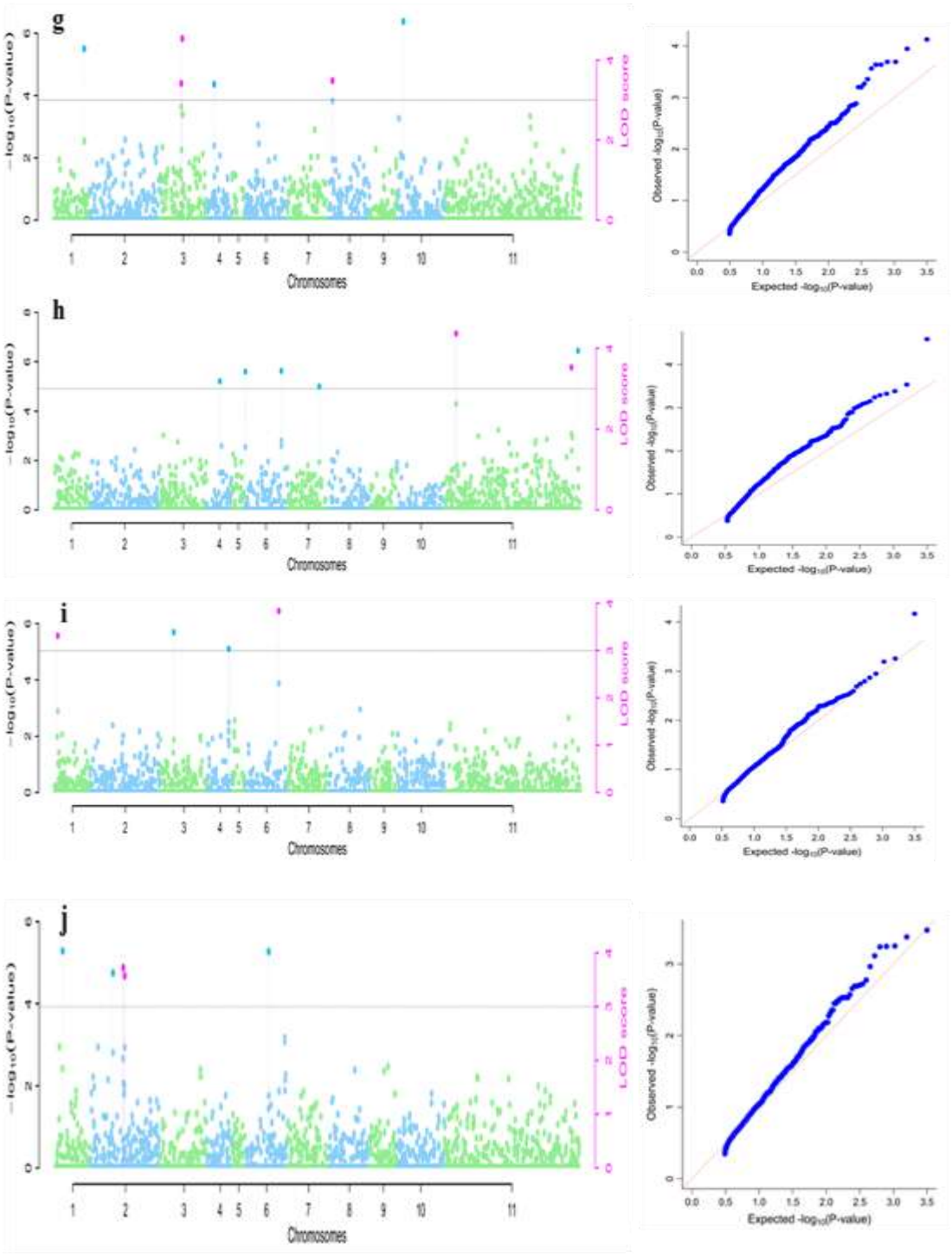


Fig 4.4: Manhattan plots and the corresponding Q-Q plots for 2018-19 (a, b, c, d, e, f, g, h, i, j) representing the identification of SNP markers associated with plant height, number of primary branches, number of secondary branches, number of pods/plant, number of seeds/ pod, 100 seed weight, YLD = yield/plot, DTM = days to maturity, Zn = grain zinc content, Fe = grain iron content respectively in2018-19. QTNs commonly identified by multiple approaches are indicated by the pink dots that are shown above dotted vertical lines, while all the QTNs identified by one single approach are indicated by the light color dots that are shown above dotted vertical lines.

For the year 2019-20, a total of 65 highly significant SNPs associated with all the traits studied were identified on different chromosomes. Out of these, 23 SNPs were identified with at least two or more than two models indicating multiple evidence for the marker-trait association. The phenotypic variance explained by these SNPs for Fe, Zn and eight yield traits ranged from 1.93E-08 % to 37.62%. For plant height, two SNPs on chromosomes 03 and 07 were identified with combination of two models. For primary branches, three SNPs on chromosomes 02, 07 and 11 were identified through combination of five models. Secondary branches had two SNPs on chromosomes 02 and 11 identified through combination of five models. Pods/plant and seeds/pod was observed to have single associated SNP identified through single model on chromosome 09 and 11, respectively. For 100-seed weight, two SNPs on chromosomes 02 and 06 were identified with the combination of three models. For yield/plot, five SNPs were identified on chromosomes 03, 05, 06, 08 and 11 with combination of six models. Days to maturity were found to have four SNPs on chromosomes 01, 06 and 11 identified through combination of two models. Chromosome 03 had single SNP for days to 50% flowering identified through single model. For grain Zn content four SNPs on chromosomes 02, 06, 08 and 11 were identified through combination of six models. Grain Fe content had single SNP on chromosome 01 identified through combination of five models. The SNP position with ID and combination of models associated were given in Table 4.16.

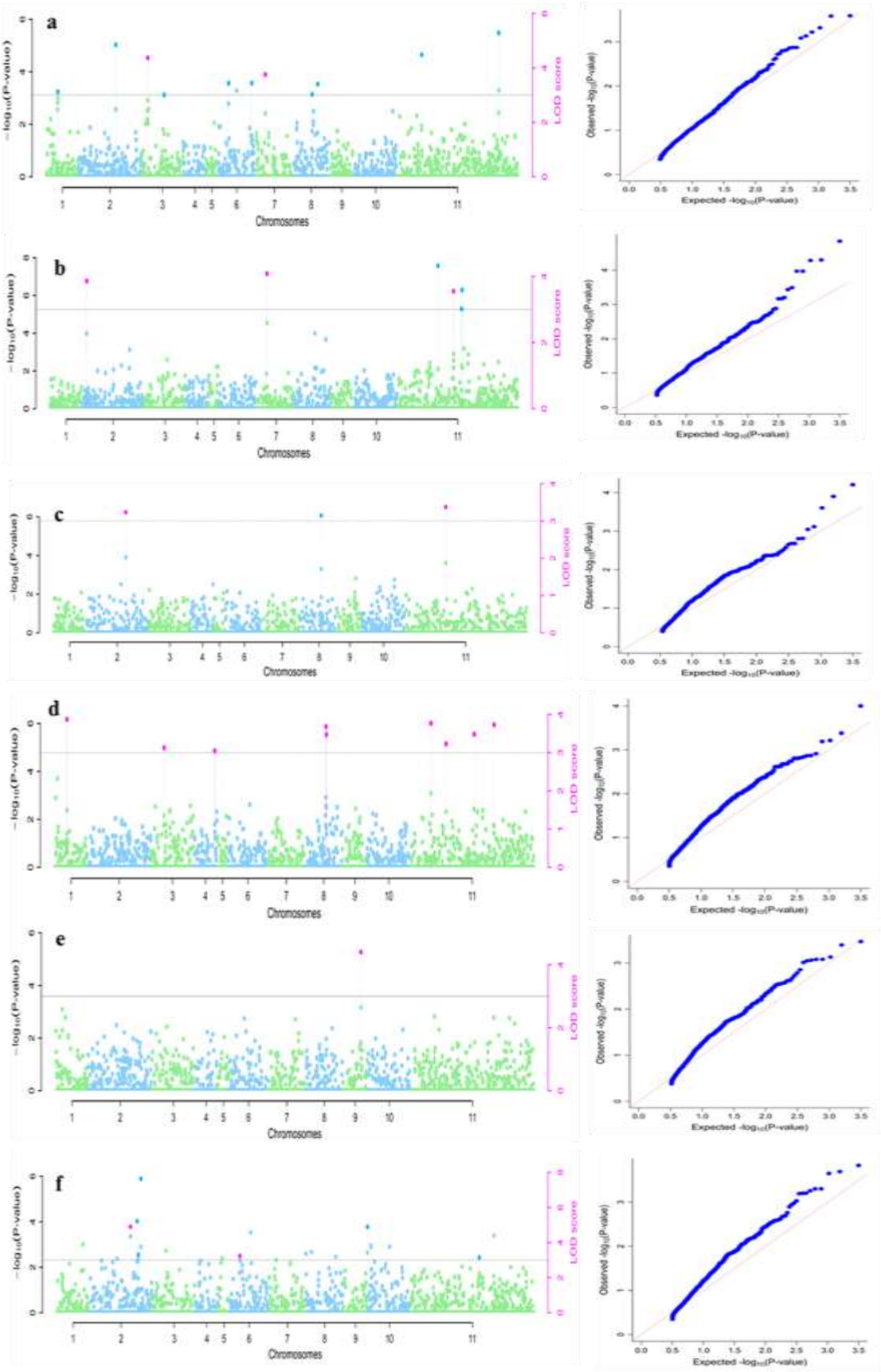
Table 4.8: Summary of SNPs associated with Fe, Zn and yield component traits in pigeonpea for year 2019-20

Trait[$]	Chromosome	Marker position (bp)	LOD score	r2 (%)	MAF	allele	Method
DF	S003_23566777	23566777	3.2357	6.92	0.4646	G/A	ISIS EM-BLASSO
DM	S001_5809512	5809512	3.5709	10.57 – 17.43	0.196	G/A	mrMLM FASTmrMLM
	S006_491518	491518	3.4309	3.20 – 5.38	0.468	A/G	mrMLM FASTmrMLM
	S011_21755950	21755950	3.9846	11.35 – 14.90	0.3	C/T	mrMLM FASTmrMLM
	S011_20003	20003	3.8791	17.68 – 23.92	0.292	G/T	mrMLM FASTmrMLM
PH	S003_5326147	5326147	3.84 - 4.89	6.48 - 20.57	0.0984	G/A	pLARmEB ISIS EM-BLASSO
	S007_3754975	3754975	3.28 – 4.23	1.72 - 2.85	0.374	A/G	pLARmEB ISIS EM-BLASSO
PB	S002_3959007	3959007	3.64 – 5.48	14.40 – 33.51	0.156	A/T	mrMLM FASTmrMLM pLARmEB ISIS EM-BLASSO
	S007_3754975	3754975	3.50 – 4.09	4.87 – 8.90	0.374	A/T	FASTmrMLM FASTmrEMMA ISIS EM-BLASSO
	S011_23250593	23250593	3.55	9.56 – 10.39	0.374	A/T	FASTmrMLM ISIS EM-BLASSO
SB	S002_20711967	20711967	3.05 – 3.35	7.53E-10 -31.23	0.1024	G/A	FASTmrMLM pLARmEB pKWmEB ISIS EM-BLASSO

Trait[$]	Chromosome	Marker position (bp)	LOD score	r2 (%)	MAF	allele	Method
	S011_17612314	17612314	3.14 – 3.70	1.93E-08 -19.43	0.0945	G/C	FASTmrMLM FASTmrEMMA pLARmEB ISIS EM-BLASSO
PD	S011_24186823	24186823	3.4799	17.79	0.4252		ISIS EM-BLASSO
SP	S009_8056636	8056636	4.4069	27.43	0.228		mrMLM
SW	S002_20883066	20883066	4.21 – 4.90	21.30 – 22.30	0.315	G/T	FASTmrMLM pLARmEB ISIS EM-BLASSO
	S006_5090275	5090275	3.23	11.67 – 12.22	0.4055	G/A	FASTmrMLM pLARmEB
YLD	S005_3498765	3498765	3.94 – 4.39	15.30 – 23.72	0.16	G/A	mrMLM FASTmrMLM pLARmEB pKWmEB ISIS EM-BLASSO
	S008_9314904	9314904	3.11 – 3.55	5.51 – 12.44	0.38	C/T	mrMLM FASTmrMLM pLARmEB ISIS EM-BLASSO
	S011_18372986	18372986	3.06 – 3.82	7.05 – 13.24	0.376	A/G	mrMLM FASTmrMLM FASTmrEMMA pLARmEB pKWmEB ISIS EM-BLASSO

Trait[$]	Chromosome	Marker position (bp)	LOD score	r2 (%)	MAF	allele	Method
	S003_25128142	25128142	3.06 – 3.21	11.58 – 11.78	0.2874	C/T	FASTmrMLM pLARmEB ISIS EM-BLASSO
	S006_8901373	8901373	3.02 – 3.43	8.79 – 21.52	0.2874	G/A	FASTmrMLM pLARmEB pKWmEB ISIS EM-BLASSO
Zn	S002_28882891	28882891	3.11 – 4.37	4.35 – 7.24	0.3	A/T	mrMLM pLARmEB ISIS EM-BLASSO
	S006_17877070	17877070	3.05 – 8.44	19.79 – 37.62	0.108	A/G	mrMLM FASTmrMLM pLARmEB pKWmEB ISIS EM-BLASSO
	S008_14511248	14511248	3.38 – 3.60	6.13 – 8.43	0.244	C/T	mrMLM FASTmrEMMA ISIS EM-BLASSO
	S011_5127283	5127283	3.74 – 5.85	10.67 – 12.01	0.24	A/C	mrMLM pLARmEB ISIS EM-BLASSO
Fe	S001_1147602	1147602	3.05 – 4.38	6.60E-10 -37.78	0.148	C/T	mrMLM FASTmrMLM FASTmrEMMA pLARmEB ISIS EM-BLASSO

DTF= days to 50% flowering, DTM = days to maturity, PH = plant height, PB = number of primary branches, SB = number of secondary branches, PD = number of pods/plant, SP = number of seeds/pod, SW = 100-seed weight, YLD = yield/plot, Zn = grain zinc content, Fe = grain iron content.


a
b
c
d
e
f
−log10(P-value)
LOD score
Chromosomes
Observed -log10(P-value)
Expected -log10(P-value)

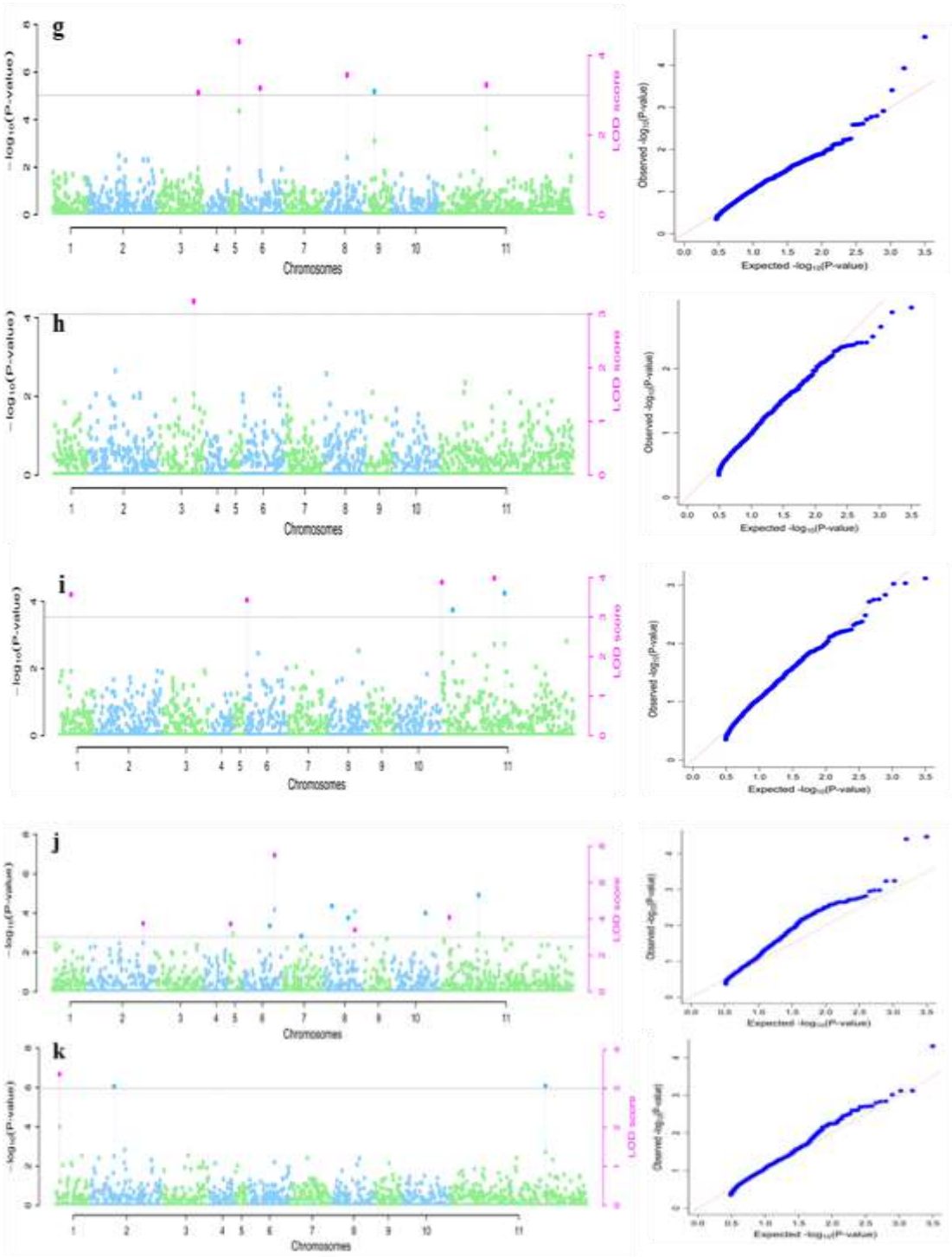


Fig 4.5: Manhattan plots and the corresponding Q-Q plots for 2019-20 (a, b, c, d, e, f, g, h, i, j, k) representing the identification of SNP markers associated with plant height, number of primary branches, number of secondary branches, number of pods/plant, number of seeds/pod, 100 seed weight, YLD = yield/plot, DTM = days to maturity, days to50% flowering, Zn = grain zinc content, Fe = grain iron content respectively in 2019-20. QTNs commonly identified by multiple approaches are indicated by the pink dots that are shown above dotted vertical lines, while all the QTNs identified by one single approach are indicated by the light color dots that are shown above dotted vertical lines.

For the pooled data of two years, a total of 56 highly significant SNPs associated with all the traits studied were identified on different chromosomes. Out of these, 25 SNPs were identified with at least two or more than two models indicating multiple evidence for the marker trait association (Table 4.17). The phenotypic variance explained by these SNPs for Fe, Zn and nine yield traits ranged from 1.15E-08 % to 38.58%. For plant height, two SNPs on chromosomes 01 and 11 were identified with combination of four models. Primary branches had single associated SNP on chromosome 10 identified through combination of two models. Secondary branches were found to have two SNPs identified through combination of five models on chromosomes 02 and 11. For pods/plant, two SNPs on chromosomes 02 and 03 were identified through combination of three models. For seeds/pod, three SNPs on chromosomes 04, 09 and 11 were identified through combination of five models. Chromosome 02 had single associated SNP identified through three models. For yield/plot two SNPs on chromosomes 03 and 11 were identified through combination of five models. For days to maturity, three SNPs on chromosomes 06 and 11 through combination of five models were identified. For days to flowering, three SNPs on chromosomes 08 and 11 were identified through single model. For grain Zn content five SNPs were identified on chromosomes 02, 05, 06, 08 and 11 through combination of six models. Grain Fe content was observed to have four SNPs on chromosomes 01, 02 and 11 identified through four models. The SNP position with ID and combination of models associated was given in Table 4.16.

Most of the SNP loci were found to be environment-and-trait specific but few of the identified SNPs were stable and consistent across years. Some of the SNPs associated with days to maturity, yield/plot and grain iron content were observed to be consistent in 2018-19 and pooled conditions. For grain zinc content, one SNP loci S006_17877070 identified on chromosome 06 was consistent in both the years as well as across years. Numerous studies have been reported on the identification of QTLs related to yield attributing and plant type traits. Saxena *et al* (2020) reported the identification of single nucleotide marker associated with nine yield component traits in pigeonpea using two backcross populations. Most of the QTLs were found to be environment-and-trait specific and few stable QTLs were also identified. This is in general agreement with our results where we have identified different trait specific SNP loci for different environment. They have reported one SNP S7_14185076 marker on chromosome 07 associated with four traits viz., days to 50% flowering, days to75% maturity, primary branches per plant and secondary branches per plant with positive additive effect in backcross population II. Likewise in other studies, identification of QTLs for yield attributing traits and plant type have also been reported in pigeonpea by Kumawat *et al* (2012), Prabhu *et al* (2014) and Patil *et al* (2018).

Table 4.9: Summary of SNPs associated with Fe, Zn and yield component traits in pigeonpea for pooled conditions

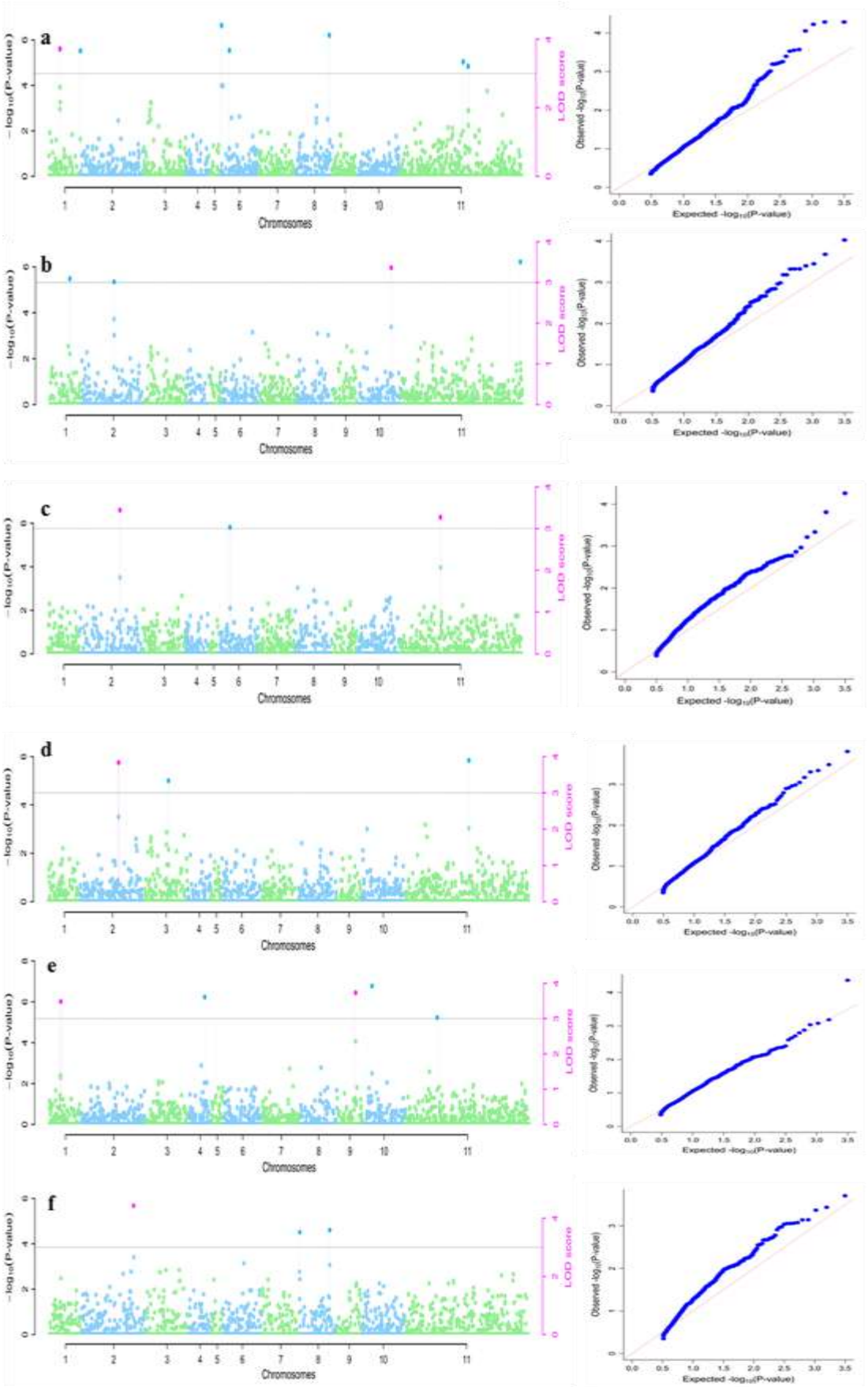
Trait[$]	Chromosome	Marker position (bp)	LOD score	r2 (%)	MAF	allele	Method
DF	S008_19259948	19259948	3.2298	11.39	0.4882	C/G	ISIS EM-BLASSO
	S011_10366132	10366132	3.3336	7.92	0.2953	T/C	ISIS EM-BLASSO
	S011_33509191	33509191	3.0338	6.99	0.3307	G/A	ISIS EM-BLASSO
DM	S006_491518	491518	3.05 – 4.07	3.36 – 6.63	0.468	A/G	mrMLM FASTmrMLM pLARmEB pKWmEB ISIS EM-BLASSO
	S011_5131233	5131233	3.16 – 3.82	15.03 –28.42	0.3307	G/A	FASTmrMLM pLARmEB pKWmEB ISIS EM-BLASSO
	S011_23395157	23395157	3.14	8.29 - 8.64	0.315	C/G	pLARmEB ISIS EM-BLASSO
PH	S001_6215375	6215375	3.43 – 3.69	7.20-13.00	0.378	A/G	FASTmrMLM FASTmrEMMA pLARmEB ISIS EM-BLASSO
	S011_24186823	24186823	3.07 - 3.51	20.44 -20.84	0.4252	C/T	FASTmrMLM ISIS EM-BLASSO

Trait[$]	Chromosome	Marker position (bp)	LOD score	r2 (%)	MAF	allele	Method
PB	S010_18104406	18104406	3.25 – 3.29	1.39E-09 -25.82	0.1654	C/T	FASTmrEMMA pKWmEB
SB	S002_20711967	20711967	3.34 – 4.28	15.50 –23.34	0.1	G/A	mrMLM FASTmrMLM pLARmEB pKWmEB ISIS EM-BLASSO
	S011_17612314	17612314	3.56 – 3.70	22.22 –28.29	0.096	G/C	mrMLM FASTmrMLM pLARmEB pKWmEB ISIS EM-BLASSO
PD	S002_20181471	20181471	3.15 – 3.34	14.46 –15.28	0.2047	A/C	FASTmrMLM pLARmEB ISIS EM-BLASSO
	S003_14308287	14308287	3.3124	12.28	0.1496	C/T	FASTmrMLM ISIS EM-BLASSO
SP	S009_8056636	8056636	3.40 – 5.27	1.15E-08 – 22.84	0.228	A/G	mrMLM FASTmrMLM FASTmrEMMA pLARmEB ISIS EM-BLASSO
	S011_12775647	12775647	3.18 – 4.50	7.46 – 14.73	0.164	C/A	mrMLM FASTmrMLM pLARmEB ISIS EM-BLASSO

Trait[$]	Chromosome	Marker position (bp)	LOD score	r2 (%)	MAF	allele	Method
	S004_10458700	10458700	3.63 – 5.14	9.05 – 17.91	0.1457	G/A	FASTmrMLM pLARmEB ISIS EM-BLASSO
SW	S002_29979442	29979442	3.01 – 4.52	17.49 –20.87	0.1181	A/T	FASTmrMLM pLARmEB ISIS EM-BLASSO
YLD	S003_11913973	11913973	3.16 – 4.15	4.38 – 7.90	0.26	C/G	mrMLM FASTmrEMMA pLARmEB pKWmEB ISIS EM-BLASSO
	S011_8160089	8160089	3.0789	14.34 -15.80	0.4843	C/A	pLARmEB ISIS EM-BLASSO
Zn	S002_28882891	28882891	3.25 – 5.01	4.38 – 10.11	0.3	A/T	mrMLM FASTmrMLM pLARmEB ISIS EM-BLASSO
	S005_1416178	1416178	3.08 – 4.18	6.89 – 9.74	0.436	T/G	mrMLM FASTmrMLM pLARmEB ISIS EM-BLASSO

Trait[$]	Chromosome	Marker position (bp)	LOD score	r2 (%)	MAF	allele	Method
	S006_17877070	17877070	5.60 – 9.94	19.65 –38.58	0.108	A/G	mrMLM FASTmrMLM pLARmEB pKWmEB ISIS EM-BLASSO
	S011_5127283	5127283	3.15 – 5.39	8.35 – 16.25	0.2362	A/C	FASTmrMLM ISIS EM-BLASSO
	S008_14511248	14511248	3.04 – 4.78	1.84E-09 – 10.95	0.2402	C/T	FASTmrEMMA pLARmEB ISIS EM-BLASSO
Fe	S001_1147602	1147602	3.31 – 3.73	18.21 –20.94	0.148	C/T	mrMLM ISIS EM-BLASSO
	S002_10735749	10735749	3.65 – 4.26	12.06 –21.80	0.396	T/G	mrMLM FASTmrMLM pLARmEB ISIS EM-BLASSO
	S002_16766436	16766436	3.00 – 3.97	7.04 – 9.43	0.248	A/G	mrMLM FASTmrMLM pLARmEB
	S011_33449156	33449156	3.10 – 3.33	10.14 –13.59	0.1811	G/A	FASTmrMLM pLARmEB

[$]PH =plant height, PB = number of primary branches, SB = number of secondary branches, PD = number of pods/plant, SP = number of seeds/pod, SW = 100 seed weight, YLD = yield/plot, DTF = days to 50% flowering, DTM = days to maturity, Zn = grain zinc content, Fe = grain iron content.


a
b
c
d
e
f
−log10(P-value)
LOD score
Chromosomes
1 2 3 4 5 6 7 8 9 10 11
Observed -log10(P-value)
Expected -log10(P-value)
0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5

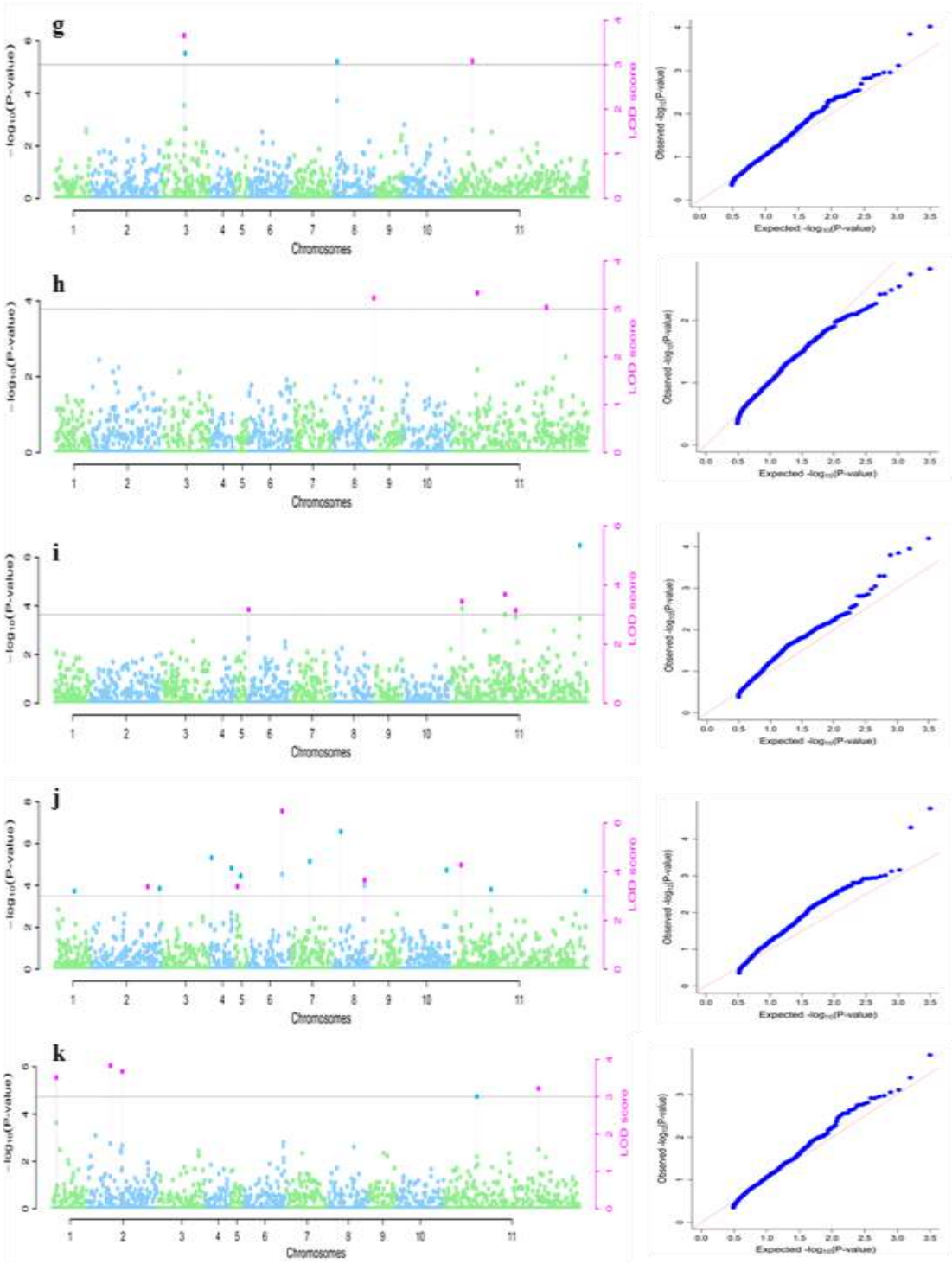


Fig 4.6: Manhattan plots and the corresponding Q-Q plots for pooled data (a, b, c, d, e, f, g, h, i, j, k) representing the identification of SNP markers associated with plant height, number of primary branches, number of secondary branches, number of pods/plant, number of seeds/pod, 100 seed weight, YLD = yield/plot, DTM = days to maturity, days to 50% flowering, Zn = grain zinc content, Fe = grain iron content respectively in pooled data. QTNs commonly identified by multiple approaches are indicated by the pink dots that are shown above dotted vertical lines, while all the QTNs identified by one single approach are indicated by the light color dots that are shown above dotted vertical lines.

4.2.3 Prediction of Candidate genes

For the identification of candidate genes underlying the association signals, highly significant SNPs associated with target trait were selected. The candidate regions of associated SNPs were defined by the average LD decay distance or the LD block. DNA sequence from 150 kb genomic region on either side of significant SNPs was considered as confidence interval for identification of putative candidate genes (Fig 4.9). The pigeonpea reference genome was *C. cajan*_V1.0 'Asha', the functional annotations and tissue specific expression of genes located in the candidate regions were obtained from (https://www.ncbi.nlm.nih.gov/genome/annotation_euk/Cajanus_cajan/101/). Based on the pigeonpea genomic annotations and expression data, potential candidate genes were predicted. The putative candidate genes and their biological functions were shown in Table 4.18.

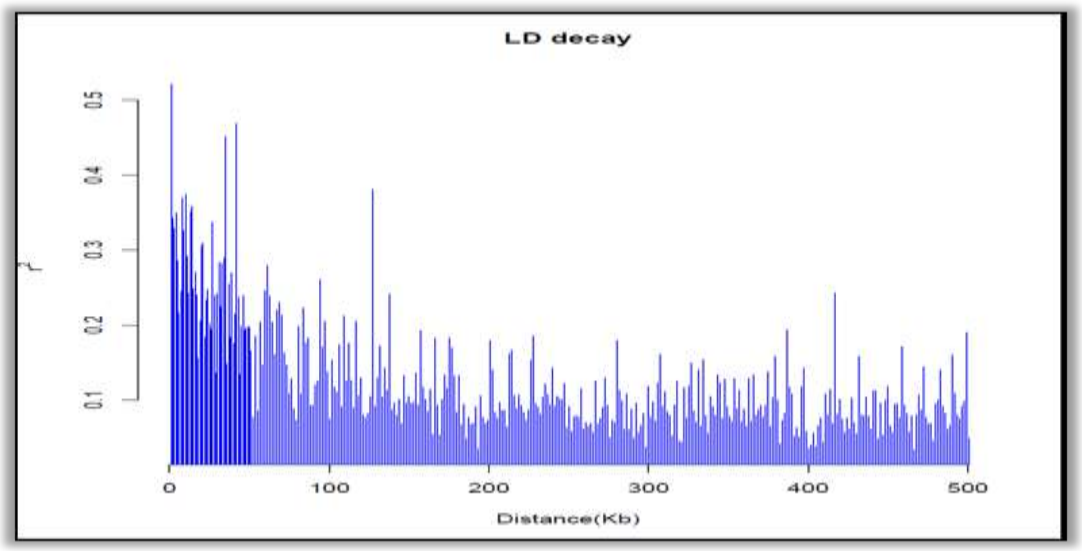


Fig.4.7: LD decay plot showing LD decay in pigeonpea germplasm

4.2.3.1 Candidate genes for plant growth and development contributing towards seed yield

In the present study, significant SNP loci identified through two or more than two models were used for the prediction of candidate genes. For seed yield six putative candidate genes were identified in the candidate region of SNP loci S003_11913973 mapped on chromosome 03, showing directly or indirectly their role towards the growth and development of floral organ and grain formation. Lectin receptor-like kinases (Lectin RLKs) are a large family of receptor-likekinases with an extracellular legume lectin-like domain and their role in various functions, such as stress responses, disease resistance, hormone signaling, and

legume–rhizobium symbiosis. Mutation in one Lectin RLK gene led to male sterility in *Arabidopsis* (Wan *et al* 2008). Protein Transcription factor MYB86 was found to be expressed in stems, seeds and flowers and weakly expressed in roots and leaves. Noji *et al* (1998) reported the role of plant myb related genes in flavonoid biosynthesis, trichome development and cell shape determination. Further, they analysed the expression of Atmyb3 gene in Arabidopsis that was showing strong expression in floral tissue only, suggesting the role of protein in development of floral tissue. Shuai *et al* (2002) reported the role of *LATERAL ORGANBOUNDARIES* (*LOB*) gene in *Arabidopsis* in plant-specific processes. Loss-of-function *LOB* mutants have no detectable phenotype under standard growth conditions suggesting that *LOB* is functionally redundant or required during growth under specific environmental conditions. Ectopic expression of *LOB* leads to alterations in the size and shape of leaves and floral organs and causes male and female sterility. Raffinose oligosaccharides are major soluble carbohydrates in seeds and other tissues of plants. They are synthesized in leaves, roots, and tubers of a range of plant species. In seeds of higher plants, they are of almost ubiquitous occurrence. Peterbauer *et al* (2002) reported on the purification, characterization, and heterologous expression of a multifunctional stachyose synthase from developing pea (*Pisum sativum* L.) seeds. Vespa *et al* (2004) reported only one specific partner of AtFKBP12, namely AtFIP37 (FKBP12 interacting protein 37 kD) in *Arabidopsis*. Knockout mutants of AtFIP37show an embryo-lethal phenotype that is caused by a strong delay in endosperm development and embryo arrest. It has been observed that gene is expressed during embryogenesis and throughout plant development. Sagasser *et al* (2002) reported the role of *transparent testa 1* mutant (*tt1*) in yellow seed colour *Arabidopsis thaliana* due to lack of condensed tannin pigments in the seed coat. *WIP* genes may play important roles in regulating developmental processes, including the control of endothelium differentiation.

4.2.3.2 Candidate genes for plant height

Four predicted candidate genes were identified in the candidate region of SNP loci S001_6215375 which was mapped on chromosome 01. Receptor-likeprotein kinase required for cell elongation during vegetative growth and controls ectopic-lignin accumulation in cellulose-deficient mutant backgrounds. Guo *et al* (2009) hypothesized that RLKs (HERCULES1, THESEUS1, and FERONIA) are involved in BR-regulated processes and demonstrated that they were required for cell elongation during vegetative growth as *herk1 the1* double and *fer*RNAi mutants displayed striking dwarf phenotypes in *Arabidopsis*. Brown *et al* (2005) reported secondary cell wall–specific cellulose synthase genes IRREGULAR XYLEM1 (IRX1) and IRX3in. These genes are likely to define entirely novel processes in secondary cell wall formation. Zhao *et al* (2011) demonstrated two paralogous single Myb domain genes, MYB-RELATEDPROTEIN 1 (MYR1) and MYB-RELATED PROTEIN 2

(MYR2), and their roles as repressors of responses to decreased light intensity in *Arabidopsis*. Homozygousmyr1 myr2 double mutants flowered early under low light intensities and additionally exhibited increases in leaf angle, petiole length, and apical dominance. Their results suggested that the ability ofMYR1 and MYR2 to repress flowering and organ elongation is at least partly due to their negative effect on levels of bioactive GA.

4.2.3.3 Candidate genes for other yield component traits

For secondary branches, single putative candidate gene associated with SNP loci (S002_20711967) was identified on chromosome 02. ER-derived vesicles protein ERV14 could regulate export of the bud site and axial growth sites selection protein AXL2 and possibly other secretory proteins from the endoplasmic reticulum in COPII-coated vesicles, seems to be required for axial budding pattern in haploid cells. Powers *et al* (1998) reported that haploid cells that lack Erv14p are viable but display a modest defect in bud site selection because a transmembrane secretory protein, Axl2p, is not efficiently delivered to the cell surface. Axl2pis required for selection of axial growth sites and normally localizes to nascent bud tips or the mother bud neck.

For pods/plant, three putative candidate genes in the candidate region of significantly associated SNP loci S002_20181471 and S003_14308287 were identified on chromosomes 02 and 03 respectively. Beta-fructofuranosidase, insoluble isoenzyme 1 may play a role in sucrose partitioning during seed development and in stress response. Hirose *et al* (2002) cloned a cDNA*OsCIN1* for a cell wall invertase from developing grains of rice. The deduced amino acid sequence showed typical features of the cell wall invertases including a β-fuctosidase motif and a cysteine catalytic site. *OsCIN1 is* expressed in sink and source – leaves, roots and in panicles. During the course of grain filling in the caryopses *OsCIN1* transcript is detectable only in the very early stage of their development when the cell wall invertase activity is highest and the increase in the caryopses length is rapid suggests that during the early stage of caryopses development, *OsCIN1* is important for supplying a carbon source to developing filial tissues. Martin *et al* (2001) reported that cytokinins are essential hormones for plant growth cytokinins regulate a host of developmental events in whole plants such as leaf expansion, bud formation, promotion of seed germination, delay of senescence, and chloroplast formation. Zeatin is a naturally occurring cytokinin. Biosynthesis and metabolism studies of zeatin have been directed mostly at the *trans* isomer, although *cis*-zeatin and its riboside occur as major components in some plant species. Based on the sequence of the gene *ZOG1*encoding a *trans*-zeatin *O*-glucosyl transferase from *Phaseolus*, a *cis*-zeatin-specific *O*-glucosyl transferase was isolated from maize suggests that *cis*-zeatin and derivatives may be more important in cytokinin homeostasis.

Table 4.10: List of putative candidate genes identified for yield and component traits

Trait	SNP	Locus	Protein	Function
YLD	11913973	11822615	Lectin-domain containing receptor kinase A4.2	Required during pollen development
		11876079	Transcription factor MYB86	Expressed in stems, flowers and seeds. Weakly expressed in leaves and roots
		11985734	Protein LATERAL ORGAN BOUNDARIES	Ectopic expression of LOB leads to alterations in the size and shape of leaves and floral organs and causes male and female sterility.
		12040743	Stachyose synthase	Oligosaccharides of the raffinose family play a protective role in maturation drying of seeds.
		12049020	FKBP12-interacting protein of 37 kDa	Essential protein required during endosperm development and embryogenesis
		5086787	Protein TRANSPARENT TESTA 1	Plays a role in the regulatory network controlling flavonoid accumulation in endothelium cells during seed development
PH	6215375	6079136	Receptor-like protein kinase THESEUS 1	Receptor-like protein kinase required for cell elongation during vegetative growth. Controls ectopic-lignin accumulation in cellulose-deficient mutant backgrounds.
		6213160	Laccase-4	Lignin degradation and detoxification of lignin-derived products. Required for secondary xylem cell wall lignification.
		6258593	Myb-like protein J	Acts redundantly with MYR2 as a repressor of flowering and organ elongation under decreased light intensity. Represses gibberellic acid (GA)-dependent responses and affects levels of bioactive GA
		6245493	ATPase 5, plasma membrane-type	The plasma membrane H^+ ATPase of plants and fungi generates a proton gradient that drives the active transport of nutrients by H^+-symport. The resulting external acidification and/or internal alkinization may mediate growth responses

Trait	SNP	Locus	Protein	Function
SB	20711967	20655463	ER-derived vesicles protein ERV14	Could regulate export of the bud site and axial growth sites selection protein AXL2 and possibly other secretory proteins from the endoplasmic reticulum in COPII-coated vesicles. Seems to be required for axial budding pattern in haploid cells
PD	20181471	20078152	Beta-fructofuranosidase, insoluble isoenzyme 1	May play a role in sucrose partitioning during seed development and in stress response.
	14308287	14206484	Auxin efflux carrier component 1	Acts as a component of the auxin efflux carrier. Seems to be involved in the basipetal auxin transport. Mediates the formation of auxin gradient which is required to ensure correct organogenesis.
		14402701	Zeatin O-glucosyl transferase	May regulate active VS. Storage forms of cytokinins and could have an impact on seed growth.
SP	8056636	8066555	NAC domain-containing protein 18	Regulates embryogenesis by regulating the development and degeneration of ovule integuments, a process required for inter tissue communication between the embryo and the maternal integument
	10458700	10431172	Protein ULTRAPETALA 2	Specifically expressed during the reproductive developmental stage. Expressed in embryonic shoot apical meristems, in inflorescence and floral meristems, and in developing stamens, carpels and ovules.

For number of seeds/pod, two putative candidate genes were identified in the candidate region of significantly associated SNP loci S009_8056636 and 10458700 on chromosomes 09 and 03 respectively. Kunieda *et al* (2008) reported NAC-REGULATED SEEDMORPHOLOGY1 and -2 caused aberrant seed shapes in *Arabidopsis thaliana*. Double knockout mutant nars1 nars2 exhibited abnormally shaped seeds; moreover, neither nars1 nor nars2 produced abnormal seeds, indicating that NARS1 and NARS2 redundantly regulate seed morphogenesis. These results indicate that NARS1and NARS2 regulate embryogenesis by regulating the development and degeneration of ovule integument.

Carles *et al* (2004) defined the *ULTRAPETALA1* (*ULT1*) gene as a key negative regulator of cell accumulation in *Arabidopsis* shoot and floral meristems, because mutations in*ULT1* cause the enlargement of inflorescence and floral meristems, the production of supernumerary flowers and floral organs, and delay in floral meristem termination. *ULT1*and *ULT2* are expressed coordinately in embryonic shoot apical meristems, in inflorescence and floral meristems, and in developing carpels, stamens and ovules. Down regulation of both ULT genes can lead to shoot apical meristem arrest shortly after germination, revealing a requirement for ULT activity in early development.

4.3 Validation of KASP markers in biparental population

The SNP markers which were found to show significant marker-trait associations in GWAS analysis and were polymorphic between the parents (AL1449 & MN-1) were further validated in biparental $F_{2:3}$ population. The list of markers converted to KASP (Kompetitive allele specific marker) markers is given in Table 4.19. The validation was carried out by converting the associated SNP markers to KASP markers.

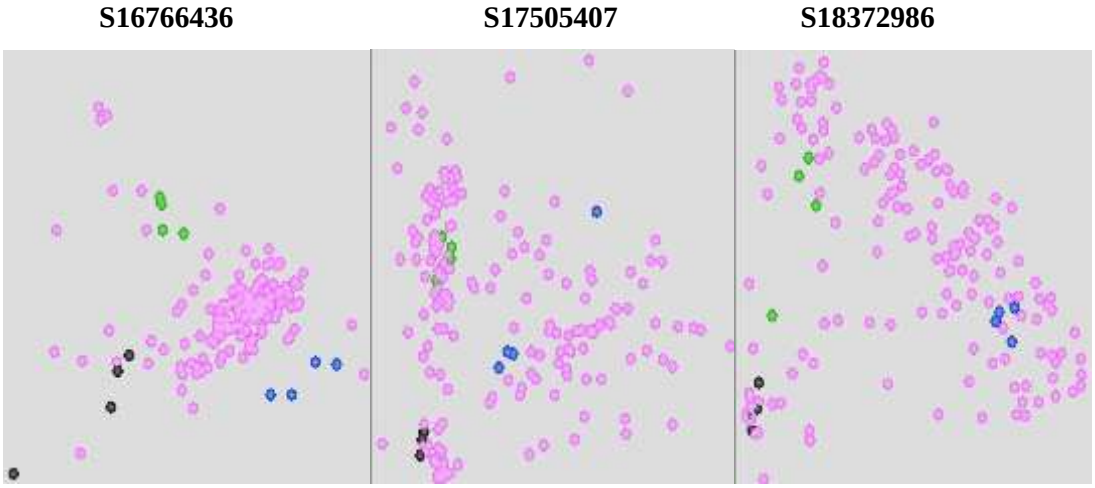


Fig 4.8: Graphical output of KASP markers using KlusterCaller software. Genotyped samples marked green are homozygous for allele reported with HEX those marked blue are homozygous for allele reported with FAM and those marked pink are segregating alleles and dots in black colour are NTC(Non-template control).

Table 4.11: List of SNP markers converted to KASP markers. Each marker carried a set of three primers i.e. two allele specific primers and one common primer

SNP ID	Trait	SNP	Marker	Allele
S16766436	Fe	A/G	**GAAGGTCGGAGTCAACGGATT**TGATGTCTCCTTTAGGTCCTTATTTAGT**G**	Hex
			GAAGGTGACCAAGTTCATGCTTGATGTCTCCTTTAGGTCCTTATTTAGT**A**	Fam
			AGGTTGTAGCCATCAATCTCCTACCTTACT	common
S14511248	Zn	C/T	**GAAGGTCGGAGTCAACGGATT**GATGTATTGTCGACCGGAGCTGTCCC**G**	Hex
			GAAGGTGACCAAGTTCATGCTGATGTATTGTCGACCGGAGCTGTCCC**A**	Fam
			CAGTTGGCTCGGCTGGCAAGTAGTC	common
S18372986	YLD	A/G	**GAAGGTCGGAGTCAACGGATT**TAAAAACTAGAGAAATGTAAAGCAAATA**A**	Hex
			GAAGGTGACCAAGTTCATGCTTAAAAACTAGAGAAATGTAAAGCAAATA**G**	Fam
			GTAGTTTCTGTTGCTTAAACCTGCCATCAC	common
S6405903	SW	G/A	**GAAGGTCGGAGTCAACGGATT**ATGGGGATGTTGGTGATGAGGCTTAA**G**	Hex
			GAAGGTGACCAAGTTCATGCTATGGGGATGTTGGTGATGAGGCTTAA**A**	Fam
			ATCCTATCAGAGCATGCCCCACTAAAG	common
S17505407	PB	A/G	**GAAGGTCGGAGTCAACGGATT**CTCTAAGAAGTAAACTAAATTATGCCACTCT**A**	Hex
			GAAGGTGACCAAGTTCATGCTCTCTAAGAAGTAAACTAAATTATGCCACTCT**G**	Fam
			GGTTGATATGAAGTGAGGTCAACTTATGGT	common

A total of five KASP markers were used for validation in population for different traits. Out of these five, three KASP markers for traits *viz.*, grain Fe content, Primary branches and yield / plot were observed to show validated results with parents having homozygous alleles for the traits and segregating alleles in the $F_{2:3}$ population.

CHAPTER V

SUMMARY

The present study was carried out with the objectives of genome-wide association studies to identify QTLs associated with Fe, Zn and yield related traits in pigeonpea germplasm and validation of QTLs in bi-parental population developed by crossing contrasting genotypes for Fe, Zn and yield attributing traits. One hundred and seventy-eight germplasm lines available with the pulses section of Department of Plant Breeding and Genetics were evaluated for grain Fe and Zn content and yield component traits. The experiment was carried out in a randomized complete block design with two replications during 2018-19 and2019-20. Observations were recorded for traits *viz.*, grain Fe content, grain Zn content, days to 50% flowering, days to maturity, plant height, number of primary branches, number of secondary branches, pods/plant, seeds/pod, 100-seed weight and yield/plot. Crosses between AL 1449 and MN 1 were attempted during 2018-19 and $F_{2:3}$ bi-parental population was developed for validation of identified QTLs in the population.

There was significant variability observed among genotypes for all the traits in both the years. Significant genotype-by-environment interaction was also observed for traits *viz.*, days to 50% flowering, days to maturity, pods/plant and seeds/pod, indicating the role of environment in the manifestation of phenotype. The mean performance of the genotypes was significantly different for year 2018-19 and 2019-20 for all the traits. This is an indication of sufficient variability for the traits under study. A wide range of variability was observed for all the traits *viz*., days to 50% flowering, days to maturity, plant height, number of primary branches, number of secondary branches, pods/plant, seeds/pod, yield/plot, grain Fe content and grain Zn content in the germplasm. Skewness and kurtosis measured the distribution of data and frequency of outliers present in the dataset. For the traits like days to maturity, numberof primary branches, number of secondary branches, pods/plant and yield/plot, the distribution was observed to be positively skewed and platykurtic in both the years and pooled across the years indicated the symmetrical distribution with no outliers in the data set. The information on distribution using skewness and kurtosis gave insights into the nature of gene action. Based on skewness values, traits like days to 50% flowering, plant height, seeds/pod and 100 seed weight were observed to exhibit duplicate (additive × additive) gene action controlling these traits. Other traits like days to maturity, number of primary and secondary branches, pods/plant, yield/plot exhibited complementary gene interaction. Grain Fe and Zn content showed complementary as well as duplicate gene interaction for their expression. Coefficient of variation for plant height, primary branches, secondary branches, pods/plant, yield/plot, grain Fe content and grain Zn content was more than 10%, indicating more phenotypic variation as compared to other traits studied.

Genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) for the traits like yield/plot and pods/plant were consistently high. For number of primary and secondary branches, grain Fe and grain Zn content GCV and PCV showed consistently moderate values, while for days to 50% flowering, days to maturity, plant height, seeds/pod and 100-seed weight, the GCV and PCV values were consistently low for both the years as well as in pooled data across two years. The value of genotypic coefficient of variation and phenotypic coefficient of variation components facilitates to assess the extent of genetic variation present in a population for the given trait. High estimates of heritability were observed for traits like days to 50% flowering, days to maturity, pods/ plant, 100-seed weight and yield/plot which were consistent in both the years as well as across two years pooled data. High heritability indicates the scope of genetic improvement of traits through selection. However, selection based on the high heritability values does not represent the amount of genetic progress for selecting individuals. So, estimates of genetic advance were considered along with heritability. High heritability in conjugation with high genetic advance indicates predominance of additive gene effects and effectiveness of selection for that trait. Yield/plot followed by pods/plant exhibited high heritability along with high genetic advance, showing additive gene effect. Therefore, these traits can be considered as effective traits for selection in breeding programme. Other traits like days to 50% flowering, days to maturity, plant height, number of primary and secondary branches, grain Fe content and grain Zn content recorded moderate to high heritability along with moderate genetic advance, indicating the selection to be effective for these traits. These observations indicated that heritability was due to additive gene effects and selection could be effective in early generations. It was likely to accumulate more additive genes or these traits may be used as selection criteria in pigeonpea breeding programme

Correlation coefficient is a statistical measure used to find out the degree and direction of relationship between two or more than two variables. The yield/ plot exhibited positive and significant high correlation with number of primary branches, number of secondary branches and pods/ plant for the year 2018-19, 2019-20 and pooled across two years, respectively. Moderate value of positive and significant correlation was observed for 2018-19, 2019-20 and pooled across two years, respectively between grain Fe and Zn content. It showed that with the increase in the grain Fe content, there would be simultaneously increase in the grain Zn content. Therefore selection for one trait will be effective in selecting the other and vice-versa. Hence, it depicted that direct selection for these traits could lead to the development of high yielding pigeonpea genotypes. The highest positive direct effect contributing to seed yield/ plot was observed due to pods/ plant followed by number of primary branches and number of secondary branches. Pods/ plant, number of primary

branches, number of secondary branches and seeds/ pod were identified as major components of seed yield and could be considered as selection criteria for increasing the seed yield of pigeonpea.

Based on the phenotypic performance ten promising genotypes *viz.*, H 005, H 0512, H 0535, AL 997, IC 245176, AL 1487, AL 997, MN 5 and IC 3977 surpassing the check AL 882 for seed yield were identified. All the genotypes identified were showing consistently high seed yield across the years. Four genotypes *viz.*, AL 1721, AL 1477, AL 1490 and AL 1396 were found to be showing consistently high grain Fe content in both the years as well as across the years and could be used as a donor parents for transferring high grain Fe content in breeding programme. For grain Zn content, five genotypes *viz.*, AL1449, IC 245176, AL 1313, ICPL 9308 and AL 1430 were identified as promising in both the years as well as across the years. These identified genotypes could be used as donor parents for high grain Zn content in breeding programme for enhancing the nutritional status of pigeonpea.

For association between phenotype and genotype, multi–locus methods which capture small effect loci in complex quantitative traits were used. The main reason for applying methods is to get benefitted from the algorithms of different models and support one by the other. A total of 69 highly significant SNPs associated with all the traits were identified, except days to flowering. Out of these, 30 SNPs were identified with combination at least of two or more than two models, indicating multiple evidence for the marker trait association for the year 2018-19. For the year 2019-20, a total of 65 highly significant SNPs associated with all the traits were identified on different chromosomes. Out of these 23, SNPs were identified with combination of at least two or more than two models. For the pooled data of two years, a total of 56 highly significant SNPs associated with all the traits studied were identified on different chromosomes. Out of these, 25 SNPs were identified with combination of atleast two or more than two models. Highly significant SNPs associated with target trait were selected to search candidate genes in their candidate regions. The candidate regions were defined by the average LD decay distance or the LD block. Biological function of the identified candidate genes revealed their role in plant growth and development, organogenesis, pollen development and source-sink relationship. These genes were found to be present on chromosome 02, 03 and 09.

For the validation of KASP markers, the SNP markers which were found to show significant marker trait associations in GWAS analysis and were polymorphic between the parents AL 1449 & MN-1, were used. Five KASP markers for traits viz., grain Fe content, grain Zn content, seeds / pod, secondary branches and plant height were generated and validated in the biparental population. Out of these five, three KASP markers were observed

to show validated results with parents having homozygous alleles for the traits and segregating alleles in the $F_{2:3}$ population.

In conclusion, the present study found promising genotypes for grain Fe, grain Zn content and yield attributing traits which can be used as donor parents in pigeonpea breeding programme. The significant SNPs associated with grain iron, grain zinc content and yield traits can be used to develop markers to be used in selection of desirable genotypes having allele of interest and indirectly for marker assisted selection (MAS) in rapid generation advancement programme.

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ANNEXURE I

Adjusted means for diverse set of germplasm lines for the year 2018-19

S. No.	Treatment	DF	DM	PH	PB	SB	PD	SP	SW	YLD	Fe	Zn
1	AL292	103.5	133.5	248.5	9.0	15.0	113.5	33.5	8.15	141.0	31.1	31.7
2	AL303	82.5	101.5	219.5	13.0	17.5	133.5	35.0	8	215.0	24.1	25.4
3	AL304	103.0	123.5	224.5	17.5	18.0	125.5	18.0	8	220.0	22.3	23.9
4	AL307	97.5	113.5	203.5	15.5	23.0	139.0	34.5	8.05	200.0	23.0	22.1
5	AL311	97.0	119.5	219.5	16.0	17.5	123.0	34.0	7.2	177.5	29.6	27.7
6	AL986	98.5	131.5	219.5	15.5	23.0	229.5	32.5	7.3	395.0	35.9	36.7
7	AL997	109.0	124.5	242.5	23.0	30.0	255.0	35.0	8.5	492.5	29.3	31.7
8	AL1313	103.0	124.5	224.5	13.0	15.0	118.5	30.0	7.9	187.5	32.9	36.7
9	AL1396	102.5	121.5	221.5	15.0	23.0	129.0	37.0	7.3	215.0	37.0	33.3
10	AL1430	103.5	122.5	221.5	17.5	26.5	218.5	35.0	9.45	415.0	29.4	33.7
11	AL1449	97.0	120.5	211.5	16.5	20.5	144.5	37.5	8.4	277.5	37.6	51.5
12	AL1452	103.0	114.5	209.5	17.0	24.5	270.0	39.0	8.55	425.0	27.2	29.7
13	AL1455	102.5	117.5	219.5	12.0	15.5	120.0	37.0	7.55	272.5	30.1	20.9
14	AL1459	87.0	110.5	205.5	11.5	20.0	166.0	35.5	8.75	347.5	33.6	28.3
15	AL1465	98.0	109.5	220.5	9.0	13.5	103.0	34.5	7.35	137.5	30.9	29.0
16	AL1466	97.0	121.5	225.5	11.0	17.5	120.0	34.5	8.55	162.5	30.0	28.5
17	AL1471	97.5	126.5	219.5	12.0	17.5	125.0	37.0	8.4	267.5	27.4	23.4
18	AL1474	98.0	127.5	202.5	12.0	15.0	116.5	35.0	8.65	210.0	26.3	20.0
19	AL1476	103.5	129.0	192.5	14.0	20.5	128.0	37.0	8.3	290.0	33.6	18.3
20	AL1477	91.5	114.0	196.5	17.0	22.5	200.0	38.5	8.1	372.5	40.1	22.0
21	AL1486	87.5	121.0	203.5	10.5	15.0	122.5	36.5	8.7	232.5	35.2	19.7
22	AL1487	96.5	127.0	204.5	20.0	27.5	263.0	40.0	8.7	470.0	33.0	22.8
23	AL1490	97.5	134.0	210.5	11.0	17.0	122.0	38.0	8.4	187.5	38.8	24.3
24	AL1491	97.0	118.0	236.5	14.0	20.0	217.0	37.0	9.45	342.5	26.8	23.2
25	AL1508	109.0	119.0	266.5	12.5	20.0	125.0	31.0	8.65	232.5	35.2	30.9
26	AL1533	97.0	119.0	228.5	14.0	21.5	215.0	32.5	8.3	305.0	31.1	26.6
27	AL1538	110.5	119.0	202.5	14.0	21.0	162.5	35.5	7.8	262.5	25.2	17.5
28	AL1543	81.5	128.0	210.5	10.0	17.0	124.0	38.0	8.35	231.5	25.8	23.0
29	AL1554	103.0	121.0	193.5	10.0	13.5	120.5	35.0	8.35	227.5	25.3	27.7
30	AL1584	97.0	114.0	224.5	9.5	12.5	114.0	36.5	8.7	172.5	25.9	23.0
31	AL1592	110.5	122.0	209.5	13.0	18.5	148.5	37.0	6.75	265.0	17.1	18.5
32	AL1594	111.0	113.0	171.5	8.5	12.5	90.0	38.5	7.35	130.0	19.7	30.1
33	AL1627	103.0	111.0	223.5	17.5	24.5	243.0	34.0	7.6	415.0	21.4	18.7
34	AL1676	103.5	121.0	237.5	13.5	21.5	230.5	39.5	8.5	400.0	27.0	26.3
35	AL1721	104.5	112.0	200.5	17.0	23.5	275.5	38.5	8.6	465.0	42.2	30.3

S. No.	Treatment	DF	DM	PH	PB	SB	PD	SP	SW	YLD	Fe	Zn
36	AL1725	109.0	110.0	151.5	15.0	20.0	228.0	35.5	6.85	362.5	28.3	32.7
37	AL1730	109.5	113.0	223.5	15.0	20.5	186.5	40.0	6.7	362.5	31.2	27.5
38	AL1740	103.0	119.0	203.5	11.0	21.0	208.0	37.0	9.55	275.0	26.8	25.8
39	AL1747	98.5	110.0	184.5	8.0	15.0	138.0	36.5	8.45	232.5	25.8	23.0
40	AL1759	97.5	122.0	162.5	10.0	21.5	136.0	36.5	8.45	210.0	22.7	27.6
41	AL1790	87.5	118.0	188.5	12.0	21.0	115.0	35.0	7.25	185.0	17.3	30.0
42	AL1801	87.0	113.0	238.5	17.0	26.5	246.5	37.5	8.3	385.0	23.1	27.5
43	AL1813	109.5	123.0	230.5	14.5	21.5	135.5	37.0	6.7	375.0	18.6	22.1
44	AL1820	108.5	113.0	214.5	9.5	15.5	122.5	40.5	6.85	190.0	23.9	24.8
45	AL1823	97.5	113.0	229.5	11.0	15.5	126.0	38.0	8.55	225.0	25.1	22.1
46	AL1835	87.0	121.0	201.5	9.5	15.0	107.5	36.5	8.05	205.0	20.5	19.9
47	AL1847	97.5	126.0	238.5	9.0	12.0	96.5	32.0	8.25	142.5	24.4	25.3
48	AL1848	104.5	131.0	225.5	11.0	17.5	145.5	38.0	7.7	252.5	18.9	22.7
49	AL1849	97.5	136.0	244.5	15.0	20.5	161.5	34.5	8.35	262.5	25.0	26.8
50	AL1853	104.0	113.0	249.5	10.0	15.5	119.0	34.0	8.3	230.0	25.6	23.1
51	AL1856	104.5	139.0	225.5	19.0	26.0	257.5	31.0	7.8	395.0	22.9	21.0
52	IC3977	105.5	104.0	195.5	21.0	25.0	280.0	36.5	8.8	435.0	25.6	22.9
53	IC245139	104.5	134.0	235.5	12.0	13.5	161.5	35.0	7.65	215.0	24.4	20.9
54	IC245176	97.5	134.0	216.5	17.0	26.5	263.0	38.0	8.35	425.0	30.3	38.5
55	IC245183	110.0	104.0	231.5	12.0	16.5	200.5	38.0	7.85	230.0	23.0	26.8
56	IC245186	109.5	134.0	237.5	13.0	16.0	138.0	33.5	8.35	225.0	27.9	29.6
57	IC245219	109.0	118.0	224.5	13.0	15.0	116.0	39.0	8	222.5	25.4	28.1
58	IC245245	108.0	118.0	231.5	10.0	13.5	109.5	35.0	8.3	172.5	24.5	28.8
59	IC245268	104.5	131.0	227.5	11.0	14.5	187.5	37.0	7.15	220.0	28.8	32.0
60	IC245273	110.5	123.0	241.5	10.0	15.0	180.0	37.0	8.7	242.5	20.2	23.4
61	IC245294	96.0	106.0	216.5	9.0	12.5	105.5	35.0	7.95	152.5	18.1	20.0
62	IC245314	104.0	114.0	230.5	15.0	22.5	213.5	34.0	8.65	342.5	16.5	16.0
63	IC245315	97.0	124.0	221.5	21.0	27.5	242.0	36.5	7.65	472.5	19.4	18.2
64	IC245497	96.5	139.0	240.5	16.0	21.5	218.5	33.0	9.1	330.0	20.1	19.3
65	IC245507	103.5	107.0	218.5	13.5	17.0	219.0	36.0	8.2	342.5	16.4	16.3
66	IC245523	109.0	119.0	210.5	9.0	14.0	199.5	38.0	8	220.0	18.5	20.1
67	IC245524	108.0	114.0	228.5	16.0	22.0	228.0	38.0	8.15	360.0	23.4	20.5
68	IC245525	104.5	134.0	205.5	12.0	16.5	179.0	33.0	8.55	230.0	22.2	26.5
69	IC245533	104.0	111.0	208.5	17.0	24.0	242.5	34.0	8.4	385.0	21.6	26.7
70	IC245560	110.5	108.0	232.5	12.0	16.0	119.5	35.0	8.3	172.5	29.0	25.2
71	AH061	109.5	124.0	239.5	12.5	22.0	209.0	36.0	8.5	330.0	24.8	22.4
72	AH067	104.0	116.0	225.5	14.5	21.0	223.0	38.5	6.1	320.0	28.0	19.7
73	AH0612	97.0	122.0	201.5	19.0	22.0	246.0	37.0	8.35	415.0	29.1	21.9

S. No.	Treatment	DF	DM	PH	PB	SB	PD	SP	SW	YLD	Fe	Zn
74	AH091	97.5	124.0	197.5	15.0	21.0	238.0	34.0	8.45	400.0	18.3	14.1
75	AH093	96.5	127.0	219.5	17.5	24.0	245.0	37.0	8.65	400.0	22.8	16.2
76	AH0911	97.0	139.0	229.5	17.0	21.0	213.0	40.0	7.65	325.0	27.1	18.1
77	AH1501	109.5	125.0	210.5	10.0	14.0	139.0	40.0	6.45	215.0	23.9	19.3
78	AH151	108.5	108.0	216.5	15.0	20.5	186.5	37.0	7.5	287.5	26.4	25.2
79	AH1502	109.0	139.0	224.5	9.0	12.5	104.0	35.0	7.65	162.5	21.1	21.5
80	AH1506	109.5	103.0	231.5	12.0	19.5	184.0	37.5	7.15	237.5	26.5	24.3
81	AH1507	107.5	138.0	213.5	9.5	14.0	175.5	38.5	7.3	232.5	24.1	21.5
82	AH1508	104.0	144.0	205.5	12.0	16.5	211.0	38.5	8.15	252.5	15.9	18.2
83	AH1516	109.5	142.0	203.5	7.5	11.5	93.0	31.0	7.1	152.5	20.3	24.2
84	AH1519	108.0	139.0	202.5	10.0	12.0	91.0	33.0	6.25	162.5	22.9	17.2
85	AH1525	108.5	144.0	213.5	12.5	14.0	219.0	41.0	7.5	330.0	14.5	12.8
86	H005	104.5	119.0	211.5	21.0	26.0	299.0	36.0	7.55	545.0	28.8	22.8
87	H013	97.0	119.0	211.5	12.5	17.0	206.0	33.0	7.7	310.0	30.3	32.2
88	H0118	104.5	144.0	201.5	15.0	22.5	213.5	34.5	8.65	285.0	23.1	27.0
89	H0137	104.0	139.0	204.5	12.0	21.0	179.5	33.0	7.5	270.0	25.3	21.6
90	H0265	103.5	102.0	195.5	16.5	23.0	247.5	35.0	7.85	387.5	26.3	24.6
91	H0328	109.5	126.0	194.5	10.0	14.0	189.0	29.0	7.55	237.5	27.7	24.2
92	H0329	87.5	136.0	228.5	17.0	22.5	229.0	37.0	7.95	365.0	19.5	15.3
93	H0420	97.5	118.0	199.5	10.0	15.5	121.0	37.0	6.85	190.0	19.9	16.9
94	H057	97.0	111.0	182.5	8.5	11.0	197.0	37.5	8.5	285.0	27.1	23.6
95	H0512	104.5	110.0	198.5	23.5	31.5	342.5	41.0	7.6	520.0	29.9	21.6
96	H0535	97.0	119.0	194.5	23.5	28.0	322.0	40.5	7.55	495.0	24.7	32.7
97	H0571	109.5	120.0	186.5	12.5	19.0	223.0	41.5	7.15	262.5	23.8	24.6
98	H493	104.5	127.0	203.5	12.0	16.5	184.5	42.5	8.4	220.0	21.3	25.4
99	H6121	98.5	131.0	199.5	11.0	15.0	113.5	37.0	7.65	172.5	26.0	28.1
100	H9332	105.5	123.0	198.5	12.5	21.0	203.0	37.0	8.3	257.5	26.7	26.2
101	ICP8947	105.5	119.0	222.5	11.0	15.0	175.0	37.0	6.75	222.5	23.4	20.8
102	ICPL2037	110.5	122.0	208.5	15.0	22.0	204.5	35.0	8.1	320.0	22.0	19.5
103	ICP9202	104.5	133.0	205.5	12.0	19.0	188.0	39.0	8.25	295.0	13.6	21.7
104	ICP9900	117.5	131.0	221.5	13.0	22.5	204.5	34.5	8.65	250.0	14.4	14.0
105	ICP11250	97.0	131.0	192.5	18.0	21.0	208.5	40.0	8.65	287.5	19.6	24.5
106	ICPL9308	104.5	137.0	203.5	15.0	21.0	208.0	39.0	8.65	322.5	23.7	35.6
107	ICPL98015	97.5	111.0	198.5	18.5	27.0	261.5	39.0	8.45	477.5	19.4	24.9
108	ICPL2033	110.5	124.0	220.5	9.0	10.0	75.5	35.0	5.75	85.0	17.6	28.2
109	ICPL920	109.5	139.0	190.5	15.0	23.5	206.5	35.5	7.35	292.5	20.4	22.1
110	Paras	109.0	123.0	225.5	13.5	21.0	185.5	41.0	8.35	232.5	22.8	21.1
111	PantA37	108.5	105.0	235.5	12.0	17.5	184.5	39.0	8.05	212.5	21.0	23.7

S. No.	Treatment	DF	DM	PH	PB	SB	PD	SP	SW	YLD	Fe	Zn
112	PantA51	110.0	127.0	222.5	15.5	23.0	231.5	41.0	7.1	372.5	23.7	30.7
113	PantA163	109.0	126.0	217.5	10.5	20.0	195.0	40.0	7.15	220.0	22.6	23.4
114	PantA174	109.5	108.0	200.5	9.5	20.0	184.0	38.0	7.05	212.5	29.2	25.9
115	PantA234	110.5	131.0	211.5	10.0	18.0	170.0	37.5	8	190.0	25.6	22.4
116	PantA251	97.0	115.0	211.5	17.0	15.0	98.0	37.0	8.25	162.5	17.3	19.1
117	PantA252	111.5	110.0	223.5	10.5	23.0	188.0	40.0	8.55	220.0	31.1	24.3
118	PantA855	109.0	110.0	221.5	14.0	16.5	233.5	37.0	8.25	377.5	27.3	22.6
119	Pusa84	108.5	115.0	208.5	14.5	21.0	199.0	38.0	7.3	285.0	24.5	23.9
120	Pusa991	97.5	132.0	226.5	9.0	13.5	140.5	35.0	8.75	182.5	24.0	33.0
121	Pusa992	109.0	121.0	205.5	11.0	16.0	177.5	30.0	8.45	215.0	23.3	30.4
122	P994	109.5	113.5	222.5	15.0	19.0	217.0	33.0	6.35	285.0	27.3	25.6
123	P2121	97.0	114.5	227.5	9.0	13.0	104.0	39.0	6.6	162.5	27.0	26.4
124	P226	108.5	122.5	198.5	14.5	15.0	166.0	39.0	6.9	197.5	24.8	24.9
125	P20016	109.5	121.5	207.5	16.5	23.0	212.0	41.0	6.2	377.5	21.0	16.0
126	P2002	111.5	133.5	149.5	12.0	16.0	110.5	35.0	8.65	160.0	22.9	19.2
127	P2003	97.5	130.5	207.5	13.0	18.0	146.5	36.0	8.5	187.5	19.0	16.4
128	P2007	111.0	112.5	199.5	13.0	19.0	156.0	38.5	8.95	205.0	17.8	15.0
129	CORG105	97.0	122.5	203.5	8.5	11.0	93.0	40.0	7.9	142.5	21.6	21.4
130	CORG111	109.0	126.5	207.5	15.0	22.0	222.0	40.0	8.05	362.5	18.8	13.6
131	CORG9704	99.0	121.5	204.5	13.0	20.0	219.5	38.0	8.9	227.5	12.5	9.0
132	CORG6012	109.0	117.5	201.5	13.0	17.0	209.5	36.0	8.15	290.0	17.8	11.4
133	MTH103	109.5	120.5	215.5	10.0	15.0	96.0	34.5	9.35	162.5	16.9	15.6
134	MN1	97.0	145.5	197.5	7.5	11.5	81.5	41.0	8.1	120.0	17.3	10.5
135	MN5	109.5	140.5	211.5	17.0	23.0	239.0	37.0	8.7	452.5	11.6	16.0
136	CIPB5120	108.5	144.5	216.5	13.0	21.0	189.5	39.0	8.85	225.0	9.1	22.5
137	V10099	109.5	132.5	172.5	15.0	22.5	208.5	38.0	7.9	325.0	9.2	17.0
138	T21	109.0	147.5	201.5	15.0	21.0	209.5	38.0	6.3	320.0	17.3	18.9
139	TAT108	109.5	125.5	189.5	15.0	18.5	217.5	37.0	8.2	327.5	19.1	22.4
140	AP1371	108.5	126.5	195.5	11.0	17.0	185.0	38.0	9	217.5	21.4	27.5
141	AF370	108.0	135.5	230.5	8.5	11.0	99.5	34.0	8.1	160.0	23.5	21.8
142	Manak	108.5	126.5	178.5	11.0	15.0	106.5	38.0	7.1	165.0	23.1	23.3
143	AL1922	97.0	116.5	182.5	14.0	21.5	252.0	37.0	6.15	300.0	23.9	21.6
144	AL2087	97.0	134.5	184.5	11.0	15.0	184.0	37.5	8.35	222.5	22.6	22.5
145	AL2091	87.0	122.5	129.5	13.0	22.0	171.5	39.0	8.85	220.0	27.6	24.4
146	AL2025	75.5	112.5	186.5	11.0	15.0	125.5	31.5	8.2	172.5	31.1	19.7
147	AL2207	97.0	123.5	195.5	13.0	22.5	236.0	35.5	6.75	290.0	25.5	27.8
148	IC245350	111.0	112.5	173.5	17.0	21.0	227.0	39.0	8.3	265.0	23.4	25.2
149	AL1781	97.0	111.5	184.5	13.0	18.0	136.0	39.0	6.15	232.5	21.3	24.6

S. No.	Treatment	DF	DM	PH	PB	SB	PD	SP	SW	YLD	Fe	Zn
150	H948	111.0	121.5	216.5	21.0	27.0	250.5	37.0	7.25	420.0	24.8	24.2
151	H200014	110.5	113.5	196.5	15.0	20.5	204.0	38.0	7.1	257.5	28.1	25.5
152	AL1789	109.5	120.5	203.5	11.0	15.0	119.0	36.0	7.3	170.0	22.2	26.8
153	AH069	109.0	143.5	194.5	9.0	11.5	107.5	36.0	8.8	150.0	24.4	26.7
154	AH063	97.0	134.5	185.5	10.0	15.0	101.5	36.0	7.8	162.5	30.8	30.2
155	AL1525	82.0	118.5	188.5	13.0	18.0	174.5	36.0	8.25	222.5	26.4	21.6
156	AL1760	87.5	118.5	186.5	11.0	14.0	103.0	37.0	7.5	152.5	23.5	25.8
157	AH0930	97.5	145.5	200.5	11.5	14.0	106.5	36.0	9.45	157.5	23.4	27.6
158	PUSA2001	97.0	140.5	180.5	13.0	21.0	132.5	37.5	8.8	177.5	24.4	26.0
159	AH0941	109.5	122.5	191.5	17.0	23.0	199.5	36.0	7.95	325.0	22.4	22.9
160	AL1846	97.0	133.5	188.5	12.5	20.5	111.0	38.0	7.3	305.0	28.1	23.0
161	AL1782	92.5	125.5	210.5	17.0	24.0	213.0	34.5	8.5	327.5	24.5	27.2
162	AL2170	96.5	135.5	200.5	13.0	19.0	187.5	32.5	7.55	242.5	28.5	32.3
163	AL2133	98.5	138.5	201.5	13.0	16.0	183.0	32.0	7	215.0	24.1	32.3
164	VLA1	97.0	134.5	192.5	15.0	19.0	228.5	33.0	7.1	267.5	23.9	22.5
165	PAU881	92.5	139.0	218.5	17.0	23.0	171.0	36.0	7.05	315.0	28.9	22.5
166	PADT16	82.5	114.5	139.5	9.0	11.0	76.0	28.0	7.05	120.0	24.5	24.2
167	AL2132	97.5	124.5	195.5	10.0	13.0	102.5	34.5	8.95	160.0	24.8	26.1
168	AL2128	96.5	112.5	187.5	7.5	10.5	90.5	33.0	6.15	137.5	28.6	23.7
169	Sarita	88.5	118.5	183.5	17.0	19.0	125.0	34.5	7.7	252.5	22.6	21.4
170	AL2131	99.0	112.5	205.5	8.5	11.0	92.0	32.5	9	125.0	27.7	27.9
171	AL882	81.5	135.0	183.5	12.0	14.0	156.5	37.0	7.75	355.0	26.6	29.5
172	AL2062	108.5	147.5	208.5	15.0	17.0	218.0	36.0	7.4	230.0	23.8	29.2
173	PBR2	97.0	117.5	212.5	9.5	12.5	99.0	36.0	7.1	132.5	25.1	24.0
174	PANTA250	117.0	145.5	223.5	11.0	13.0	108.5	28.0	8.8	165.0	23.3	22.6
175	Pant37	109.0	110.5	198.5	9.0	11.0	94.0	36.0	9	137.5	22.8	26.5
176	AH092	97.0	109.5	217.5	10.5	12.5	93.0	37.0	8.35	150.0	22.2	24.7
177	Pant234	111.0	143.5	215.5	10.0	11.5	95.5	35.0	6.4	125.0	18.9	18.7
178	AL2204	79.0	130.5	99.5	9.0	11.0	69.5	35.0	7.15	117.5	23.4	20.9
	CD (5%)	4.02	2.94	1.02	3.58	4.51	29.92	3.58	0.48	39.15	7.90	6.66

ANNEXURE II

Adjusted means for diverse set of germplasm lines for the year 2019-20

S. No.	Treatment	DF	DM	PH	PB	SB	PD	SP	SW	YLD	Fe	Zn
1	AL292	105.0	133.5	243.5	14.0	26.5	109.5	38.0	8.4	165.0	28.5	33.0
2	AL303	84.0	104.5	211.0	13.0	19.5	120.0	35.0	8.3	200.0	26.5	26.0
3	AL304	105.0	122.5	230.5	18.5	24.5	120.5	31.5	8.7	209.0	21.5	25.0
4	AL307	99.0	116.5	208.0	17.5	21.5	132.0	31.0	9.1	215.0	24.5	26.5
5	AL311	84.0	111.5	215.5	14.5	20.0	121.5	32.5	9.0	167.5	34.0	35.0
6	AL986	99.0	132.5	215.0	16.0	24.0	230.0	35.0	8.8	397.5	31.5	32.0
7	AL997	111.0	122.5	239.0	20.5	27.5	232.5	32.0	8.3	465.0	29.5	31.5
8	AL1313	84.0	111.5	218.5	12.0	22.5	113.5	36.0	9.1	182.5	34.0	35.0
9	AL1396	84.0	111.5	217.5	13.0	18.5	131.5	37.5	8.1	217.5	35.0	33.0
10	AL1430	84.0	111.5	216.5	17.0	22.0	225.0	33.5	9.5	405.0	30.5	35.5
11	AL1449	97.0	119.5	215.5	14.5	22.5	138.5	37.0	8.9	265.0	32.5	43.0
12	AL1452	105.0	114.5	215.5	15.5	22.0	239.5	35.0	8.7	415.0	30.5	32.5
13	AL1455	84.0	111.5	214.5	12.5	14.0	110.0	34.5	8.3	242.5	29.5	24.0
14	AL1459	89.0	111.5	210.0	15.5	17.5	175.0	35.0	9.0	360.0	31.0	33.0
15	AL1465	99.0	111.5	216.5	10.0	16.0	104.0	37.5	6.8	140.5	32.0	35.5
16	AL1466	99.0	119.5	220.0	9.5	14.5	116.5	32.5	8.8	152.5	30.5	33.0
17	AL1471	84.0	114.5	214.0	9.0	14.0	126.0	36.0	8.5	235.0	33.5	29.5
18	AL1474	84.0	114.5	207.5	12.5	15.0	121.5	31.0	8.8	225.0	29.5	22.0
19	AL1476	84.0	125.5	189.0	14.0	24.0	129.5	33.5	8.2	270.0	33.5	21.0
20	AL1477	84.0	111.5	202.0	15.0	19.0	178.0	37.0	8.5	337.5	38.0	24.5
21	AL1486	84.0	119.5	209.5	9.5	13.5	108.0	35.0	8.3	215.0	33.5	25.0
22	AL1487	84.0	125.5	211.0	18.0	23.5	252.5	37.0	8.7	470.0	35.0	25.5
23	AL1490	84.0	127.5	206.5	14.5	16.0	117.5	32.0	9.7	140.0	35.5	29.5
24	AL1491	84.0	111.5	231.5	14.5	20.0	217.5	35.5	9.2	340.0	28.5	26.0
25	AL1508	111.0	122.5	261.0	13.0	21.0	130.0	37.0	8.1	240.0	29.5	31.0
26	AL1533	84.0	114.5	223.5	15.5	21.5	217.5	36.5	8.5	310.0	30.5	25.5
27	AL1538	84.0	103.5	207.0	15.5	22.5	175.0	29.0	7.7	270.0	29.0	19.0
28	AL1543	84.0	122.5	116.0	9.5	13.0	122.0	34.5	9.0	225.0	23.0	20.0
29	AL1554	105.0	125.5	199.5	9.0	11.5	121.5	31.5	9.7	215.0	26.0	32.5
30	AL1584	84.0	105.5	219.0	9.0	11.0	109.5	33.0	9.3	162.5	26.5	26.5
31	AL1592	111.0	125.5	206.5	12.5	16.5	140.5	38.0	7.1	255.0	19.5	22.0
32	AL1594	111.0	116.5	178.0	9.5	14.0	103.0	37.0	7.4	145.0	27.0	29.5

S. No.	Treatment	DF	DM	PH	PB	SB	PD	SP	SW	YLD	Fe	Zn
33	AL1627	105.0	114.5	217.5	16.0	20.0	218.0	37.5	7.5	372.5	26.5	26.5
34	AL1676	105.0	122.5	231.5	13.5	23.5	207.0	43.0	8.5	367.5	27.5	24.0
35	AL1721	105.0	114.5	206.0	13.0	22.5	264.0	37.0	8.3	467.5	35.0	28.5
36	AL1725	111.0	114.5	158.0	12.0	15.0	220.0	36.0	7.1	337.5	30.5	32.5
37	AL1730	111.0	114.5	218.0	12.0	18.0	175.5	44.0	8.3	322.5	33.5	29.5
38	AL1740	105.0	122.5	210.5	12.5	21.0	211.0	29.0	9.8	285.0	32.0	28.0
39	AL1747	99.0	111.5	189.0	9.0	16.0	141.5	35.5	8.7	237.5	23.5	26.0
40	AL1759	77.0	111.5	169.0	11.0	21.5	153.0	33.5	8.3	232.5	26.0	29.0
41	AL1790	76.5	111.5	193.5	10.5	21.0	105.5	35.0	9.0	172.5	40.0	31.0
42	AL1801	86.5	114.5	233.0	14.5	21.0	223.0	34.0	9.0	355.0	21.5	22.0
43	AL1813	108.5	122.5	229.5	11.0	20.5	128.0	37.0	6.8	360.0	22.0	27.5
44	AL1820	108.5	114.5	209.0	10.5	16.5	125.5	29.0	6.3	195.0	22.0	22.0
45	AL1823	96.5	111.5	224.0	10.0	14.0	124.5	36.5	8.2	227.5	23.5	26.0
46	AL1835	86.5	122.5	207.5	10.5	16.5	115.0	33.5	8.1	215.0	24.5	25.0
47	AL1847	75.5	114.5	235.0	9.0	13.0	107.5	38.0	8.2	167.5	23.5	27.5
48	AL1848	103.5	132.5	222.0	12.0	16.0	137.0	34.0	8.6	240.0	23.5	22.0
49	AL1849	74.5	127.5	239.0	14.0	18.5	145.0	37.0	8.4	250.0	27.0	29.0
50	AL1853	103.5	114.5	244.5	12.0	13.5	114.0	33.5	8.6	215.0	27.5	25.5
51	AL1856	74.5	129.5	220.5	14.5	23.5	232.5	36.5	7.6	355.0	21.5	24.0
52	IC3977	103.5	105.5	190.0	20.0	22.0	265.5	34.5	8.7	420.0	25.0	25.5
53	IC245139	103.5	133.5	231.5	16.0	16.0	187.5	39.0	8.6	232.5	25.0	25.5
54	IC245176	96.5	133.5	212.5	14.0	24.5	250.5	36.5	8.3	410.0	29.5	35.0
55	IC245183	108.5	106.5	227.5	13.0	20.0	209.0	34.5	8.4	252.5	25.5	30.5
56	IC245186	108.5	132.5	234.0	15.0	20.5	164.5	35.5	8.8	262.5	29.5	29.5
57	IC245219	108.5	119.5	218.0	10.0	13.5	110.5	34.5	8.1	205.0	29.0	30.0
58	IC245245	108.5	119.5	227.5	9.0	10.0	102.5	35.0	8.1	142.5	21.0	26.5
59	IC245268	103.5	132.5	223.5	10.0	13.0	165.0	35.0	7.2	217.5	24.5	32.5
60	IC245273	108.5	122.5	237.5	11.0	18.0	191.5	36.0	8.8	260.0	26.5	27.0
61	IC245294	96.5	106.5	211.0	10.0	14.0	113.5	37.0	8.6	162.5	19.0	24.0
62	IC245314	103.5	114.5	227.0	13.5	20.5	203.5	36.0	8.4	327.5	15.0	17.0
63	IC245315	96.5	122.5	216.5	16.5	21.0	211.5	35.0	7.5	442.5	19.0	20.5
64	IC245497	81.5	127.5	235.5	14.5	21.5	207.0	37.0	9.3	315.0	20.5	18.0
65	IC245507	103.5	106.5	215.5	12.0	16.0	212.5	34.5	8.1	330.0	18.0	16.5
66	IC245523	108.5	120.0	206.5	12.0	18.0	215.0	36.5	8.7	262.5	17.0	21.0

S. No.	Treatment	DF	DM	PH	PB	SB	PD	SP	SW	YLD	Fe	Zn
67	IC245524	108.5	112.0	223.0	15.0	19.5	213.5	33.5	9.2	330.0	23.5	22.0
68	IC245525	74.5	115.0	209.5	9.5	12.5	153.0	34.0	8.8	190.0	20.5	28.0
69	IC245533	103.5	112.0	210.5	16.0	21.0	235.0	32.0	8.1	370.0	19.0	26.0
70	IC245560	108.5	107.0	227.0	10.5	13.5	111.5	35.0	7.7	160.0	30.5	28.5
71	AH061	108.5	123.0	236.0	15.5	22.0	217.0	35.0	8.1	345.0	23.5	25.0
72	AH067	103.5	115.0	219.5	11.0	19.0	206.5	32.0	6.3	295.0	25.0	22.0
73	AH0612	96.5	124.0	196.0	17.5	23.0	239.5	33.0	8.4	400.0	29.5	24.0
74	AH091	81.5	112.0	193.5	15.0	21.0	234.0	33.0	9.1	397.5	24.0	14.0
75	AH093	96.5	128.0	215.5	16.0	22.5	235.0	35.0	9.3	387.5	22.5	15.5
76	AH0911	81.5	133.0	224.5	14.0	17.0	192.5	37.5	7.7	245.0	23.5	18.0
77	AH1501	108.5	130.0	206.5	11.0	18.5	189.0	33.0	6.6	257.5	21.0	21.0
78	AH151	108.5	112.0	211.0	15.0	20.0	183.5	35.0	8.1	295.0	25.5	26.5
79	AH1502	81.5	128.0	219.0	10.0	14.5	105.5	36.0	7.5	172.5	25.0	28.0
80	AH1506	108.5	107.0	228.0	13.0	20.0	188.0	32.5	7.5	232.5	25.5	27.0
81	AH1507	81.5	128.0	208.0	10.5	16.0	182.0	27.5	8.0	232.5	26.5	23.0
82	AH1508	81.5	128.0	210.5	12.0	18.5	220.0	35.0	8.7	260.0	18.5	17.0
83	AH1516	81.5	112.0	199.5	10.0	13.0	98.0	36.0	7.4	165.0	19.5	22.5
84	AH1519	81.5	126.0	207.0	11.0	16.0	105.0	34.0	6.9	177.5	20.5	23.0
85	AH1525	74.5	123.0	209.5	12.5	12.5	214.0	37.0	8.4	332.5	25.5	18.5
86	H005	81.5	107.0	207.5	15.0	21.0	274.0	38.0	7.4	500.0	28.5	28.0
87	H013	96.5	123.0	208.0	16.0	22.5	220.0	38.0	9.2	342.5	34.0	32.0
88	H0118	74.5	128.0	207.5	13.5	19.0	208.5	28.0	8.7	277.5	25.0	28.5
89	H0137	81.5	123.0	208.0	9.0	20.0	156.0	37.0	8.0	247.5	29.0	25.5
90	H0265	103.5	107.0	189.5	14.0	18.5	216.0	33.0	7.0	330.0	26.0	27.5
91	H0328	108.5	128.0	188.5	11.0	16.0	186.0	36.0	8.3	225.0	28.5	25.0
92	H0329	81.5	133.0	223.0	13.5	20.5	216.5	37.0	9.6	340.0	24.5	20.5
93	H0420	96.5	120.0	204.5	9.0	12.5	103.0	33.0	6.5	150.0	22.5	18.5
94	H057	96.5	112.0	180.5	15.0	17.0	222.0	37.0	8.8	325.0	29.0	25.0
95	H0512	103.5	112.0	193.0	21.0	29.5	315.5	37.0	8.4	507.5	31.5	22.5
96	H0535	96.5	120.0	189.0	24.5	28.0	332.5	34.5	8.2	497.5	26.0	31.0
97	H0571	108.5	123.0	190.0	11.0	15.0	212.5	34.5	8.6	232.5	26.5	26.0
98	H493	74.5	120.0	208.5	11.0	14.5	176.5	39.0	8.1	215.0	22.5	33.0
99	H6121	96.5	133.0	194.0	9.5	13.0	89.0	38.0	6.3	147.5	21.5	27.5
100	H9332	103.5	128.0	193.0	13.5	22.5	213.0	33.0	8.4	257.5	27.0	26.5

S. No.	Treatment	DF	DM	PH	PB	SB	PD	SP	SW	YLD	Fe	Zn
101	ICP8947	103.5	123.0	218.5	13.0	15.0	184.0	38.0	7.1	237.5	20.0	25.0
102	ICPL2037	108.5	123.0	203.5	11.0	19.0	201.0	33.0	9.1	297.5	22.5	23.0
103	ICP9202	103.5	133.0	210.5	15.5	22.5	207.0	37.0	9.0	337.5	15.5	20.5
104	ICP9900	116.5	133.0	216.5	12.0	20.5	162.5	38.0	9.0	190.0	15.5	17.0
105	ICP11250	96.5	133.0	189.5	15.0	17.0	187.5	35.5	8.7	275.0	23.0	28.0
106	ICPL9308	81.5	120.0	208.0	14.0	20.0	286.5	33.0	9.4	287.5	26.5	37.0
107	ICPL9801	96.5	115.0	192.5	16.5	24.5	215.0	37.0	9.0	422.5	18.5	27.5
108	ICPL2033	74.5	107.0	216.5	8.0	11.0	82.0	40.0	6.4	87.5	15.5	31.5
109	ICPL920	81.5	130.0	186.5	11.5	22.5	199.5	33.0	7.9	282.5	24.5	25.5
110	Paras	108.5	128.0	220.5	15.5	19.5	184.5	41.0	8.9	232.5	29.0	21.0
111	PantA37	108.5	107.0	232.5	13.0	19.0	198.0	35.0	8.0	235.0	24.0	24.5
112	PantA51	108.5	128.0	218.5	13.0	21.0	207.5	39.0	8.1	352.5	26.5	35.0
113	PantA163	108.5	128.0	212.0	11.5	20.0	208.0	41.0	8.8	242.5	26.5	31.0
114	PantA174	108.5	112.0	206.0	10.5	21.0	191.5	38.0	7.5	227.5	31.5	29.0
115	PantA234	85.0	112.0	208.5	12.5	20.5	200.0	31.0	8.2	232.5	20.5	26.0
116	PantA251	100.0	120.0	206.0	18.5	21.5	105.5	36.0	9.0	195.0	24.5	22.5
117	PantA252	112.0	115.0	220.0	9.5	21.5	178.5	35.0	8.2	205.0	23.5	24.0
118	PantA855	112.0	112.0	217.5	12.0	15.5	205.5	37.0	8.3	335.0	29.0	30.0
119	Pusa84	112.0	120.0	204.0	12.5	19.0	184.0	35.0	7.5	277.5	26.5	32.0
120	Pusa991	100.0	133.0	223.0	8.5	10.0	121.5	34.5	8.2	152.5	23.5	33.0
121	Pusa992	112.0	123.0	209.5	13.0	17.0	189.5	37.0	8.5	247.5	29.5	26.0
122	P994	112.0	112.0	217.0	14.0	19.0	158.5	35.0	7.1	265.0	29.5	29.5
123	P2121	100.0	112.0	223.5	11.5	14.5	105.5	37.0	7.3	170.0	30.0	29.0
124	P226	112.0	123.0	193.0	11.0	17.0	177.5	35.0	7.0	217.5	27.5	21.5
125	P20016	112.0	123.0	212.0	15.0	21.0	203.5	37.0	7.1	365.0	25.5	18.5
126	P2002	114.0	133.0	155.5	13.0	18.0	122.5	35.0	9.5	172.5	22.0	21.0
127	P2003	100.0	133.0	213.5	12.0	15.5	132.0	37.0	8.4	170.0	22.5	18.5
128	P2007	112.0	115.0	194.0	11.0	15.0	173.0	33.0	9.2	189.5	21.5	17.5
129	CORG105	100.0	123.0	208.5	9.0	10.0	80.5	31.0	8.3	132.5	26.0	24.5
130	CORG111	112.0	128.0	211.5	14.0	11.0	197.0	36.0	8.5	302.5	23.5	19.0
131	CORG9704	100.0	123.0	200.5	15.0	20.0	239.0	38.0	9.5	242.5	15.0	10.0
132	CORG6012	112.0	120.0	207.5	13.0	17.0	204.0	35.0	8.4	205.0	19.5	9.0
133	MTH103	112.0	123.0	210.0	11.0	16.0	103.0	35.0	9.7	167.5	20.0	18.0
134	MN1	78.0	133.0	192.0	8.0	12.5	90.0	38.0	8.5	132.5	16.0	9.5

S. No.	Treatment	DF	DM	PH	PB	SB	PD	SP	SW	YLD	Fe	Zn
135	MN5	78.0	120.0	206.5	21.0	27.5	256.5	39.0	9.9	482.5	11.0	18.0
136	CIPB5120	78.0	120.0	211.0	11.0	22.0	187.5	35.0	8.7	232.5	10.5	22.5
137	V10099	112.0	133.0	177.5	13.0	21.0	189.0	35.0	8.3	312.5	11.0	20.0
138	T21	85.0	136.0	200.0	13.0	20.0	201.0	35.0	8.5	300.0	23.0	21.5
139	TAT108	112.0	126.0	185.5	13.0	15.0	213.0	40.0	8.4	322.5	24.5	25.5
140	AP1371	112.0	128.0	190.0	10.0	15.5	178.5	36.0	8.6	207.5	22.5	28.0
141	AF370	112.0	136.0	225.5	11.0	14.5	108.0	39.0	8.2	167.5	27.5	25.0
142	Manak	112.0	128.0	183.0	9.5	12.0	86.0	34.5	7.6	137.5	26.5	22.0
143	AL1922	100.0	120.0	187.0	11.0	18.0	224.5	39.0	7.2	267.5	23.5	18.5
144	AL2087	78.0	123.0	181.5	12.0	13.0	155.5	37.0	8.2	197.5	24.0	27.0
145	AL2091	86.0	120.0	136.0	9.0	19.0	153.5	36.0	8.4	200.0	29.5	29.0
146	AL2025	78.0	115.0	190.5	10.0	13.5	105.0	35.0	8.2	155.0	30.5	25.5
147	AL2207	78.0	107.0	190.0	15.0	19.0	209.0	40.5	8.5	262.5	26.5	27.0
148	IC245350	112.0	115.0	178.0	12.5	17.0	212.5	36.0	8.6	235.0	26.5	27.0
149	AL1781	100.0	112.0	185.0	7.0	11.5	55.0	33.5	6.6	82.5	24.5	28.5
150	H948	112.0	123.0	211.5	17.0	23.0	239.0	37.0	7.5	420.0	28.5	30.0
151	H200014	112.0	115.0	191.0	11.0	17.0	191.0	40.0	8.8	237.5	26.5	21.0
152	AL1789	112.0	123.0	208.5	12.0	13.5	106.5	30.0	8.7	160.0	27.0	31.0
153	AH069	85.0	123.0	188.5	10.0	13.0	111.5	35.0	8.7	162.5	22.0	26.0
154	AH063	85.0	120.0	190.5	10.0	15.0	102.0	33.0	7.6	162.5	27.5	33.5
155	AL1525	85.0	120.0	185.0	11.0	17.0	166.5	37.0	8.4	222.5	29.5	23.0
156	AL1760	86.0	115.0	191.5	10.5	17.0	102.0	35.0	7.5	160.0	25.0	26.0
157	AH0930	78.0	128.0	197.5	10.0	12.0	101.0	43.0	9.9	150.0	24.5	29.5
158	PUSA2001	85.0	120.0	186.5	14.0	19.0	123.0	31.5	8.8	167.5	28.5	24.0
159	AH0941	112.0	123.0	187.5	15.0	21.0	195.0	34.0	8.2	325.0	25.0	23.5
160	AL1846	100.0	136.0	184.5	17.0	22.5	218.5	34.5	8.3	337.5	30.0	19.5
161	AL1782	85.0	120.0	216.0	13.0	22.5	214.0	35.0	8.3	332.5	27.0	27.0
162	AL2170	78.0	120.0	206.5	12.5	18.0	174.5	35.0	9.5	227.5	30.5	34.0
163	AL2133	78.0	123.0	208.0	11.0	13.0	165.0	30.0	8.4	140.0	28.5	30.5
164	VLA1	85.0	127.0	188.5	13.0	15.0	199.5	38.0	9.7	235.0	25.5	26.0
165	PAU881	94.5	139.5	224.5	17.0	20.0	177.0	40.0	8.2	315.0	31.0	24.5
166	PADT16	85.0	116.0	135.5	9.5	12.5	78.5	33.0	7.1	135.0	29.0	25.0
167	AL2132	100.0	125.0	189.5	9.0	12.0	94.5	35.0	9.7	150.0	25.5	28.5
168	AL2128	100.0	114.0	192.0	12.0	17.0	101.5	35.0	6.5	172.5	30.0	28.5

S. No.	Treatment	DF	DM	PH	PB	SB	PD	SP	SW	YLD	Fe	Zn
169	Sarita	86.0	114.0	192.0	13.0	17.0	110.0	36.0	7.2	195.0	27.5	27.0
170	AL2131	100.0	111.0	211.0	11.0	13.0	92.5	29.0	9.2	137.5	23.5	24.0
171	AL882	83.0	133.0	180.0	11.5	15.0	165.5	32.0	8.9	365.0	29.0	28.0
172	AL2062	78.0	119.0	213.0	13.0	16.0	168.5	37.0	9.1	187.5	22.5	30.0
173	PBR2	100.0	119.0	207.5	9.5	12.5	92.0	33.0	10.0	135.0	28.0	24.5
174	PANTA250	116.0	132.0	217.5	11.5	14.0	120.5	35.0	9.4	165.0	28.5	26.5
175	Pant37	112.0	111.0	193.5	9.5	12.0	88.5	36.0	8.8	127.5	19.0	27.5
176	AH092	100.0	111.0	212.0	13.0	15.0	103.0	35.0	8.1	162.5	18.5	27.0
177	Pant234	78.0	111.0	210.0	11.0	13.0	109.5	38.0	7.0	160.0	17.5	19.0
178	AL2204	78.0	106.0	105.5	10.0	12.0	71.5	34.5	8.5	140.0	27.5	19.5
	CD (5%)	4.41	1.04	23.50	3.80	5.54	25.43	2.14	0.60	43.30	2.69	1.95

ANNEXURE III

Adjusted means for diverse set of germplasm lines for the pooled data conditions of both the years

S. No.	Treatment	DF	DM	PH	PB	SB	PD	SP	SW	YLD	Fe	Zn
1	AL292	104.3	133.5	246.0	11.5	20.8	111.5	35.8	8.3	153.0	29.8	32.3
2	AL303	83.3	103.0	215.3	13.0	18.5	126.8	35.0	8.1	207.5	25.3	25.7
3	AL304	104.0	123.0	227.5	18.0	21.3	123.0	24.8	8.3	214.5	21.9	24.5
4	AL307	98.3	115.0	205.8	16.5	22.3	135.5	32.8	8.6	207.5	23.8	24.3
5	AL311	90.5	115.5	217.5	15.3	18.8	122.3	33.3	8.1	172.5	31.8	31.4
6	AL986	98.8	132.0	217.3	15.8	23.5	229.8	33.8	8.1	396.3	33.7	34.3
7	AL997	110.0	123.5	240.8	21.8	28.8	243.8	33.5	8.4	478.8	29.4	31.6
8	AL1313	93.5	118.0	221.5	12.5	18.8	116.0	33.0	8.5	185.0	33.4	35.9
9	AL1396	93.3	116.5	219.5	14.0	20.8	130.3	37.3	7.7	216.3	36.0	33.1
10	AL1430	93.8	117.0	219.0	17.3	24.3	221.8	34.3	9.5	410.0	29.9	34.6
11	AL1449	97.0	120.0	213.5	15.5	21.5	141.5	37.3	8.7	271.3	35.1	47.2
12	AL1452	104.0	114.5	212.5	16.3	23.3	254.8	37.0	8.6	420.0	28.9	31.1
13	AL1455	93.3	114.5	217.0	12.3	14.8	115.0	35.8	7.9	257.5	29.8	22.4
14	AL1459	88.0	111.0	207.8	13.5	18.8	170.5	35.3	8.9	353.8	32.3	30.7
15	AL1465	98.5	110.5	218.5	9.5	14.8	103.5	36.0	7.1	139.0	31.5	32.3
16	AL1466	98.0	120.5	222.8	10.3	16.0	118.3	33.5	8.7	157.5	30.2	30.7
17	AL1471	90.8	120.5	216.8	10.5	15.8	125.5	36.5	8.5	251.3	30.5	26.5
18	AL1474	91.0	121.0	205.0	12.3	15.0	119.0	33.0	8.7	217.5	27.9	21.0
19	AL1476	93.8	127.3	190.8	14.0	22.3	128.8	35.3	8.2	280.0	33.5	19.7
20	AL1477	87.8	112.8	199.3	16.0	20.8	189.0	37.8	8.3	355.0	39.1	23.3
21	AL1486	85.8	120.3	206.5	10.0	14.3	115.3	35.8	8.5	223.8	34.3	22.4
22	AL1487	90.3	126.3	207.8	19.0	25.5	257.8	38.5	8.7	470.0	34.0	24.1
23	AL1490	90.8	130.8	208.5	12.8	16.5	119.8	35.0	9.0	163.8	37.1	26.9
24	AL1491	90.5	114.8	234.0	14.3	20.0	217.3	36.3	9.3	341.3	27.6	24.6
25	AL1508	110.0	120.8	263.8	12.8	20.5	127.5	34.0	8.4	236.3	32.4	30.9
26	AL1533	90.5	116.8	226.0	14.8	21.5	216.3	34.5	8.4	307.5	30.8	26.1
27	AL1538	97.3	111.3	204.8	14.8	21.8	168.8	32.3	7.8	266.3	27.1	18.2
28	AL1543	82.8	125.3	163.3	9.8	15.0	123.0	36.3	8.7	228.3	24.4	21.5
29	AL1554	104.0	123.3	196.5	9.5	12.5	121.0	33.3	9.0	221.3	25.6	30.1
30	AL1584	90.5	109.8	221.8	9.3	11.8	111.8	34.8	9.0	167.5	26.2	24.8
31	AL1592	110.8	123.8	208.0	12.8	17.5	144.5	37.5	6.9	260.0	18.3	20.2

S. No.	Treatment	DF	DM	PH	PB	SB	PD	SP	SW	YLD	Fe	Zn
32	AL1594	111.0	114.8	174.8	9.0	13.3	96.5	37.8	7.4	137.5	23.4	29.8
33	AL1627	104.0	112.8	220.5	16.8	22.3	230.5	35.8	7.5	393.8	23.9	22.6
34	AL1676	104.3	121.8	234.5	13.5	22.5	218.8	41.3	8.5	383.8	27.3	25.1
35	AL1721	104.8	113.3	203.3	15.0	23.0	269.8	37.8	8.5	466.3	38.6	29.4
36	AL1725	110.0	112.3	154.8	13.5	17.5	224.0	35.8	7.0	350.0	29.4	32.6
37	AL1730	110.3	113.8	220.8	13.5	19.3	181.0	42.0	7.5	342.5	32.3	28.5
38	AL1740	104.0	120.8	207.0	11.8	21.0	209.5	33.0	9.7	280.0	29.4	26.9
39	AL1747	98.8	110.8	186.8	8.5	15.5	139.8	36.0	8.6	235.0	24.6	24.5
40	AL1759	87.3	116.8	165.8	10.5	21.5	144.5	35.0	8.4	221.3	24.3	28.3
41	AL1790	82.0	114.8	191.0	11.3	21.0	110.3	35.0	8.1	178.8	28.6	30.5
42	AL1801	86.8	113.8	235.8	15.8	23.8	234.8	35.8	8.7	370.0	22.3	24.8
43	AL1813	109.0	122.8	230.0	12.8	21.0	131.8	37.0	6.8	367.5	20.3	24.8
44	AL1820	108.5	113.8	211.8	10.0	16.0	124.0	34.8	6.6	192.5	23.0	23.4
45	AL1823	97.0	112.3	226.8	10.5	14.8	125.3	37.3	8.4	226.3	24.3	24.1
46	AL1835	86.8	121.8	204.5	10.0	15.8	111.3	35.0	8.1	210.0	22.5	22.4
47	AL1847	86.5	120.3	236.8	9.0	12.5	102.0	35.0	8.2	155.0	24.0	26.4
48	AL1848	104.0	131.8	223.8	11.5	16.8	141.3	36.0	8.1	246.3	21.2	22.4
49	AL1849	86.0	131.8	241.8	14.5	19.5	153.3	35.8	8.4	256.3	26.0	27.9
50	AL1853	103.8	113.8	247.0	11.0	14.5	116.5	33.8	8.5	222.5	26.5	24.3
51	AL1856	89.5	134.3	223.0	16.8	24.8	245.0	33.8	7.7	375.0	22.2	22.5
52	IC3977	104.5	104.8	192.8	20.5	23.5	272.8	35.5	8.8	427.5	25.3	24.2
53	IC245139	104.0	133.8	233.5	14.0	14.8	174.5	37.0	8.1	223.8	24.7	23.2
54	IC245176	97.0	133.8	214.5	15.5	25.5	256.8	37.3	8.3	417.5	29.9	36.7
55	IC245183	109.3	105.3	229.5	12.5	18.3	204.8	36.3	8.1	241.3	24.2	28.6
56	IC245186	109.0	133.3	235.8	14.0	18.3	151.3	34.5	8.6	243.8	28.7	29.5
57	IC245219	108.8	118.8	221.3	11.5	14.3	113.3	36.8	8.1	213.8	27.2	29.0
58	IC245245	108.3	118.8	229.5	9.5	11.8	106.0	35.0	8.2	157.5	22.7	27.7
59	IC245268	104.0	131.8	225.5	10.5	13.8	176.3	36.0	7.2	218.8	26.7	32.3
60	IC245273	109.5	122.8	239.5	10.5	16.5	185.8	36.5	8.7	251.3	23.3	25.2
61	IC245294	96.3	106.3	213.8	9.5	13.3	109.5	36.0	8.3	157.5	18.6	22.0
62	IC245314	103.8	114.3	228.8	14.3	21.5	208.5	35.0	8.5	335.0	15.8	16.5
63	IC245315	96.8	123.3	219.0	18.8	24.3	226.8	35.8	7.6	457.5	19.2	19.3
64	IC245497	89.0	133.3	238.0	15.3	21.5	212.8	35.0	9.2	322.5	20.3	18.6
65	IC245507	103.5	106.8	217.0	12.8	16.5	215.8	35.3	8.1	336.3	17.2	16.4

S. No.	Treatment	DF	DM	PH	PB	SB	PD	SP	SW	YLD	Fe	Zn
66	IC245523	108.8	119.5	208.5	10.5	16.0	207.3	37.3	8.3	241.3	17.8	20.6
67	IC245524	108.3	113.0	225.8	15.5	20.8	220.8	35.8	8.7	345.0	23.4	21.3
68	IC245525	89.5	124.5	207.5	10.8	14.5	166.0	33.5	8.7	210.0	21.3	27.2
69	IC245533	103.8	111.5	209.5	16.5	22.5	238.8	33.0	8.3	377.5	20.3	26.3
70	IC245560	109.5	107.5	229.8	11.3	14.8	115.5	35.0	8.0	166.3	29.7	26.8
71	AH061	109.0	123.5	237.8	14.0	22.0	213.0	35.5	8.3	337.5	24.2	23.7
72	AH067	103.8	115.5	222.5	12.8	20.0	214.8	35.3	6.2	307.5	26.5	20.8
73	AH0612	96.8	123.0	198.8	18.3	22.5	242.8	35.0	8.4	407.5	29.3	22.9
74	AH091	89.5	118.0	195.5	15.0	21.0	236.0	33.5	8.8	398.8	21.2	14.0
75	AH093	96.5	127.5	217.5	16.8	23.3	240.0	36.0	9.0	393.8	22.7	15.8
76	AH0911	89.3	136.0	227.0	15.5	19.0	202.8	38.8	7.7	285.0	25.3	18.1
77	AH1501	109.0	127.5	208.5	10.5	16.3	164.0	36.5	6.5	236.3	22.5	20.2
78	AH151	108.5	110.0	213.8	15.0	20.3	185.0	36.0	7.8	291.3	26.0	25.8
79	AH1502	95.3	133.5	221.8	9.5	13.5	104.8	35.5	7.6	167.5	23.0	24.7
80	AH1506	109.0	105.0	229.8	12.5	19.8	186.0	35.0	7.3	235.0	26.0	25.7
81	AH1507	94.5	133.0	210.8	10.0	15.0	178.8	33.0	7.7	232.5	25.3	22.2
82	AH1508	92.8	136.0	208.0	12.0	17.5	215.5	36.8	8.4	256.3	17.2	17.6
83	AH1516	95.5	127.0	201.5	8.8	12.3	95.5	33.5	7.2	158.8	19.9	23.4
84	AH1519	94.8	132.5	204.8	10.5	14.0	98.0	33.5	6.6	170.0	21.7	20.1
85	AH1525	91.5	133.5	211.5	12.5	13.3	216.5	39.0	8.0	331.3	20.0	15.7
86	H005	93.0	113.0	209.5	18.0	23.5	286.5	37.0	7.5	522.5	28.6	25.4
87	H013	96.8	121.0	209.8	14.3	19.8	213.0	35.5	8.5	326.3	32.2	32.1
88	H0118	89.5	136.0	204.5	14.3	20.8	211.0	31.3	8.7	281.3	24.0	27.8
89	H0137	92.8	131.0	206.3	10.5	20.5	167.8	35.0	7.7	258.8	27.1	23.5
90	H0265	103.5	104.5	192.5	15.3	20.8	231.8	34.0	7.4	358.8	26.1	26.1
91	H0328	109.0	127.0	191.5	10.5	15.0	187.5	32.5	7.9	231.3	28.1	24.6
92	H0329	84.5	134.5	225.8	15.3	21.5	222.8	37.0	8.8	352.5	22.0	17.9
93	H0420	97.0	119.0	202.0	9.5	14.0	112.0	35.0	6.7	170.0	21.2	17.7
94	H057	96.8	111.5	181.5	11.8	14.0	209.5	37.3	8.7	305.0	28.0	24.3
95	H0512	104.0	111.0	195.8	22.3	30.5	329.0	39.0	8.0	513.8	30.7	22.0
96	H0535	96.8	119.5	191.8	24.0	28.0	327.3	37.5	7.9	496.3	25.4	31.8
97	H0571	109.0	121.5	188.3	11.8	17.0	217.8	38.0	7.9	247.5	25.1	25.3
98	H493	89.5	123.5	206.0	11.5	15.5	180.5	40.8	8.3	217.5	21.9	29.2
99	H6121	97.5	132.0	196.8	10.3	14.0	101.3	37.5	7.0	160.0	23.8	27.8

S. No.	Treatment	DF	DM	PH	PB	SB	PD	SP	SW	YLD	Fe	Zn
100	H9332	104.5	125.5	195.8	13.0	21.8	208.0	35.0	8.4	257.5	26.8	26.4
101	ICP8947	104.5	121.0	220.5	12.0	15.0	179.5	37.5	6.9	230.0	21.7	22.9
102	ICPL2037	109.5	122.5	206.0	13.0	20.5	202.8	34.0	8.6	308.8	22.3	21.3
103	ICP9202	104.0	133.0	208.0	13.8	20.8	197.5	38.0	8.6	316.3	14.5	21.1
104	ICP9900	117.0	132.0	219.0	12.5	21.5	183.5	36.3	8.8	220.0	14.9	15.5
105	ICP11250	96.8	132.0	191.0	16.5	19.0	198.0	37.8	8.7	281.3	21.3	26.3
106	ICPL9308	93.0	128.5	205.8	14.5	20.5	247.3	36.0	9.0	305.0	25.1	36.3
107	ICPL9801	97.0	113.0	195.5	17.5	25.8	238.3	38.0	8.7	450.0	18.9	26.2
108	ICPL2033	92.5	115.5	218.5	8.5	10.5	78.8	37.5	6.1	86.3	16.6	29.9
109	ICPL920	95.5	134.5	188.5	13.3	23.0	203.0	34.3	7.6	287.5	22.5	23.8
110	Paras	108.8	125.5	223.0	14.5	20.3	185.0	41.0	8.6	232.5	25.9	21.1
111	PantA37	108.5	106.0	234.0	12.5	18.3	191.3	37.0	8.0	223.8	22.5	24.1
112	PantA51	109.3	127.5	220.5	14.3	22.0	219.5	40.0	7.6	362.5	25.1	32.8
113	PantA163	108.8	127.0	214.8	11.0	20.0	201.5	40.5	8.0	231.3	24.5	27.2
114	PantA174	109.0	110.0	203.3	10.0	20.5	187.8	38.0	7.3	220.0	30.4	27.5
115	PantA234	97.8	121.5	210.0	11.3	19.3	185.0	34.3	8.1	211.3	23.1	24.2
116	PantA251	98.5	117.5	208.8	17.8	18.3	101.8	36.5	8.6	178.8	20.9	20.8
117	PantA252	111.8	112.5	221.8	10.0	22.3	183.3	37.5	8.4	212.5	27.3	24.2
118	PantA855	110.5	111.0	219.5	13.0	16.0	219.5	37.0	8.3	356.3	28.2	26.3
119	Pusa84	110.3	117.5	206.3	13.5	20.0	191.5	36.5	7.4	281.3	25.5	27.9
120	Pusa991	98.8	132.5	224.8	8.8	11.8	131.0	34.8	8.5	167.5	23.8	33.0
121	Pusa992	110.5	122.0	207.5	12.0	16.5	183.5	33.5	8.5	231.3	26.4	28.2
122	P994	110.8	112.8	219.8	14.5	19.0	187.8	34.0	6.7	275.0	28.4	27.6
123	P2121	98.5	113.3	225.5	10.3	13.8	104.8	38.0	7.0	166.3	28.5	27.7
124	P226	110.3	122.8	195.8	12.8	16.0	171.8	37.0	6.9	207.5	26.2	23.2
125	P20016	110.8	122.3	209.8	15.8	22.0	207.8	39.0	6.6	371.3	23.3	17.2
126	P2002	112.8	133.3	152.5	12.5	17.0	116.5	35.0	9.1	166.3	22.5	20.1
127	P2003	98.8	131.8	210.5	12.5	16.8	139.3	36.5	8.5	178.8	20.7	17.5
128	P2007	111.5	113.8	196.8	12.0	17.0	164.5	35.8	9.1	197.3	19.7	16.2
129	CORG105	98.5	122.8	206.0	8.8	10.5	86.8	35.5	8.1	137.5	23.8	23.0
130	CORG111	110.5	127.3	209.5	14.5	16.5	209.5	38.0	8.3	332.5	21.2	16.3
131	CORG9704	99.5	122.3	202.5	14.0	20.0	229.3	38.0	9.2	235.0	13.8	9.5
132	CORG6012	110.5	118.8	204.5	13.0	17.0	206.8	35.5	8.3	247.5	18.6	10.2
133	MTH103	110.8	121.8	212.8	10.5	15.5	99.5	34.8	9.5	165.0	18.4	16.8

S. No.	Treatment	DF	DM	PH	PB	SB	PD	SP	SW	YLD	Fe	Zn
134	MN1	87.5	139.3	194.8	7.8	12.0	85.8	39.5	8.3	126.3	16.6	10.0
135	MN5	93.8	130.3	209.0	19.0	25.3	247.8	38.0	9.3	467.5	11.3	17.0
136	CIPB5120	93.3	132.3	213.8	12.0	21.5	188.5	37.0	8.8	228.8	9.8	22.5
137	V10099	110.8	132.8	175.0	14.0	21.8	198.8	36.5	8.1	318.8	10.1	18.5
138	T21	97.0	141.8	200.8	14.0	20.5	205.3	36.5	7.4	310.0	20.1	20.2
139	TAT108	110.8	125.8	187.5	14.0	16.8	215.3	38.5	8.3	325.0	21.8	23.9
140	AP1371	110.3	127.3	192.8	10.5	16.3	181.8	37.0	8.8	212.5	22.0	27.8
141	AF370	110.0	135.8	228.0	9.8	12.8	103.8	36.5	8.2	163.8	25.5	23.4
142	Manak	110.3	127.3	180.8	10.3	13.5	96.3	36.3	7.3	151.3	24.8	22.7
143	AL1922	98.5	118.3	184.8	12.5	19.8	238.3	38.0	6.7	283.8	23.7	20.1
144	AL2087	87.5	128.8	183.0	11.5	14.0	169.8	37.3	8.3	210.0	23.3	24.8
145	AL2091	86.5	121.3	132.8	11.0	20.5	162.5	37.5	8.6	210.0	28.6	26.7
146	AL2025	76.8	113.8	188.5	10.5	14.3	115.3	33.3	8.2	163.8	30.8	22.6
147	AL2207	87.5	115.3	192.8	14.0	20.8	222.5	38.0	7.6	276.3	26.0	27.4
148	IC245350	111.5	113.8	175.8	14.8	19.0	219.8	37.5	8.4	250.0	24.9	26.1
149	AL1781	98.5	111.8	184.8	10.0	14.8	95.5	36.3	6.4	157.5	22.9	26.5
150	H948	111.5	122.3	214.0	19.0	25.0	244.8	37.0	7.4	420.0	26.6	27.1
151	H200014	111.3	114.3	193.8	13.0	18.8	197.5	39.0	7.9	247.5	27.3	23.2
152	AL1789	110.8	121.8	206.0	11.5	14.3	112.8	33.0	8.0	165.0	24.6	28.9
153	AH069	97.0	133.3	191.5	9.5	12.3	109.5	35.5	8.7	156.3	23.2	26.4
154	AH063	91.0	127.3	188.0	10.0	15.0	101.8	34.5	7.7	162.5	29.2	31.9
155	AL1525	83.5	119.3	186.8	12.0	17.5	170.5	36.5	8.3	222.5	27.9	22.3
156	AL1760	86.8	116.8	189.0	10.8	15.5	102.5	36.0	7.5	156.3	24.2	25.9
157	AH0930	87.8	136.8	199.0	10.8	13.0	103.8	39.5	9.7	153.8	24.0	28.5
158	PUSA2001	91.0	130.3	183.5	13.5	20.0	127.8	34.5	8.8	172.5	26.4	25.0
159	AH0941	110.8	122.8	189.5	16.0	22.0	197.3	35.0	8.1	325.0	23.7	23.2
160	AL1846	98.5	134.8	186.5	14.8	21.5	164.8	36.3	7.8	321.3	29.1	21.2
161	AL1782	88.8	122.8	213.3	15.0	23.3	213.5	34.8	8.4	330.0	25.7	27.1
162	AL2170	87.3	127.8	203.5	12.8	18.5	181.0	33.8	8.5	235.0	29.5	33.1
163	AL2133	88.3	130.8	204.8	12.0	14.5	174.0	31.0	7.7	177.5	26.3	31.4
164	VLA1	91.0	130.8	190.5	14.0	17.0	214.0	35.5	8.4	251.3	24.7	24.2
165	PAU881	93.5	139.3	221.5	17.0	21.5	174.0	38.0	7.6	315.0	30.0	23.5
166	PADT16	83.8	115.3	137.5	9.3	11.8	77.3	30.5	7.1	127.5	26.7	24.6
167	AL2132	98.8	124.8	192.5	9.5	12.5	98.5	34.8	9.3	155.0	25.2	27.3

S. No.	Treatment	DF	DM	PH	PB	SB	PD	SP	SW	YLD	Fe	Zn
168	AL2128	98.3	113.3	189.8	9.8	13.8	96.0	34.0	6.3	155.0	29.3	26.1
169	Sarita	87.3	116.3	187.8	15.0	18.0	117.5	35.3	7.5	223.8	25.0	24.2
170	AL2131	99.5	111.8	208.3	9.8	12.0	92.3	30.8	9.1	131.3	25.6	25.9
171	AL882	82.3	134.0	181.8	11.8	14.5	161.0	34.5	8.3	360.0	27.8	28.7
172	AL2062	93.3	133.3	210.8	14.0	16.5	193.3	36.5	8.2	208.8	23.1	29.6
173	PBR2	98.5	118.3	210.0	9.5	12.5	95.5	34.5	8.6	133.8	26.5	24.2
174	PANTA250	116.5	138.8	220.5	11.3	13.5	114.5	31.5	9.1	165.0	25.9	24.6
175	Pant37	110.5	110.8	196.0	9.3	11.5	91.3	36.0	8.9	132.5	20.9	27.0
176	AH092	98.5	110.3	214.8	11.8	13.8	98.0	36.0	8.2	156.3	20.4	25.8
177	Pant234	94.5	127.3	212.8	10.5	12.3	102.5	36.5	6.7	142.5	18.2	18.9
178	AL2204	78.5	118.3	102.5	9.5	11.5	70.5	34.8	7.8	128.8	25.4	20.2
	CD (5%)	15.12	11.05	11.75	3.05	4.07	26.96	5.11	0.90	36.44	4.74	3.91

ANNEXURE IV

Total number of processed reads and total number of reads with RAD-tags -

Treatment	Total No. of reads	No. of reads with RadTags
1	903374	888772
2	679444	639932
3	942724	926062
4	824722	805944
5	771410	755566
6	910662	892734
7	838254	823548
8	938278	921682
9	875244	858720
10	963942	946898
11	1504328	1483086
12	1879792	1855536
13	2015098	1985500
14	1556838	1536638
15	1551766	1531610
16	1939030	1892840
17	2196674	2144558
18	1417096	1395550
19	1496522	1471030
20	1385942	1352428
21	1879438	1842862
22	1795724	1759688
23	1613484	1549180
24	1629962	1598398
25	1279964	1254786
26	2238472	2192448
27	2178506	2132328
28	2669456	2619030
29	1973506	1930240
30	855024	831038
31	2527134	2469270
32	2523768	2472524
33	1516638	1474852
34	2173322	2120538
35	1298614	1264074

Treatment	Total No. of reads	No. of reads with RadTags
36	1259586	1231026
37	2114014	2074824
38	2487404	2444330
39	2386104	2321084
41	1912160	1864276
43	1749480	1712858
45	1594014	1563194
46	1195786	1170870
47	1270654	1243742
48	1697908	1656574
49	858126	833374
50	2882262	2732622
51	2184460	2146674
52	991254	971798
53	1499950	1473612
54	1467684	1441750
55	1196968	1173446
56	758962	741780
57	2118292	2080896
58	1783158	1741184
59	1784108	1751768
60	1127490	1075720
61	1899618	1865318
62	1727028	1702706
63	1304082	1279832
64	1225744	1207154
65	2285406	2209848
66	1120888	1073870
67	1741064	1704438
68	1472726	1440862
69	1833914	1795980
70	2152936	2100674
71	1514556	1481806
72	1818866	1778482
73	1972728	1928428

Treatment	Total No. of reads	No. of reads with RadTags
74	1924406	1883740
75	1659868	1622076
76	1564304	1531042
77	1951072	1898168
78	2349588	2294012
79	2049360	1996186
80	1164930	1128586
81	1506172	1422664
82	1347744	1316388
83	1761422	1710202
84	1713866	1670726
85	1422836	1384158
86	1430724	1396218
87	1143468	1116348
88	1782780	1739952
89	1545678	1507106
90	1736856	1696192
91	1381708	1347098
92	1500982	1461114
93	1616404	1578220
94	1456560	1395704
95	1306590	1276832
96	1621374	1583694
97	1550058	1509534
98	2689566	2628328
99	2060444	2016772
100	9735982	9291296
101	2158106	2114586
102	2427312	2380696
103	2141166	2093888
104	1519816	1483570
105	1581508	1544452
106	1771308	1731618
107	1546604	1511010
108	1287206	1259866
109	2179944	2134094
110	1739276	1705944
111	2295162	2252012

Treatment	Total No. of reads	No. of reads with RadTags
112	2036220	1995082
113	1486490	1453964
114	1801538	1765920
115	1515558	1459490
116	1881000	1844328
117	1589924	1558224
118	1920534	1880616
119	1154668	1128090
120	1960086	1916918
121	1436810	1407194
122	1693062	1658370
123	1760164	1723346
124	1765100	1727144
125	2029716	1984130
126	1791098	1749818
127	1618646	1584052
128	1607500	1565138
129	1612468	1573688
130	1563776	1525622
131	1807048	1766800
132	1775702	1733896
133	1494082	1461874
134	1267910	1242768
135	1238728	1213974
136	1129058	1088978
137	1434714	1409080
138	1062980	1045576
139	1026442	987574
140	1327160	1305920
141	1222546	1203876
142	1194380	1172316
143	1453326	1427606
144	1467100	1445066
145	1369360	1347924
146	1347362	1325408
147	1515778	1492710
148	2334242	2299346
149	1698904	1672922

Treatment	Total No. of reads	No. of reads with RadTags
150	2181018	2148680
151	1597770	1570454
152	1892402	1864018
153	2254696	2220594
154	2689564	2647996
155	2669570	2629464
156	2430568	2396504
157	1875262	1846856
158	6216448	6128294
159	5769216	5673412
160	5132468	5053790
161	4439056	4374558
162	5328012	5253750
163	1626688	1603728
164	1649576	1625876
165	1835080	1811772
166	1306758	1286606
167	1806568	1776650

Treatment	Total No. of reads	No. of reads with RadTags
168	2436182	2366164
169	1811776	1739504
170	1910556	1858170
171	1841684	1787702
172	1876256	1803250
173	2350430	2299118
174	1736828	1708120
175	2577322	2537718
176	2270612	2231924
177	2054672	2018440
178	1903802	1871530
179	1846424	1808076
180	2542506	2494998
181	1972912	1934568
182	1718784	1684586
183	1830334	1789618
184	1557176	1488824
185	1479742	1435238

VITA

Name of the student	:	Harpreet Kaur
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EDUCATIONAL QUALIFICATION

Bachelor degree	**:**	**B.Sc. Agriculture (Hons.)**
University and year of award	:	Punjab Agricultural University, Ludhiana
OGPA/OCPA/% marks	:	7.77/10.00
Master's Degree	**:**	**M.Sc. Plant Breeding and Genetics**
OCPA	:	7.66/10.00
Title of Master's Thesis	:	Assessment of Quality Protein Maize Inbred lines and their use in crosses for conversion of elite inbred lines
Ph.D. Degree	**:**	**Ph.D. Plant Breeding and Genetics**
OCPA/OCPA% marks	:	8.37/10.00
Title of Ph.D. Dissertation	:	Genome-wide Association mapping for Fe, Zn and yield traits in Pigeonpea
Awards/Distinctions/Scholarships	:	Awarded with University Merit Scholarship during Ph.D. Degree Programme (2017-2020)