GENETIC VARIATION AND PRINCIPAL COMPONENT ANALYSIS FOR AGRONOMIC AND GRAIN QUALITY TRAITS IN MNP-2 RECOMBINANT INBRED LINE (RIL) POPULATION OF BREAD WHEAT (*Triticum aestivum*)

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BANARAS HINDU UNIVERSITY

THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF DEGREE OF

Master of Science (Agriculture)
in
Genetics and Plant Breeding

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Dedicated to

My Parents

Mr. Krishna Shyam Maharjan

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Dear Sir,

I have great pleasure in forwarding the thesis entitled **"Genetic variation and principal component analysis for agronomic and grain quality traits in MNP-2 recombinant inbred line (RIL) population of bread wheat (*Triticum aestivum*)"** submitted by **Ms. RASHILA MAHARJAN, I.D. No. 20412GPB029, Enrollment Number-429469**, in partial fulfilment of the requirements for the degree of **Master of Science (Agriculture)** in **Genetics and Plant Breeding,** Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi and placing on record that she has completed the requisite residential requirements as contained in the statute of the University.

I certify that the entire scheme of investigation presented herein was planned and carried out solely by the candidate under my guidance and supervision. The data presented in the thesis, to the best of my knowledge and belief, are genuine and original.

Thanking you.

Forwarded by

(Prof. B. Sinha)
Head of Department

Yours faithfully,

(Dr. Sandeep Sharma)
Supervisor/Advisor

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Place*: Varanasi* ***(Rashila Maharjan)***

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LIST OF SYMBOLS AND ABBREVIATIONS

%	:	Percentage
@	:	at the rate
et al.	:	Co authors [at all ii]
F1	:	First filial generation
F2	:	Second filial generation
df	:	degree of freedom
RIL	:	Recombinant Inbred Line
ANOVA	:	Analysis of Variance
SE	:	Standard Error
SD	:	Standard Deviation
CV	:	Coefficient of Variation
GCV	:	Genetic Coefficient of Variation
PCV	:	Phenotypic Coefficient of variation
QTL	:	Quantitative Trait Loci
PPVFRA	:	Protection of Plant Variety and Farmers Right Act
CIMMYT	:	International Maize and Wheat Improvement Center
SPAD	:	Soil Plant Analysis Development
IRRI	:	International Rice Research Institute
H2	:	Broad sense heritability
GA	:	Genetic Advance
PCA	:	Principal Component Analysis

Chapter I

INTRODUCTION

Wheat is one of the most important staple food crops in the world. The wheat crop belongs to family Poaceae and genus Triticum. Triticum consists of six biological species at diploid, tetraploid, and hexaploidy level (Dvorak, 2001). Among these three species *Triticum aestivum* (Bread wheat), *Triticum durum* (Macaroni wheat), and *Triticum dicoccum* (Emmer wheat) are commonly cultivated, occupying 95%, 4% and 1% of total wheat cultivated area. *T. aestivum* is a hexaploid species with chromosome no (2n=6x=42), whereas *T. durum* is tetraploid (2n=4x=28) and *T. dicoccum* is diploid (2n=2x=14). It is a self-pollinating crop believed to have originated from South West Asia.

Owing to its large area coverage and high productivity and eminent wheat has been described as the 'King of cereals'. It serves as a staple food for more than 30% of world population (Lobell *et al*., 2011). The global wheat production acreage and volume were 225.06 million ha and 778.6 million metric tons respectively in the marketing year 2020-2021 whereas global per capita wheat consumption was 67.6kg/year (Shahbandeh, 2022). In India wheat ranks second after rice in terms of both area and production, occupying 30.55 m ha area and production of 107.86 mt with average national productivity of 3508 Kg/ha. The major wheat-producing states in India are Uttar Pradesh, Punjab, Haryana, Madhya Pradesh, Rajasthan, Himachal Pradesh, Jammu, and Kashmir. These states contribute nearly about 99.5% of country's total production. Uttar Pradesh is the leading state in terms of both area and production with 1.08 m ha land coverage and 33.8 million metric tons/year respectively.

Wheat is mainly composed of carbohydrate, moderate amount of protein and some amount of vitamins and minerals. On average it contains 78.10% carbohydrate, 14.7% protein, 2.1% fat, 2.1% vitamins and minerals (Davis *et al*, 1980). Thiamine, Riboflavin, Niacin, Folate are the vitamins found in wheat. Minerals like iron and zinc are present, some amount of trace elements selenium and magnesium are also found. Wheat kernels are the storehouse of nutrients. Endosperm that occupies 83% of kernel

weight stores the majority of proteins (about 72%) and is the source of white flour. It also contains substantial amount of carbohydrates, vitamins and iron. The outer brown layer of wheat is the bran that is about 14.5% of grain weight contains more amount of fiber, potassium, magnesium, calcium, phosphorus and niacin. Bran is included during milling for whole wheat flour. The germ or embryo is rich in protein, fat and vitamin B-complex. The outer layer of endosperm and aleurone layer has more proteins, vitamins and phytic acid than inner endosperm which is mainly composed of starch. Outer layer is being removed while making white flour thus large amount of nutrients are lost along. (kumar *et al*, 2011).

However, in majority of the developing countries cereals such as rice, wheat, maize and cassava are the primary source of food (Nishida *et al*., 2004). High consumption of these cereal-based foods with low Zn content and absence of variation in food intake leading to imbalanced diet is the major reason of Zn deficiency in human population. This problem is aggravated by growing cereal crops on potentially Zn deficient soils. A widespread Zn deficiency in humans occurs mainly in the regions where soils have Zn deficiency problem and cereals are major source of daily calorie intake. Micronutrient malnutrition (insufficient dietary intake of micronutrients like Iron (Fe), Zinc (Zn), Iodine (I), and vitamin A) threatens more than 4 billion people, predominantly in developing countries (Stein, 2010). Children are mostly affected by this hidden hunger and more than 11% of child mortality under 5 years of age is due to micronutrient deficiency (Black *et al*., 2004). About 2.4% and 1.9% of total global burden of diseases is caused due to Fe and Zn disorder, the two most widespread nutritional disorder (Rodgers *et al*., 2004).

Iron deficiency causes anemia in children leads to impaired physical growth, problems in mental development and learning capacity (Bouis, 2003). Severe Iron deficiency during pregnancy may compromise the health of both mother and child, also causing complications during and even after the child birth. Iron deficiency has also been associated with prematurity, intrauterine growth restrictions and compromised offspring neurodevelopment like autism, schizophrenia, lower recognition memory, slower speed of processing and poorer bonding that persists in spite of postnatal iron repletion (Georgieff, 2020). Zinc deficiency causes retarded growth, skeletal

abnormalities, delayed wound healing, increased abortion risks and diarrhoea (Salgueiro *et al*., 2002). Children are highly sensitive to Zn deficiency and causes nearly 4,50,000 child deaths every year (Black *et al*., 2008)

Traditionally, this nutrient deficiency problems have been attempted to rectify through nutrient supplementation programs, but it fails to meet its goal set by the international health organizations due to lacks of external funds, low purchasing power of people, inaccessible market and health care system, and lack of awareness about long term health benefits of these nutrient supplements (Gilani and Nasim, 2007). Recently, a complimentary solution to mineral malnutrition has been approached which is known as 'Biofortification' (Graham, 2001; Bouis, 2003). According to WHO, Biofortification is the process by which the nutritional value of food crops is enhanced by various methods. Biofortification differs from conventional fortification in the sense that it aims to increase nutrient levels in crops during plant growth rather than through manual means during processing of the crops (Dwyer *et al*, 2014). The common approaches being used in biofortification process are agronomic, conventional and transgenic biofortification processes. However, Conventional plant breeding biofortification is very feasible and most adoptable. It exploits high vitamin or mineral containing genotypes available in the gene pool and is crossed with commercial variety to release a new variety that have the desired nutrient and agronomic traits (Saltzman *et al*., 2013).

Thus, being most important staple food and rich source of nutrients, a minute variation in production due to various biotic and abiotic stress could show greater impact on food security and public health. However continuous effort from plant breeders and agronomists towards development of new cultivars with higher yield and tolerance to various stresses is significant and noteworthy. A better understanding of available and exploitable genetic variability and their heritability patterns is a pre-requisite for any crop improvement programs. Genetic diversity is defined as those parameters which quantifies the magnitude of genetic variability present within a population. Each species has their own genetic background and within same species too the individuals have their unique genetic makeup which set them different from any other individual. Differences and similarities among various related species are governed by these diverse genes which is the prime reason behind their distinguish

traits. These variations present within a species serves a vital role as a natural source for breeding. Many traits get lost as a result of domestication, continuous inbreeding and sole selection for yield. As a result, some traits related to abiotic, biotic stress that were previously present in its wild types gets masked. With the loss in diversity these important genes also might get lost. (Hughes, 2008). Further, creating new variation through crossing and continuous selections to generate variability for required traits, to use them for various genetic and inheritance studies facilitates better understanding of trait and in designing efficient breeding programs. There are many kinds of populations have been defined and developed, like F_2, RIL, NIL, DH for various inheritance and genetic mapping studies. But RIL's being dynamic and holds relatively higher variation present in F_2 population for various traits. Recombinant Inbred lines (RILs) also known as F_2 derived lines are the homozygous lines obtained by crossing two diverse parent lines followed by continuous selfing of F_2 plants for 6-8 generation through single seed descent method (Singh,2015). Recombination during meiotic phase create a divergent mosaic of genome in each offspring at F_2 generation. Consecutive selfing helps fix these variations into a line providing a number of recombinant inbred lines with a diverse genotype. These RILs show different phenotypes as per its gene expressed. The genetically distinct RILs that vary quantitatively in their phenotypes can be used as mapping population to map a quantitative trait loci (Pollard, 2012).

The RILs are designed as per the objective of the breeder, the first step being selection of parents. Generally, such parent strains are selected those which show divergence in trait of interest. The parents are assayed for phenotype, marker availability and variance is established before the investment of resources into construction of RILs. The strains should consist of enough no of markers. The construction of design for developing RILs depend on a no factors such as number of RILs to be produced, no of inbreeding past F_2 generation, cost/benefit analysis etc. The no of cross to be made depends on size of RILs to be developed. Large population is favored over small one given that a large population show more variability for different traits and precision will be higher. The no of parent crosses to be made for 'N' no of RILs are $4N/B^2$, where, B is brood size however, it is recommended to have more no of crosses for practical reasons. The obtained F1 is selfed and F_2 population is obtained

which is the segregating generation. These F2 are inbred for no of generations to obtain homozygosity. (Pollard, 2012)

Recombinant Inbred lines were one of the first types used as mapping population. They are highly preferred and conveniently developed lines for genetic mapping because to locate genes a population segregating for such genes is required. Since the RILs have been developed from F2 segregating generation they consist of maximum variability among genotypes but same parental background. The advantage of using RILs as mapping population is that they can be maintained and replicated over different locations. (Singh, 2015).

Therefore, the present study utilizes 232 RIL population developed by CIMMYT, named MNP-2 from diverse parents SOKOLL//W15.92/WBLL1 and CIANO T 79, which were differing significantly for spot blotch resistance. Though it was developed mainly for mapping spotblotch resistance but it tends to have recombination for other traits also. Hence, the present study was conducted with following objectives, to assess the variability present in these RIL for agronomic and quality traits like grain Zn and Fe, so that the same RIL can also be used for mapping other traits.

Objectives of the study

1. To assess the genetic variation and heritability for agronomic and quality traits (grain Zn and Fe content) in MNP-2 RIL population.
2. To study the correlation and association patterns between measured traits
3. Performing principal component analysis to check the association between lines and traits.

Chapter II

REVIEW OF LITERATURE

In present investigation titled "Genetic variation and principal component analysis for agronomic and grain quality traits in MNP-2 recombinant inbred line (RIL) population of bread wheat (*Triticum aestivum*)" an attempt was made to study the genetic variability present in wheat recombinant inbred lines for agronomic and grain Iron and Zinc concentration. A brief review of previous studies on variability and trait association patterns are presented below.

2.1 Genetic variability

Genetic variability refers to the variation present for a given trait over different genotypes within the species or population. Variation in alleles with varied frequency occurs at individual or species level may have originated through evolution of mutation, genetic drift, natural selection etc. It is the source of diversity. The presence of variation for different traits makes it possible to make use of naturally available sources for any crop improvement breeding program of interest. Exploring genetic diversity is essential for exploiting heterosis or developing productive recombinants. Some of the studies made in genetic diversity of wheat are presented below:

Huang *et al* (2002) collected 998 accessions of bread wheat from 68 countries of five continents and used a set of 24 microsatellite markers to assess the genetic diversity. They detected 470 alleles with an average allele number of 18.1 per locus. The highest number of alleles per locus was found in B genome with 19.9 whereas A and D genome had 17.4 and 16.5 respectively. Greater genetic variation was observed in non-centromeric regions than centromeric regions of chromosome. It was also seen that alleles for each locus were present in regular two or three base pair steps which indicates that variation occurred step by step continuously during evolution. Among all the accessions collected, those from the Near East and Middle East exhibited highest genetic diversity.

Oury *et al*. (2006) directed an experiment on genetic variability of wheat with four trials to illustrate the concentration of grain magnesium (Mg), zinc (Zn) and iron

(Fe) and study the impact of genotype by environment (G × E) interactions. They collected genotypes from old French landraces, worldwide germplasm collection and elite breeding lines (modern cultivars). The concentration of Mg was from 600 to 1400ppm, some exotic varieties reached the highest of 1890ppm. ANOVA table for Mg showed high genotype effects and moderate G × E interactions. Zn concentration ranged from 15 to 35 ppm with some exotic varieties containing 43ppm. Zn concentration variability was significantly affected by genotype but also had a high G × E interactions. It was also observed that Mg and Zn were positively correlated thus selection for Mg would be reliable for Zn selection. Fe concentration varied from 20 to 60 ppm with some non-adapted material reaching 88 ppm. No significant genotype effect was observed for Fe, G × E interactions was pretty high.

Morgounov *et al*. (2007) evaluated 66 spring and winter common wheat genotypes from Central Asian national breeding programs for iron and zinc concentration in grains and found that both Fe and Zn showed large variation among genotypes. Fe ranging from 25 mg/kg to 56 (mean 38 mg/kg) and Zn from 20 mg/kg to 39 mg/kg (mean 28 mg/kg). Spring cultivar contained more Fe concentration than winter wheat whereas winter wheat showed higher Zn grain concentration than spring genotypes. Within spring wheat, they found a strongly significant positive correlation between Fe and Zn. Grain protein content was also significantly ($P < 0.001$) correlated with grain Zn and Fe content. However, there was a strong, significant, negative correlations between Fe and plant height, and Fe and glutenin content. Similar correlation coefficients were observed for Zn. In winter wheat, significant positive correlations were found between Fe and Zn, and between Zn and sulfur (S). Both Fe and Zn had negative correlation with Manganese (Mn) and phosphorus (P). They also found that genotype effect largely controlled Fe concentration whereas Zn concentration was highly dependent on location effect. Finally, Morgounov and his coworkers concluded 7 promising genotypes (2 spring and 5 winter genotypes) for increasing Fe and Zn concentration as well as enhancing concentration of promoters of Zn bioavailability, such as S containing amino acids.

Ali *et al*. (2008) examined genetic variability in 70 exotic wheat cultivars for 8 characters i.e., number of productive tillers per plant, fertility percent, plant height,

spike length, number of spikelets per spike, number of grains per spike, test weight, yield. There was a significant difference among the studied characters. The estimates of GCV and PCV were high for no of productive tillers per plant, yield and number of grains per spike. All characters except fertility percent showed high heritability with high genetic advance.

Majumder *et al*. (2008) studied the genetic variability and correlation of grain yield with its component features in twenty spring wheat genotypes. Genotypic and Phenotypic variances were significant for all the studied traits. Slightly higher phenotypic variance suggested some amount of environmental influence. However, the influence was minimal so the variance was attributed to genotypic difference. Grains per spike, plant height, harvest index, 1000 kernel weight and yield showed high heritability with high genetic advance.

Joshi *et al*. (2010) conducted a multilocation trail in 14 environments of eastern Gangetic plains (including Banaras Hindu University) for 20 wheat genotypes developed by CIMMYT to access genetic diversity in relation to micronutrient content (iron and zinc) and determine Genotype × environment interaction for zinc and iron concentration. It was found that GE was significant for all four traits. Response to grain zinc and iron was observed to vary significantly according to location. Maximum temperature and nutrient content in 30-60cm soil depth were also significant determinant for zinc and iron concentration. Hence future breeding programs need to consider environmental factors as a huge Impact factor while breeding for biofortification.

Yadav and coworkers (2011) recorded a significant variation for characters like days to 50% heading, days to physiological maturity, tillers per plant, plant height, spike length, grains per spike, 1000 grain weight, grain yield and biomass yield plant. Except for no of grains per spike and plant height all of the traits had a high heritability. No of Tillers per plant and grain yield per plant demonstrated significantly high heritability along with genetic advance, indicating a reliable trait during selection process.

Deoraj *et al*. (2016) reported significant variability in F2 generation of twenty-one crosses made between seven parents and two checks. Highest GCV and PCV were observed for number of productive tillers per plant and number of spikelets per spike;

whereas, days to maturity and days to 50% flowering had the least GCV and PCV. High heritability coupled with high genetic advance as per cent of mean was seen for no of productive tillers per meter, length of spike, 1000 grain weight and grain yield which suggested that additive gene was in effect for expression of these characters.

Chimdesa *et al*. (2017) analyzed 21 varieties and 4 promising lines using randomized complete block design, 3 replications of plants were grown and 14 characters were studied. Data showed that the varieties differed significantly for 8 traits under study. Genotypic coefficient of variation (GCV) ranged from 4.59 (days to physiological maturity) to 13.76% (grain yield per hectare), while phenotypic coefficient of variation (PCV) ranged from 5.03% (days to physiological maturity) to 20.85% (grain yield per hectare). Broad Sense Heritability and genetic advance as percent of mean (GAM) varied from 33.33% (tillers per plant) to 84.67% (peduncle length) and 8.66% (days to physiological maturity) to 18.74% (grain yield per hectare) respectively.

Jamil *et al*. (2017) evaluated 60 wheat genotypes in RCBD design with three replications to estimate genotypic coefficient of variance (GCV) and phenotypic coefficient of variance (PCV) for several yield contributing parameters. They observed high estimates for grain yield and thousand kernel weight. Most noteworthy genetic advance was obtained for grain yield. thousand kernel weight and grain yield per plant showed high heritability with high genetic advance.

Balfourier *et al*. (2019) acquired a set of 4506 different landraces and cultivars from 105 different countries that were genotyped with single nucleotide polymorphism of high density to study the phylogenic history of spread, adaptation and selection. They found that continuous selection and domestication has led to highly unbalanced germplasm compared to the ancestral diversity. The germplasm from Asia contributes highest amount of worldwide diversity that still remains unexploited.

Mourad *et al*. (2020) acquired 103 spring wheat varieties from different countries of five continents to study genetic diversity of useful agronomic traits that will be of value in breeding programs, marker assisted selection (MAS), Genome Wide Association studies (GWAS) etc. All the cultivars were genotyped with the help of 36,720 GBS SNPs (genotyping by sequencing derived SNPs) distributed throughout

the genome. On the basis of ancestral kinship the genotypes were divided into 3 different subpopulations. AMOVA analysis showed a significant variation among the subpopulations, the first group being the most diverse one. On genomic level, the highest linkage disequilibrium (LD) was found in D genome and among chromosome 2D, 5A, 7B showed highest LD with value 0.08, 0.07 and 0.05, respectively. Thus, the diverse genotype can be explored for use in upcoming future programs and LD hotspots can be investigated for more interesting genes.

2.2 Correlation and Principal component analysis (PCA)

Waqar-Ul-Haq *et al*. (2010) evaluated 10 different wheat genotypes to explore correlation between yield and different morphological parameters. Spike length, no of spike per plant, no of spikelets per spike, no of tillers per plant, thousand kernel weight all showed significantly positive correlation with grain yield per plant whereas days to heading, days to maturity and plant height were not significant with grain yield.

Khodadi *et al.* (2011) utilized 36 winter wheat genotypes to determine genetic diversity and cluster the genotypes through Euclidean square distance and principal component analysis. Cluster analysis employing the first five main components of PCA that defined 97% of total variation, revealed six distinct genotype groupings, with the greatest genetic distance between the Sardari and Vorona/Kauz genotypes.

Rymuza *et al*. (2012) applied PCA on spring wheat to assess the characteristic diversity. The recorded correlations demonstrate that the relationship between wheat attributes and the growth system is dependent. The PCA made it possible to study complex entangled relation between variables by redefining seven initial characteristics into three new variables, which carried over 75 percent of the information from the direct sowing input data and nearly 80 percent for conventional tillage. The 1000 kernel weight and grain yield demonstrated the greatest discriminatory capacity, which diversified the given wheat genotypes.

Farshadfar *et al*. (2013) evaluated genetic parameters of agronomic, and morpho-physiological indicators in relation to drought tolerance. The plants were crossed in 6×6 diallel cross in randomized block design and three replication. Plant height (PH), peduncle length (PL), number of tillers per plant (NTP), thousand kernel

weight (TSW), no of days to flowering (DTF), relative water content (RWC), stomatal conductance (SC), chlorophyll fluorescence (CHF), and yield were all found to be significantly different between genotypes under stress. Both general combining ability (GCA) and specific combining ability (SCA) showed substantial differences in PH and PL, demonstrating the participation of both additive and non-additive gene action in their inheritance. SCA showed considerable differences in RWC and SC, indicating that non-additive gene action was prevalent in their inheritance.

Naik *et al*. (2015) worked with 78 bread wheat genotypes to study 8 traits in relation to yield and protein content in grains. They observed Genetic Coefficient of Variance (GCV) for protein content was 7.56 and that for grain yield was 52.38. High heritability was obtained for sedimentation value, plant height and no of days to heading. The sedimentation value and grain yield per plot also showed high genetic advance.

Dutamo *et al*. (2015) figured positive correlation between no of productive tillers per plot, spike length, no of spikelets per spike, thousand grain weight, biomass with grain yield. the observation was made on the basis of data collected from 4 germplasm of bread wheat planted along with 4 standard checks in an augmented design.

Ojha *et al*. (2018) examined 20 diverse wheat genotypes to figure out correlation among 11 contributing characters. Among the 11 characters, number of spikes/m^2, spike weight, no of grains per spike, days to heading and test weight executed positive correlation on grain yield. the investigation suggests that number of spikes per m^2 and spike weight can be used as an indirect criterion to select for economic yield.

Škrbić *et al*. (2005) performed PCA on 14 wheat cultivars obtained from Siberian regions. They used Atomic absorption Spectrophotometer (AAS) to quantify trace elements and compare among cultivars of different regions. Four major components were discovered to account for 87.2 percent of the total variation in the data. The plot of component loadings revealed substantial groupings for various microelement concentrations. The component ratings revealed that the wheat-growing regions of Serbia are similar.

McDonald *et al*. (2008) analyzed zinc concentration in regards to grain yield. They found a negative correlation between yield and Zn content. They suggested that is probably due to dilution effect. The increase in grain yield in majorly due to more no of grains in terminal spikelets which do not receive enough Zn from vegetative tissues.

Khan *et al*. (2013) conducted an experiment on durum wheat to distinguish the direct and indirect effects morphological characters have on grain yield. there was significant variation for all the studied characters. Grain yield was directly influenced by maturity days, number of spikes/m^2, and 1000-grain weight. The number of grains per spike had a direct favorable effect as well, but it was minor. Maturity days and 1000-grain weight were used to determine the indirect effect of heading days and plant height on grain yield. In order to choose durum wheat genotypes, focus should be placed on heading days and plant height, as well as 1000-grain weight, number of spikes/m^2, and number of grains/spike.

Beheshtizadeh *et al.* (2013) performed principal component analysis (PCA) on 11 morphological traits observed in 18 bread wheat cultivars. Four significant components accounted for nearly 76 percent of the overall variation, according to PCA. The PC1 accounted for 38% of total variation between features and was correlated with spike yield and their components in a substantial way. As a result, this factor was labelled as spike seed yield. Other components no of tillers, grain weight, and grain yield accounted for 15, 12, and 11 percent of variation.

Liu *et al*. (2014) studied the relation between Fe and Zn by using 655 samples of wheat. They observed that grain Fe and Zn content was lower in high yielding types. Grain Fe and Zn concentrations were reduced by 0.9–2.1 mg/kg for every 1.0 t/ha increase in grain production. They also found that, in both spring and winter wheat, grain Fe concentration was correlated significantly positive with Zn concentration. The Fe concentration increased by 0.6 mg/kg in spring varieties and 0.3 mg/kg in winter varieties with every 1 mg/kg rise in grain Zn

2.3 Genetic studies in wheat recombinant inbred lines

Boukhatem *et al*. (2002) analyzed a set of 98 F_8 recombinant inbred lines descended from Camp × Michigan Amber and 114 lines from cross Opata85 × synthetic

hexaploidy. These RILs were used to identify quantitative trait loci (QTLs) responsible for yellow rust resistance. Yellow rust is a common disease in cold-weather locations that can have a significant impact on grain productivity. Pathogen evolution has overcome the resistance provided by major specific genes. Camp Remy is one among few varieties that has maintained its resistance. Two and five QTLs were found in these two RIL populations, respectively. The role of the centromeric region of chromosome 2B and the telomeric areas of chromosomes 2AL and 7DS in lasting yellow rust resistance has been underlined in this study. Resistance to various infections is linked to the same chromosomal areas.

Epstein *et al*. (2002) investigated 130 RILs obtained from cross between varieties Kanto 107 and Bai Huo to determine key component responsible for cohesive, springy, and resilient white salty noodles. Using texture profile analysis, the three Waxy genes gave a unique chance to tie granule bound starch synthase (GBSS) gene dosage to the texture of noodles. The variation was significantly high for all characters that defines noodle texture. Waxiness was found to pe positively correlated with soft, cohesive, springy noodles whereas noodles made with normal starch flour was hard, adhesive and chewy. Thus, it was proposed that starch composition plays a role in texture of white salted noodles.

Shindo *et al*. (2003) studied the recombinant inbred lines obtained from a cross between *Triticum aestivum* (cv. Chinese spring) and *Triticum spelta* var *duhameliamum* to examine genetic segregation of heading traits. The experiment was carried out in two environments control and field and they tried to explore effect of vernalization, photoperiod and narrow sense earliness on heading along with the interaction between them. Photoperiod and earliness were found to correlated with heading time. 38 linked markers were detected for the three factors and heading among the RILs. The time of heading in parental lines were significantly different, vernalization with long day condition was most effective for heading.

Hanocq *et al*. (2004) worked on an important adaptive trait that causes variation in agronomic characters, the earliness in heading with the help of RIL population derived from French varieties ‘Renan’ and ‘Recital’. The goal of this study was to use an experimental strategy that combined field trials and controlled growing conditions

to find and map QTL for all three traits vernalization, photoperiod sensitivity and intrinsic earliness. A map based on 194 lines and 254 markers was previously created, spanning around 77 percent of the genome. There were 13 QTL with a LOD>2.5 found globally, with four controlling Photoperiod Sensitivity, five controlling Vernalization Requirement, and four controlling Intrinsic Earliness. Two large photoperiod sensitive QTL were found on chromosomes 2B and 2D, near the two key genes Ppd-B1 and Ppd-D1. Together, they explained more than 31% of the phenotypic variance. On 5A, a significant VR QTL was discovered that explained 21.8–39.6% of phenotypic variance (depending on the year). Two PS and VR QTL that had not been previously mentioned were discovered on 5A and 6D, respectively. A VR QTL previously identified in a related population on 2B was confirmed.

Sun *et al*. (2009) conducted an analysis using 131 recombinant inbred lines developed from cross between Chuan 35050 and Shannong 483. The population was evaluated for four characters thousand kernel weight (TKW), test weight (TW), kernel length (KL), kernel width (KW) in four different environments. They observed 5.9 to 26.4% phenotypic variation. The no of QTL detected for TKW, TW, KL, KW were 4, 7, 6, 3 respectively. The heritability was highest for test weight.

Ci *et al*. (2009), screened the recombinant inbred lines for cadmium tolerance and found four lines that showed most variance. They evaluated the responses of these wheat seedlings to Cd toxicity by exposing them to 50 M CdCl2 at three-leaf stage for 24 days. Except for secondary root numbers and average root diameter, most growth indices and root morphological features were reduced under Cd stress. Because of increased malondialdehyde (MDA) level and reduced superoxide dismutase (SOD) and catalase (CAT) activities in leaves, leaf cell peroxidation was enhanced by Cd. Furthermore, CAT activity was lower in the Cd-sensitive lines and higher in the tolerant lines. The photosystem II (PSII) in the leaves was damaged. In both the shoot and the root, total soluble sugar concentrations fell while free amino acid concentrations increased. We concluded that under Cd toxicity, Cd-tolerant lines accumulated less Cd in the plant and had low Cd concentration in the shoot (less Cd translocation to shoot), maintained higher CAT activity in the leaf, and had higher PS II function than Cd-sensitive lines, which could be related to their Cd tolerant capacity in the current study.

Li *et al*. (2009) crossed two commercial varieties and derived 198 recombinant inbred lines to study the genetics of grain hardness, wet gluten content, grain protein content, water absorption and sodium dodecyl sulphate sedimentation value on bread quality. These are the non-gluten factors that affect bread quality beside gluten fraction that is responsible for 30 to 60% variation in quality of bread. A genetic map was created using 255 marker loci, 250 simple sequence repeat markers, and five glutenin loci, Glu-A1, Glu-B1, Glu-D1, Glu-B3, and Glu-D3. For all attributes, a total of 73 QTLs were discovered. On chromosome 1B, a significant QTL for Grain hardiness was discovered and its 27.7% of phenotypic variation was contributed by GH. 30% of variation was due to water absorption, a major QTL on chromosome 5D. A pleiotropic effect was predicted since some QTLs for correlated traits formed QTL clusters while mapping.

Tiwari *et al*. (2009) generated recombinant inbred lines by breeding high zinc and iron efficient wild cultivar *Triticum boeticum* (pau5088) with *Triticum monococcum* (pau14087). Using an atomic absorption spectrophotometer, the Fe and Zn concentrations were determined. The RIL population's grain Fe and Zn concentrations ranged from 17.8 to 69.7 mg/kg and 19.9 to 64.2 mg/kg, respectively. Quantitative trait loci (QTL) for grain Fe and Zn accumulation were mapped using a population-wide linkage map. Two QTL for grain Fe were found on chromosomes 2A and 7A, while one QTL for grain Zn was found on chromosome 7A.

Khan *et al*. (2010) screened 14 recombinant inbred lines and 2 parent varieties Inqilab and Tatara to study the correlation and path coefficient relationship among morphological traits and grain yield. They found that days to maturity, no of tillers/m^2, and number of grains per spike were all positively correlated with grain yield. Plant height, spike length, peduncle length, peduncle extrusion, sheath length, and 1000-grain weight all had a negative connection with grain yield. In terms of the relationship between several criteria, positive correlation for genotype was 55.55% and 57.77% as for phenotypic correlation. Peduncle length had the highest direct effect on grain yield.

Chalish and Houshmand (2011) evaluated 104 recombinant inbred lines of durum wheat to study the morphological variation and estimate heritability of those characters. The genotypes along with parents and control were planted in completely

randomized design in pot experiment and augmented design in field experiment. 3 replications were made in both cases. As indicated by analysis of variance, the lines varied significantly in both pot and field conditions. Recombinant inbred lines displayed increased genetic diversity for grain production, number of seed per plant, harvest index, and number of tillers. Height, grain yield, spike length, harvest index, days to flowering, and number of seed per plant had low heritability, while height, grain yield, spike length, harvest index, days to flowering, and number of seed per plant had medium and high heritability. Grain yield was most closely related to the harvest index and the number of seeds per plant. The number of seeds per plant had a direct effect on grain yield, while the harvest index contributed indirectly to grain yield via seed quantity per plant. As a result, it appears that the quantity of seeds per plant is the most important factor in explaining variation in the studied RILs.

Li *et al*. (2012) generated two recombinant inbred lines population of wheat by crossing Weimei8 and Jimai 20 (485 inbred lines), Weimei 8 and Yannong 19 (229 inbred lines). These lines were screened at 5 different locations to detect QTL for seven quality traits that are important for determining pasta making quality. They found a total 150 QTLs spread throughout the genome, 13 of which were common to both the population that accounts for 20% and 15.29% of total QTL in 1st and 2nd population respectively. On chromosome 5A, a large QTL for GPC was discovered, accounting for 53.04 percent of the phenotypic variation. A substantial WGC QTL shared this interval as well, explaining more than 36% of phenotypic variance and being significant in two settings.

Aharizad *et al*. (2012) evaluated 94 recombinant varieties of recombinant inbred lines (descendants of cross between Roshan and Superhed#2) for multivariate analysis of genetic diversity in bread wheat. The lines were evaluated for a number of agronomical and morphological traits. Significant difference was observed from ANOVA for all the traits under study. Some traits like peduncle length, spike length number of grains per spike etc. showed higher level of genetic variance. This study discovered significant genetic variety among the genotypes studied, which could aid in future selection and breeding. The lines were divided into three categories based on cluster analysis using Ward's technique and squared Euclidean distances. Parents could

be chosen for crossing from clusters with high genetic distance in order to achieve genetic recombination and transgressive segregation in following generations. However, more research across locations and years is needed to back up the findings of this study.

Xu *et al*. (2012) utilized 182 F_{11} wheat recombination inbred lines, Xiaoyan 54 and Jing 411 used as parents, to analyze zinc, iron and protein content in two different environments to find out quantitative trait loci and their interaction. Nine additive and four epistatic QTLs were discovered, out of which six and four were effective in the two environments, respectively. On chromosomes 4B and 5A three intervals affecting 2 to 3 traits were discovered indicating a genetic basis for grain Zn, Fe, and protein concentrations.

Li *et al*. (2013) found that, In the wheat cultivar 'Clark,' quantitative trait loci (QTLs) for Hessian fly resistance (HFR), a major pest was located and was strongly connected to DNA markers for the QTLs. They developed RIL population by crossing ‘Ning7840’ and ‘Clark’. The RILs were used to construct a linkage map through single nucleotide polymorphism and simple sequence repeats marker. Two QTLs were identified on chromosome of 6B and 1A that served for resistant to GP fly biotype. The QTL on 6B is a novel wheat gene resistant to HFR that has not been reported before.

Zafarnaderi *et al*. (2013) conducted an experiment using eight recombinant inbred lines and their parental lines Roshan & Super Head on split-plot based randomized complete block design (RCBD) with three replications each replication with three irrigation levels to investigate the relationship between grain yield and agronomic traits in bread wheat under water deficit condition. The irrigation levels given were @ 80,120 and 160 mm evaporation from the pan. Grain yield, harvest index, and peduncle length all showed strong positive correlations in correlation analysis. According to a path analysis utilizing stepwise regression based on the average of irrigation factors, the most beneficial components on grain yield were the peduncle length, number of grains in spike, number of fertile tillers, and thousand grain weight. As a result, these characteristics could be employed as crucial indicators when selecting high-yielding plants.

Jia *et al*. (2013) crossed a commercial variety Nanda2419 with indigenous cultivar Wangshuibai and evaluated 230 RILs produced for yield contributing traits. Their findings corroborated some previously reported wheat QTLs while also identifying several new ones, namely QSn.nau-6D for productive tillers, QGn.nau-4B.2 for grain number, QGw.nau-4D for seed weight, QPh.nau-4B.2 and QPh.nau-4A for plant height, and QFlw.nau-5A.1 for flag leaf width. Through the Nanda2419 alleles, four QTL clusters contributed significantly to breeding goal-based trait improvement in trials conducted in distinct ecological zones.

Case *et al*. (2014) evaluated 268 RILs developed from a cross between wheat cultivars Coda and Brundage to construct a linkage map assessing seedling reaction to strip rust disease. The lines were grown in 9 different locations of U.S Pacific Northwest. With the exception of 1D, a linkage map of 2,391 polymorphic DNA markers was generated, covering all chromosomes of wheat. The most significant QTL found were two on chromosome 1B that were linked to adult plant and seedling reaction. In the seedling stage, these QTL reduced adult plant infection type from seven to two, reducing disease severity by an average of 25% and providing protection against races PST-100, PST-114, and PST-127. A single QTL on chromosome 5D was related with seedling reaction to PST-114, while two more QTL on chromosome 3B and one on chromosome 5B were related with mature plant reaction exclusively. This identification of molecular markers will allow for the precise transfer of these genes into other cultivars, ensuring that stripe rust resistance remains high.

Gande *et al*. (2014) developed recombinant inbred lines by breeding rice varieties IRRI38 And Jeerigesanna to evaluate variation in grain zinc content and identify their potential candidate gene. 8 out of 24 candidate gene showed polymorphism and all three simple sequence repeats (SSR) showed polymorphism. Four putative gene markers (OsNAC, OsZIP8a, OsZIP8c, and OsZIP4b) showed significant variation among RILs, with phenotypic variations of 4.5, 19.0, 5.1, and 10.2 percent, respectively, according to single marker analysis. Validation with 96 rice genotypes revealed three markers OsZIP8a, OsNAC, and OsZIP4b with 11.0%, 5.8%, and 4.8 % phenotypic variance, respectively.

PU *et al*. (2014) performed QTL analysis in two recombinant inbred line population to detect the genetic factors in relation with micronutrient concentration like iron, zinc, copper, manganese and selenium. They found 39 QTLs for five micronutrient concentrations in total. In both populations, five QTLs on chromosomes 2A, 3D, 4D, and 5B revealed considerable phenotypic variation for 2–3 micronutrient concentrations.

Gopalareddy *et al*. (2015) evaluated 30- recombinant inbred lines along with its parent and commercial variety PBW 343 in three different environments for two years to assess iron and zinc concentration in wheat grains. They found that environment had the most significant effect that accounted for 37.42% and 57.78% variation for Fe and Zn concentration respectively. For Fe and Zn, the genotype-environment interaction (G x E) accounted for 29.46% and 23.24% of the overall sum of squares, respectively. The G x E interaction had a relatively large magnitude. Iron (0.81) and zinc (0.71) concentrations had high heritability, indicating a non-crossover kind of interaction. The positive and relatively high correlation (0.677**) between Fe and Zn concentrations suggests that both micronutrients will improve at the same time.

Shi *et al*. (2020) utilized the recombinant inbred lines to construct a high-density genetic map and assess the genetic architecture of wheat metabolome. They analyzed the wheat kernel metabolism and researched comprehensive metabolome using widely targeted LC-MS/MS. They also studied the genetic link between metabolites and agronomic traits. There were 1260 metabolic characteristics found in all. 1005 metabolic quantitative trait loci (mQTLs) were discovered using linkage analysis and were dispersed irregularly across the genome. Twenty-four candidate genes were discovered to control the levels of various metabolites, two of which were functionally annotated as being involved in the production and modification of flavonoids by in vitro investigation. This research uses metabolomics and association analysis to better understand the metabolite agronomic trait correlation, genetic basis of wheat metabolism, which predicts the performance of a genotype and will aid wheat breeding in the future.

Singhal *et al*. (2021) reported the QTL for iron and zinc concentration in pearl millet by creating a linkage map through simple sequence repeats with the help of 210

F_6 recombinant inbred lines (parents PPMI 683 and PPMI 627). On three years of research at 3 locations, QTL analysis found a total of 22 QTLs for grain Fe and Zn, with 14 for Fe and eight for Zn. Grain Fe levels in the RIL population ranged from 36 to 114 mg/kg, while Zn levels ranged from 20 to 106 mg/kg. In this group, two constitutive QTLs for Fe and Zn was co-mapped. Bioinformatics techniques identified potential genes such as Ferritin, Al3+ Transporter, K+ Transporters, Zn2+ transporters, and Mg2+ transporters inside QTLs.

2.4 Genetic biofortification of Iron and Zinc

Cakmak and coworkers (1994) experimented on mobilization of phytosiderophores from roots of two cultivars, Zn efficient Aroona and Zinc inefficient Durati in Zinc and Iron deficient conditions. Under Zn deficiency Aroona responded well by releasing high amount of phytosiderophores (deoxymugineic acid and 3 hydroxymugineic acid) whereas phytosiderophore release in Durati was significantly lower at 25 days. However, Aroona and Durati released similarly high amount of phytosiderophores under Fe deficiency and also under both nutrient deficiency.

Velu *et al*. (2012) tested 37 advanced lines obtained from crosses of high yielding wheats with genetic resources possessing high Zn and Fe concentration like *Triticum spelta*, landraces and *Triticum dicoccum* based synthetic wheats. The trials were carried out at 9 locations in Mexico, India and Pakistan for Fe, Zn concentration, grain yield, and other traits. ANOVA test revealed significant genotypic, environmental and genotype × environment (G × E) effects. Environment had greater effect than genotype and G × E interaction. High heritability with high genetic correlations between locations were observed, many entries exceeded the target level of Zn across different environments. This provided one of the first proofs for genetic biofortification of wheat with considerably high yield as well as farmers preference.

Garcia *et al*. (2014) have reviewed on the health issues arising due to iron and zinc deficiency and evaluated the economy behind agronomic and genetic biofortification. They suggested that while agronomic biofortification is a supportive strategy to improve these micronutrients in food crops it is short term and cost inefficient whereas genetic biofortification is the reliable long-term strategy for increasing Fe and Zn in dietary food source of developing countries population. Thus,

breeding of cereal crops for nutrient enrichment holds a grand scope of research and is the best way to fortify micronutrients than other methods like supplementation, fortification or diversification.

Guzman *et al*. (2014) evaluated 141 advance lines derived from crosses between landraces and common wheat ancestors (*Aegilops tauschii*, *Triticum turgidium ssp. diccocoides*, *T. turgidium ssp. dicoccum* and *T aestivum ssp. spelta*) species with high productivity CIMMYT lines to select lines that show high Fe and Zn concentration along with high yield. Several lines showed higher micronutrient content than check i.e. (29.4 mg Fe/kg and 21.7 mg Zn/kg). some genotypes showed Zn content as high as 53 mg/kg. these advance lines obtained from Harvestplus yield trial revealed genotypes with good processing quality along with high Zn and Fe concentration.

Slamet-Loedin *et al*. (2015) have reviewed various ways of enhancing Zn and Fe content in wheat along with decrease in Cd content. Cadmium uptake and translocation has been found to be associated with divalent metal cations such as Fe and Zn. For example; drying of soil increases Zn uptake along with increase in Cd. Even a moderate amount of Cd contamination is dangerous for health. They have given several agronomic, genetic and management approaches to tackle this situation in rice.

Verma *et al*. (2016) directed an experiment to transfer 2S chromosomal fragment from *Aegilops kotschyi* into *Triticum aestivum* genome to biofortify bread wheat with high iron and zinc concentration. The material used was Wheat Ae. Kotschyi 2A/2Sk substitution line and method used was seed irradiation approach. The radiation induced hybrids were better than check variety WL711 in iron and zinc content. They recorded a 54% increase in zinc content and 65% increase in iron content with better harvest index implying that the translocation was effective.

Amiri *et al*. (2020) focused on the genetic analysis of genes GFeC and GZnC in wheat to understand the inheritance pattern of these nutritional quality traits under normal and drought stress condition. Two crosses were made using high GFeC and GZnC cultivar “Marvdasht” (high yielding) as maternal parent and “Rassoul” and “Shahpasand” as paternal parents. Six basic generations P1, P2, F1, F2, BC1, BC2 were evaluated using generation mean analysis. Mardasht × Rassoul cross showed more

response to selection for GFeC whereas Mardasht × Shahpasand had more response to selection for GZnC.

Ali *et al*. (2020) gave an insight on use of novel genomic resources like high quality wheat genome sequence, gene editing, transgenics, tilling approaches etc. to identify candidate genes controlling micronutrient content in wheat and integrate the knowledge into successful biofortification of the crop.

2.5 Transgenic approach

Goto *et al*. (1999) made the first attempt to obtain Fe enriched rice by transferring coding region of ferritin gene from Soybean to rice through Agrobacterium mediated transformation. GluB 1 (seed storage protein glutelin promoter) from rice was used as promoter. The ferretin subunits significantly increased in seed specifically in endosperm tissues. The transformed seeds showed in increasement in iron content as high as three times the untransformed control.

Holm *et al*. (2002) attempted a transgenic approach to improve bioavailability of iron and zinc in wheat. According to the researchers, phytic acid is a phosphate bound compound that hinders the iron and zinc uptake. The reduction in phytic acid concentration may help increasing iron and zinc bioavailability. They have described their work where they have introduced a phytase encoding gene from *Aspergillus niger* along with constitutive endosperm specific promoter to wheat immature embryos. The result suggested that fungal phytase can be synthesized in wheat and builds a ground for further studies but inactivation of *A. niger* phytase above 60 ℃ seems to a problem in cereal crops since cooking or baking will destroy the phytase.

Connorton *et al.* (2017) reported vacuolar iron transporter ($TaVIT_2$) found in wheat is beneficial for iron and zinc biofortification. They obtained more than 2-fold increase in iron in wheat flour by overexpressing the gene ($TaVIT_2$) under the control of endosperm-specific promoter. Phytic acid was not increased during $TaVIT_2$ overexpression and the biofortified iron was available in the white flour fraction.

Malik and Maqbool (2020) have reviewed and summarized few methods to enrich grain iron and zinc through transgenic approach. Some of them were introducing

coding sequence that codes zinc binding proteins, nicotianamide synthase gene overexpression, phytic acid reduction, NAC gene overexpression etc.

Chapter III

MATERIALS AND METHODS

The present study entitled, "Genetic variation and principal component analysis for agronomic and grain quality traits in MNP-2 recombinant inbred line (RIL) population of bread wheat (*Triticum aestivum*)." was carried out at Agriculture Research farm, Institute of Agricultural sciences, Banaras Hindu University, Varanasi during Rabi season 2021-2022. The details regarding material, methods, observation and statistics used are discussed under following headings.

3.1 Experimental location

3.2 Agroclimatic condition of the site

3.3 Experimental material

3.4 Experimental design and layout

3.5 Observations recorded

3.6 Statistical analysis

3.1 Experiment location

The experiment site of the given experiment Agriculture research farm, BHU is located in Varanasi, Uttar Pradesh. The site lies in the Indo Gangetic plains on the south eastern part of Varanasi city at 25º 18' north latitude and 82º 59' east longitude and the elevation is 75.5 meter above sea level.

3.2 Agroclimatic condition

Varanasi lies at the region of tropic of cancer hence the climate is moist subtropical with high variation in temperature in summer and winter. The summer temperature ranges between 32 to 46 and winter temperature lies between 5 to 15. The average annual rainfall is 1110mm majority of which is received during the month of July to September. The mean relative humidity lies between 40-50% but can reach upto 70% during rainy months. The soil here is fertile alluvial loam since it lies on the banks of Ganges river which is suitable for growing our experimental material. During our

experiment the temperature was about 24 during sowing, 20 at flowering and 40 during harvesting. The relative humidity was about 27-45%.

3.3 Experimental material

The materials used in this experiment were 232 RILs, named as MNP-2, which were derived from diverse parents SOKOLL//W15.92/WBLL1 and CIANO T 79. This RIL population was originally developed by International Maize and Wheat Improvement Center (CIMMYT, Mexico). Among the 232 RILs, Six lines (RIL 12, 47, 122, 123, 124, and 126) did not germinate due to unknowing reasons and they were excluded from all the analysis performed. Further two genotypes HD2967 (C1) and TAW95 (C2) were used as checks in the augmented design used.

3.4 Experimental design and layout

The 232 RIL's (MNP-2) were sown in augmented design with repeated checks HD2967 (C1) and TAW95 (C2), and parents SOKOLL//W15.92/WBLL1 and CIANO T 79. There were total 4 blocks, and block I and III were had 56 and 54 genotypes respectively, whereas block II and IV had 58 genotypes each. In all blocks two checks were repeated for comparison. Each genotype sown in 1m row with row to row distance of 22.5 cm and plant to plant distance of 5 cm. The experimental material was sown on 22nd Nov 2021. All recommended agronomic practices, crop protection measures were followed to ensure proper growth of all genotypes.

3.5 Observations recorded

3.5.1 Germination percentage (GP %)

The germination % was recorded 14 days after date of sowing. The percentage was assigned as per the visual observation.

3.5.2 Plant growth habit

On the basis of angle of culm, the plant is categorized into following classes according to the scale defined by PPVFRA, 2007.

Category	Scale
Erect	1
Semi- erect	3
Intermediate	5
Semi-prostrate	7
Prostrate	9

3.5.3 Foliage color

Depending on the visual intensity of the color of leaves, the lines are classified into following three categories (PPVFRA, 2007)

Category	Scale
Pale green	1
Green	5
Dark green	9

3.5.4 Chlorophyll content

Relative greenness of Chlorophyll content was measured with the help of SPAD meter (SPAD 502 Plus Chlorophyll Meter, Konica Minolta). It is a hand-held device that measures the absorption of red wavelength (650nm) and infrared wavelength (940nm). The difference in optical density between two wavelength is read by the meter and provides a relative numerical SPAD value. Higher the absorption, higher the SPAD value and more is the chlorophyll content. The meter is useful for instantaneous and non-destructive reading on a plant leaf. (Konica Minolta, 2009)

From each line 5 plants were chosen and SPAD value was taken from 1/3 rd portion from lower leaf base of fully emerged flag leaf. The value usually lies between 0.0 and 50.0.

3.5.5 Leaf color chart

The leaf color chart is a 15 inch long ruler shaped tool made by IRRI (IRRI leaf color chart) with 5 strips of gradient of green color,that ranges from yellowish green to

dark green. The fully emerged flag leaf is chosen from a healthy plant and the middle section of leaf is placed across the LCC and the color of leaf is compared with the panel strips in LCC and accordingly the number is assigned between 1-5.

3.5.6 Days to 50% heading (days)

The no of days taken from date of sowing to heading in 50% of total plants was recorded.

3.5.7 Plant height (cm)

Plant height of 5 plants from each line taken. The plants were measured from ground to tip of spike excluding awns and is expressed in cm. This measurement was taken at the time of maturity so that the plant has attained it full growth potential.

3.5.8 Peduncle length (cm)

The peduncle length was measured in 5 plants which were recorded for plant height, from latest node to the base of spike and was recorded to nearest cm.

3.5.9 Spike length (cm)

The spike length of 5 observational plants were taken from base of spike to the tip (excluding awns) and was recorded to nearest cm.

3.5.10 Leaf angle

The scoring of flag leaf angle is done at early grain filling stage according to PPVFRA, 2007. The angle at which the leaves are held relative to the vertical axis can lead to difference in appearance of canopy and degree of light penetration towards lower leaves. Based on the leaf angle scoring is done.

Category	Scale
Erect	1
Horizontal	2
Pendant	3

3.5.11 Lodging type

Two types of lodging can be recognized 1. Stem lodging where roots are held firmly in soil but the culm bends through internodes. 2. Root lodging here plants anchorage becomes weak and lodges from ground. The type of lodging is recorded

3.5.12 Lodging percentage (%)

The proportion of culms lodged within the plot is recorded as per visual observation. The scale ranging from 0 to 100%

3.5.13 Days to physiological maturity (days)

The spike starts drying and peduncle becomes yellowish in color, this is termed as physiological maturity. The no of days when 50% of plants in a line exhibit this characteristic is recorded and it is the days to physiological maturity.

3.5.14 Total Biomass [Biomass (grams)]

Each genotype was harvested separately and weight of total biomass of each genotype was recorded.

3.5.15 Grain yield (grams)

The spikes from each genotype were threshed separately and their total grain weight was recorded.

3.5.16 Harvest index

Harvest index is expressed as the ratio of grain yield of genotype to biological yield of that genotype

$$Harvest\ index\ \% = \left(\frac{grain\ yield}{biological\ yield}\right) \times 100$$

3.5.17 Grain iron and zinc content (ppm)

Iron and zinc content were measured with the help of XRF machine. The XRF machine works on the principle of X-ray fluorescence caused by atoms in the sample being excited. An inner shell electron of the atom is hit by a primary X-ray and the electron is ejected from the atom. Fluorescence radiation is emitted when an electron from a outer shell fills the open place. The energy difference between the two election

shells equals the fluorescence energy. As a result, the energy of this radiation is specific to the atom and can be used to determine which atom is present in the sample.

3.5.18 1000 kernel weight (grams)

1000 seeds of each genotype were counted and weight of those 1000 seeds was recorded.

3.6 Statistical Analysis

The recorded data for all agronomic and quality traits of sample plants were averaged to calculate mean values and the data was subjected to following statistical analysis.

3.6.1 Analysis of variance

The ANOVA test allows to compare more than two groups to see if there is a significant relationship between them. The F statistic (also known as the F-ratio) is a result of the ANOVA formula that allows for the study of many sets of data to identify the variability between and within samples.

In the present study Augmented ANOVA was performed for 232 RIL's (excluding those which were not germinated) with two checks in R software and all the analysis output including means, CV, GCV, PCV, heritability etc. were presented below.

To test the significance of differences among genotype of RILs, the data on mean values for different characters was analyzed as per standard statistical procedure for Augmented block design as proposed by Federer and Raghavarao (1975)

Table 3.1: Analysis of Variance (ANOVA) table for Augmented design.

Source of variation	Degree of freedom	Sum of Square	Mean Sum of Square	F-Ratio
Blocks eliminating treatment	b-1	SSB	MSSB=S_1	S_1/S_6
Treatment eliminating blocks	v-1	SSTr	MSSTr= S_2	S_2/S_6
Among tests	w-1	SSTs	MSSTs=S_3	S_3/S_6
Among checks	u-1	SSC	MSSC=S_4	S_4/S_6
Test vs checks	1	SSTc	MSSTc=S_5	S_5/S_6
Error	n-v-b-1	SSE	MSSE=S_6	
Total	n-1	TSS		

Where,

n = Number of total treatments (test genotypes + replicated checks)

b = no. of blocks

w = no. of test genotypes

u = no. of checks

v = w + u (no. of tests + no. of checks)

SSB = Sum of square of blocks

SSTr = Sum of square of treatment (genotype)

SSTs = Sum of square of tests

SSC = Sum of squares of checks

SSTc: Sum of squares of test vs checks

SSE = Sum of square due to error

TSS = Total sum of squares

MSSB = Mean sum of square of blocks

MSSTr = Mean sum of square of treatment (genotype)

MSSTs = Mean sum of square of test genotypes

MSSC = Mean sum of square of checks

MSSTc: Mean sum of squares of test vs checks

MSSE = Mean sum of square due to error

The calculated F values is compared with table F value, to identify significance at 5% and 1% level of significance.

3.6.2 Descriptive Statistics

3.6.2.1 Mean performance

It is the average of total samples collected for a particular genotype.

3.6.2.2 Range

The maximum and minimum reading within the population determines the range for that particular trait.

3.6.3 Genetic variability parameters

The phenotypic, genotypic and environmental variance (σ^2p, σ^2g and σ^2e) are obtained from the ANOVA tables according to the expected value of mean square described by Federer and Searle (1976) as follows:

$$\boldsymbol{\sigma^2g = \sigma^2p - \sigma^2e}$$

3.6.5 Phenotypic and genotypic coefficients of variation (PCV and GCV)

Phenotypic and genotypic coefficients of variation (PCV and GCV) are estimated according to Burton and Devane (1953) as follows:

$$\mathbf{GCV = (\sigma^2g / \sqrt{x}) \times 100}, \text{ where x is the mean.}$$

The estimates of P CV and GCV are categorized according to Siva Subramanian and Madhavamenon (1978) as follows:

Table 3.2: Scale for categorization of GCV and PCV (Subramanian and Menon, 1973)

Low	<10%
Moderate	10% - 20%
High	>20%

3.6.4 Broad sense heritability [H^2]

Heritability (as broad sense) for different traits were estimated by using the components of variance as per Hanson *et al*. (1956) in the following manner.

$$\mathbf{H^2(broad\ sense) = \frac{\sigma^2_g}{\sigma^2_p} \times 100}$$

Where,

σ^2_g = genotypic variance

σ^2_p = phenotypic variation

H^2 = heritability (broad sense)

As suggested by Johnson *et al* (1955), heritability values are categorized as follow:

Table 3.3: Scale for heritability (Johnson *et al*. 1955)

Low	<30%
Moderate	30% - 60%
High	>60%

3.6.5 Genetic advance as percent of mean (GAM)

It is the measurement of genetic gain under selection and it was estimated by using the formula suggested by Lush (1949), followed by Johnson *et al*. (1955) and allard (1960)

$$\textbf{Genetic Advance (GA)} = \boldsymbol{k \times \sigma_{\rho} \times h_{b}^{2}}$$

where,

k = Selection differential at 5% selection intensity which accounts to a constant value of 2.063.

h_{b}^{2} = heriatability in board sense.

σ_{ρ} = phenotypec standard deviation

$$GAM = \frac{GA}{\bar{x}} \times 100$$

Table 3.4: Scale for genetic advance as percent of mean (Johnson *et al*, 1955)

Low	<10%
Moderate	10% - 20%
High	>20%

3.6.6 Correlation studies

It was computed to find out the association between the variables measured. Pearson phenotypic correlations were worked out by using formulae

3.6.6.1 Phenotypic coefficient of correlation (r_p)

$$r(x_i.x_j)_g = \frac{Cov(x_i.x_j)_g}{\sqrt{v(x_i)_g v(x_j)_g}}$$

where,

$r(x_i.x_j)_g$ = Genotypic correlation between i^{th} and j_{th} character

$Cov(x_i.x_j)_g$ = Genotypic covariance between i^{th} and j_{th} character

$v(x_i)_g$ = Genotypic variance of i^{th} character

$v(x_j)_g$ = Genotypic variance of j^{th} character

Table 3.5: Scale for Correlation Coefficients

S.N.	Values of correlation coefficient	Scale
1	>0.65	Very strong
2	0.50 to 0.64	Moderately strong
3	0.30 to 0.49	Moderately weak
4	<0.30	Very weak

3.6.6.2 Test of significance

The calculated value of correlation coefficients was tested for its significance by comparing the observed value of correlation coefficients with the table value of 'r' given by Fisher and Yates (1963) available in standard books at (n-2) degrees of freedom as follows:

$$t = \frac{r}{\sqrt{(1 - r2)}} \times \sqrt{n - 1}$$

Where,

r = correlation coefficient

n = number of genotypes used for the pair of characters

3.7 Principal component Analysis

Principal Component Analysis (PCA) is a dimensionality-reduction approach for reducing the dimensionality of large data sets through feature extraction by transforming a large collection of variables into a smaller one that retains the majority of the information in the larger set. Smaller data sets are easier to evaluate and visualize, and machine learning algorithms can analyze data much more easily and quickly without having to deal with additional factors. PCA's basic concept is to reduce the number of variables in a data collection while retaining as much information as feasible (Abdi *et al*, 2010) It was developed by Karl Pearson in 1901 and later by Harold Hotelling in 1930s.

3.7.1 Determining principal component

1. Ensure that the range of continuous initial variables is uniformly standardized.

Standardization is done to equalize contribution of each continuous initial variable to the analysis because PCA is quite sensitive to the variances in initial variables and can cause biasness. It can be done by following formula

$$z = \frac{v - \bar{x}}{SD}$$

Where,

v = value of data points

$\bar{x}$ = mean of variable

SD = Standard deviation

Standardization will transform all variables into same scale.

2. To find correlations, compute the covariance matrix.

This step figures out how the variables in the input data set differ from the mean in relation to one another, that is it determines if there is any association between them. Thus, covariance matrix was computed in order to find these associations.

$$Cov(p,q) = \frac{\sum(P_i - \vec{P})(qi - \overline{q)}}{n-1}$$

Where,

Cov (p, q) = covariance between variables p and q

p_i = value of data p

q_i = value of data q

$\bar{p}$ = mean of p

$\bar{q}$ = mean of q

n = no of data values

$$\begin{bmatrix} Cov(p,p) & Cov(p,q) & Cov(p,r) \\ Cov(q,p) & Cov(q,p) & Cov(q,r) \\ Cov(r,p) & Cov(r,q) & Cov(r,r) \end{bmatrix}$$

Covariance matrix for 3 combinational data

3. Determine the principal components by computing the eigenvectors and eigenvalues of the covariance matrix.

When we draw a scatterplot there can form a line which best represent the random variable. This line is known as line of best fit and its direction gives the Eigenvector. Thus, Eigenvector is the direction of the covariance matrix which holds the most variance, that is our principal component. It is a unit vector that has a magnitude 1.

Eigenvalues are the coefficients applied to eigenvectors that determine the length or magnitude of the vectors.

$$Eigen\ value\ for\ PCn = Sum\ of\ square\ (distance\ for\ PCn)$$

$$Singular\ value\ for\ PCn\ = \sqrt{Eigen\ value\ for\ PCn}$$

$$Variation\ for\ PCn = \frac{Eigen\ value\ (PCn)}{n-1}$$

Where,

PC_n = n^{th} principal component

n = no of variables

The first Eigenvector is the main principal component (PC1) that accounts for greatest possible variance in the data set. The second principal component is perpendicular to the PC1 and this explains for highest variance after PC1 and similar follows. Therefore, Principal components are new variables that are created by integrating the basic variables in a linear way. The new variables (i.e., principal components) are uncorrelated as a result of these combinations, and the majority of the information from the initial variables is condensed into the principal components

4. Draw a feature vector to help select which of the main components to maintain

The feature vector is essentially a matrix with the eigenvectors of the components we want to maintain as columns. Because we only keep p eigenvectors (components) out of n, the final data set will only have p dimensions.

5. Replot the data on the axis of the principal components

In the final step the data is reoriented from its axis to the new one as directed by the principal component. This is done by using the feature vector that is formed from the covariance matrices eigenvectors.

6. Biplot Analysis

A PCA Biplot is a two-dimensional graph that depicts the relation between a numbers of variables in the newly formed principal component axes. The points within the axes are the variables and the arrow represent the direction of increase in value.

Chapter IV

EXPERIMENTAL FINDINGS

The present study has been carried out to study genetic variability, correlation among different parameters recorded and to display the pattern of similarity of the observations and of the variables as points in maps by PCA analysis. The results obtained through statistical analysis of the data are presented below under following subheadings.

4.1 Analysis of Variance (ANOVA)

4.2 Descriptive Statistics

4.3 Variability, heritability and Genetic Advance

4.4 Correlation studies

4.5 Principal Component Analysis (PCA)

4.1 Analysis of Variance (ANOVA)

The analysis of variance for 13 characters is shown in the Table 4.1. The characters under study leaf color, chlorophyll content, spike length, Peduncle length, Plant height, days to 50% maturity, yield, biomass, harvest index, were significant among the treatment genotypes (RIL + checks) revealing that there is a vast variation among genotypes for these parameters.

The traits also showed a significant difference between test genotypes and the checks which proves that these inbred lines vary from those used as checks. Fe and TKW showed significant difference between test vs check only. Zn content and days to maturity showed no significant difference. There were no variation between blocks for most of the traits suggesting homogeneity in the experiment layout. Further, only in test treatments (only 232 RILs), Biomass, DH, HI, LCC, PHT, PL, SL and SPAD showed significant variation among them.

Table 4.1: Analysis of variance (ANOVA) table

Source	Df	Biomass	DH	DM	Fe	HI	LCC	PHT	PL	SL	SPAD	TKW	YIELD	Zn
Treatment (ignoring Blocks)	227	15912.1 *	58.27 **	10.59 ns	11.83 ns	98 **	0.31 *	167.67 **	43.81 **	1.15 *	14.13 **	28.21 ns	2956.87 *	8.47 ns
Treatment: Check	1	9870.12 ns	66.13 **	91.13 **	17.61 ns	36.22 *	0.0013 ns	15.68 ns	2 ns	1.39 *	4.68 *	90.59 ns	110.48 ns	6.62 ns
Treatment: Test vs. Check	1	1287223 **	287.36 **	0.83 ns	21.63 *	70.72 **	2.07 **	2117.44 **	2486.67 **	1.67 *	39.83 **	145.16 *	123546.93 **	18.96 ns
Treatment: Test	225	10288.68 *	57.22 **	10.27 ns	11.76 ns	98.4 **	0.3 *	159.68 **	33.14 **	1.14 *	14.05 **	27.41 ns	2433.57 ns	8.43 ns
Block (eliminating Treatments)	3	5685.12 ns	2.46 ns	3.13 ns	6.65 ns	7.06 ns	0.17 ns	23.69 *	3.59 ns	0.64 *	3.1 *	13.55 ns	1754.71 ns	2.65 ns
Residuals	3	1005.46	1.12	2.46	2.05	1.74	0.02	1.72	0.41	0.06	0.28	10.87	321.73	2.34

* Significant at P <= 0.05, ** Significant at P <= 0.01

LCC= Leaf color chart**, SPAD =** SPAD Chlorophyll content **SL =** Spike length**, PL =** Peduncle length**, PHT =** Plant height**, DH =** Days to heading**, DM =** Days to physiological maturity**, HI =** Harvest index**, Fe =** Iron content**, Zn =** Zinc content**, TKW =** Thousand kernel weight.

4.2 Descriptive Statistics

The descriptive statistics of 13 traits are presented in table 4.2

Table 4.2: Mean, Range, Standard Error (SE), Standard Deviation (SD), Skewness and Kurtosis

Traits	Mean	Range		SE	SD	Variability	Skewness	Kurtosis
		Min	Max					
LCC	3.75	2.5	5	0.04	0.54	0.29	-0.19 ns	2.18 **
SPAD	46.97	29.1	55.46	0.28	4.22	14.05	-0.59 **	4.11 **
SL	10.45	7.4	14.2	0.08	1.14	1.142	0.45 **	3.58 ns
PL	50.14	31.4	66	0.4	5.98	33.14	-0.27 ns	2.88 ns
PHT	114.4	82.8	140.6	0.85	12.89	159.67	-0.25 ns	2.43 *
DH	75.03	59	93	0.5	7.53	57.21	0.01 ns	2.31 **
DM	116.3	110	126	0.21	3.15	10.27	0.67 **	3.05 ns
Yield	112.83	13.02	32.7	3.79	57.24	2433.56	0.76 **	3.9 *
Biomass	305.21	108	775.17	7.84	118.36	10288.68	1.16 **	5.04 **
HI	36.57	8.73	60.85	0.66	9.97	98.39	-0.67 **	3.45 ns
Fe	35.95	28.1	47.9	0.25	3.75	11.76	0.3 ns	3.44 ns
Zn	25.59	17.85	35.15	0.2	2.96	8.42	0.47 **	3.11 ns
TKW	37.13	23.77	50.59	0.37	5.62	27.41	-0.14 ns	2.93 ns

non significant (ns) P > 0.05; Significant at * P <= 0.05; ** P <= 0.01

LCC= Leaf color chart**, SPAD =** SPAD Chlorophyll content **SL =** Spike length**, PL =** Peduncle length**, PHT =** Plant height**, DH =** Days to heading**, DM =** Days to physiological maturity**, HI =** Harvest index**, Fe =** Iron content**, Zn =** Zinc content**, TKW =** Thousand kernel weight

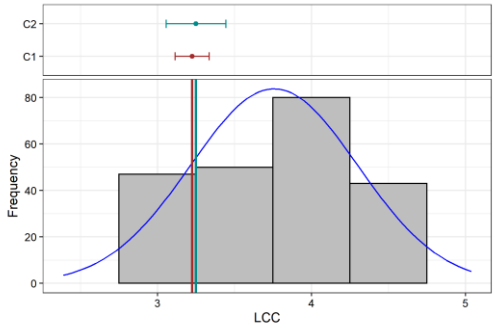
4.2.1 Check Statistics from augmented ANOVA

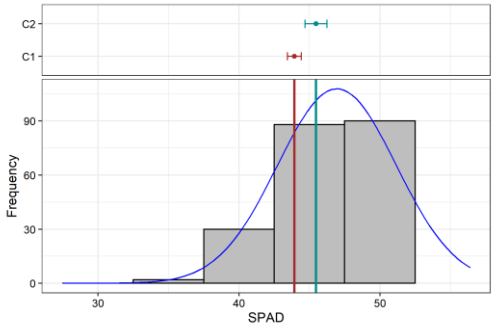
The descriptive statistics (mean, standard error (SE), minimum and maximum values) of checks C1 and C2 for all the traits are presented in Table 4.3

Table 4.3: Check statistics for 13 traits in checks C1 and C2

SN	Trait	C1				C2			
		Mean	SE	min	max	Mean	SE	min	max
1	LCC	3.23	0.11	3	3.5	3.25	0.19	2.9	3.8
2	CC	43.93	0.49	43.26	45.36	45.46	0.78	44.16	47.32
3	SL	10.40	0.29	9.6	10.84	9.57	0.30	9	10.08
4	PL	31.70	0.52	30.4	32.8	32.70	0.85	31.4	35
5	PHT	99.25	1.40	97.6	103.4	96.45	2.10	93.2	102
6	DH	84.00	0.41	83	85	78.25	0.85	76	80
7	DM	120.00	1.08	118	123	113.25	0.48	112	114
8	Yield	235.57	13.43	198	260	243	18.41	200	290
9	Biomass	748.50	30.20	660	790	678.25	27.58	609	744
10	HI	31.42	0.78	30	32.91	35.68	1.26	32.84	38.97
11	Fe	36.14	1.17	33.03	38.23	39.11	0.90	36.8	41.1
12	Zn	26.25	0.79	23.9	27.4	28.07	0.79	26	29.82
13	TKW	38.10	0.61	36.9	39.37	44.83	2.39	40	49.22

LCC= Leaf color chart**, SPAD =** SPAD Chlorophyll content **SL =** Spike length**, PL =** Peduncle length**, PHT =** Plant height**, DH =** Days to heading**, DM =** Days to physiological maturity**, HI =** Harvest index**, Fe =** Iron content**, Zn =** Zinc content**, TKW =** Thousand kernel weight


C2
C1
Frequency
0
20
40
60
80
3
4
5
LCC


C2
C1
Frequency
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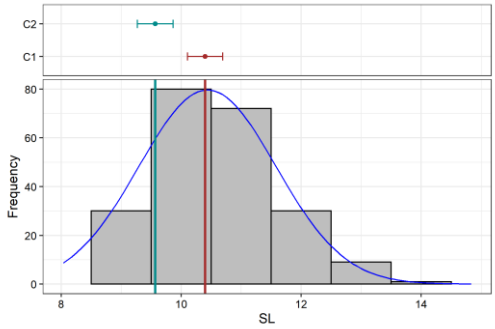
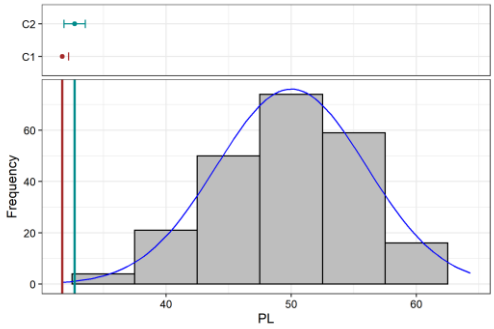
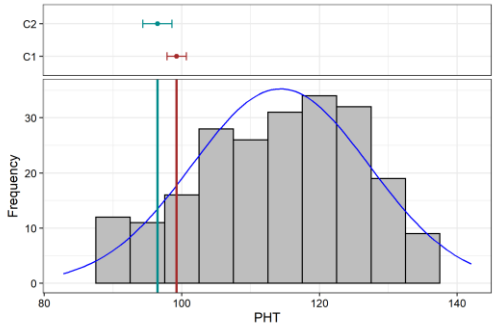
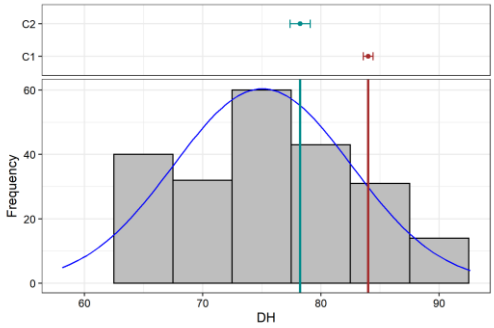
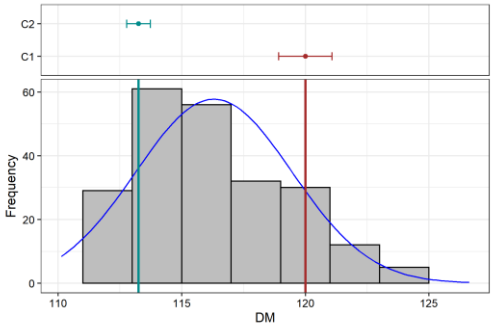
30
60
90
30
40
50
SPAD


C2
C1
Frequency
0
20
40
60
80
8
10
12
14
SL


C2
C1
Frequency
0
20
40
60
40
50
60
PL


C2
C1
Frequency
0
10
20
30
80
100
120
140
PHT


C2
C1
Frequency
0
20
40
60
60
70
80
90
DH


C2
C1
Frequency
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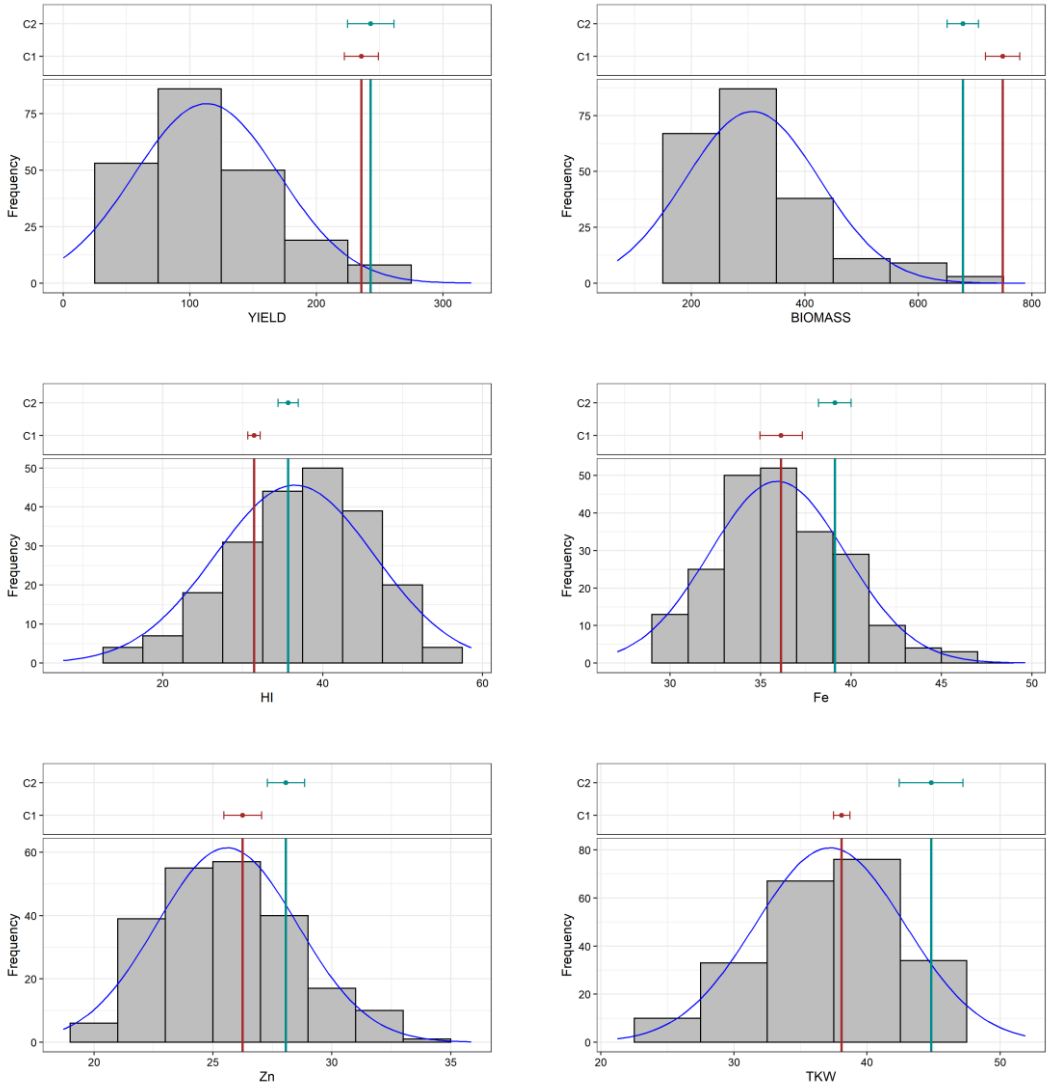
60
110
115
120
125
DM



LCC= Leaf color chart**, SPAD** = SPAD Chlorophyll content **SL** = Spike length**, PL** = Peduncle length**, PHT** = Plant height**, DH** = Days to heading**, DM** = Days to physiological maturity**, HI** = Harvest index**, Fe** = Iron content**, Zn** = Zinc content**, TKW** = Thousand kernel weight

Figure 4.1: Frequency distribution curve of 13 traits

4.3 Variability, Heritability and Genetic advance from Augmented ANOVA

The variability parameters Genetic coefficient of variability (GCV), Phenotypic Coefficient of variability (PCV), Environment coefficient of variability (ECV), Broad sense heritability (hBS), Genetic advance (GA), and Genetic advance as percent of mean (GAM) for all 13 traits are presented in Table 4.4.

Table 4.4: Genetic variability parameters, Broad sense heritability and genetic advance

Trait	Adj. mean	PV	GV	EV	GCV	PCV	ECV	hBS	GA	GAM
LCC	3.76	0.3	0.27	0.02	13.94	14.55	4.17	91.77	1.03	27.54
SPAD	46.94	14.05	13.78	0.28	7.91	7.99	1.12	98.02	7.58	16.15
SL	10.44	1.14	1.08	0.06	9.97	10.24	2.34	94.77	2.09	20.03
PL	50.01	33.14	32.73	0.41	11.44	11.51	1.29	98.75	11.73	23.45
PHT	114.32	159.68	157.96	1.72	10.99	11.05	1.15	98.92	25.79	22.56
DH	75.05	57.22	56.09	1.12	9.98	10.08	1.41	98.03	15.3	20.38
DM	116.3	10.27	7.81	2.46	2.4	2.76	1.35	76.07	5.03	4.32
Yield	113.32	2433.6	2111.8	321.73	40.55	43.53	15.83	86.78	88.32	77.94
Biomass	307.57	10289	9283.2	1005.5	31.33	32.98	10.31	90.23	188.8	61.39
HI	36.5	98.4	96.66	1.74	26.93	27.18	3.62	98.23	20.1	55.07
Fe	35.93	11.76	9.72	2.05	8.68	9.55	3.98	82.61	5.85	16.27
Zn	25.59	8.43	6.09	2.34	9.64	11.34	5.98	72.24	4.33	16.91
TKW	37.22	27.41	16.55	10.87	10.93	14.07	8.86	60.36	6.52	17.52

LCC= Leaf color chart**, SPAD =** SPAD Chlorophyll content **SL =** Spike length**, PL =** Peduncle length**, PHT =** Plant height**, DH =** Days to heading**, DM =** Days to physiological maturity**, HI =** Harvest index**, Fe =** Iron content**, Zn =** Zinc content**, TKW =** Thousand kernel weigh

1. Leaf color chart

The average score of Leaf color chart was 3.75. The highest was 5 (RIL 160,161,182) and lowest was 2.5 (RILs 74, 76). The frequency distribution was normally distributed. Medium genotypic (13.94) and phenotypic (14.55) coefficient of variability was noted. Heritability was pretty high at 91.77 and genetic advance as percent of mean was high (27.54).

2. SPAD Chlorophyll content

The SPAD reading recorded was highest at 55.46 (RIL 214) and lowest was 28.73 (RIL 108) with mean being 46.97. The distribution was negative skewness. Low genotypic (7.91) and phenotypic (7.99) coefficients of variation were noted. However, heritability was high at 98.02 and genetic advance as percent of mean was moderate (16.15).

3. Spike length

The spike length ranged from maximum 14.2 (RIL 174) to minimum 7.4 (RIL 135), the mean value was 10.45. The frequency distribution was positively skewed. Low genotypic (9.97) and phenotypic (10.24) coefficient of variability was noted. Heritability was pretty high at 94.77 and genetic advance as percent of mean was high (20.03).

4. Peduncle length

The Peduncle length ranged from maximum 66 (RIL 149) to minimum 34.6 (RIL 3), the mean value was 50.14. The frequency distribution was normal. Moderate genotypic (11.44) and phenotypic (11.51) coefficient of variability was noted. Heritability was pretty high at 98.75 and genetic advance as percent of mean was high (23.45).

5. Plant height

The Plant height ranged from maximum 140.6 (RIL 73) to minimum 82.8 (RIL 90), the mean value was 114.40. The frequency distribution was positively skewed. Medium genotypic (10.99) and phenotypic (11.01) coefficient of variability was noted. Heritability was high at 98.92 and genetic advance as percent of mean was high (22.56).

6. Days to 50% heading

The days required for 50% of plants to flower ranged from maximum 93 (RIL 39) to minimum 59 (RILs 154, 155, 157, 175), the mean value was 75.03. The frequency distribution was positively skewed. Low (9.98) genotypic and medium phenotypic (10.08) coefficient of variability was noted. Heritability was very high at 98.03 and genetic advance as percent of mean was high (20.38).

7. Days to physiological maturity

Days to physiological maturity ranged from maximum 126 (RIL 51) to minimum 110 (RIL 155), the mean value was 116.3. The frequency distribution was normal. Low genotypic (2.4) and phenotypic (2.76) coefficient of variability was noted. Heritability was high at 76.07 and genetic advance as percent of mean was low (4.32).

8. Grain Yield

The grain yield ranged from maximum 321.67gm (RIL 55) to minimum 13 gm (RIL 97, 100% lodging soon after heading), the mean value was 112.83gm. The distribution was positively skewed. High genotypic (40.55) and phenotypic (43.53) coefficient of variability was noted. Heritability was high at 86.78 and genetic advance as percent of mean was high (77.94).

9. Biomass

Biomass ranged from maximum 86.67gm (RIL 58) to minimum 108gm (RIL 98), the mean value was 305.21gm. The distribution was positively skewed. High genotypic (31.33) and phenotypic (32.98) coefficient of variability was noted. Heritability was high at 90.23 and genetic advance as percent of mean was high (61.39).

10. Harvest Index

The ranged from maximum 60.88 (RIL 73) to minimum 8.732 (RIL 219, 100% lodging soon after heading), the mean value was 36.57. The distribution was negatively skewed. High genotypic (26.93) and phenotypic (27.18) coefficient of variability was noted. Heritability was high at 98.23 and genetic advance as percent of mean was high (55.07).

11. Iron content

The Iron content ranged from maximum 47.9 (RIL 55) to minimum 28.1 (RIL 229), the mean value was 35.95. The frequency distribution was normal. Low genotypic (8.68) and phenotypic (9.55) coefficient of variability was noted. Heritability was high at 82. 61 and genetic advance as percent of mean was moderate (16.27).

12. Zinc content

The Zinc content ranged from maximum 35.15 (RIL 222) to minimum 17.9 (RIL 147), the mean value was 25.59. The distribution was positively skewed. Low genotypic (9.64) and Medium phenotypic (11.34) coefficient of variability was noted. Heritability was high at 72.24 and genetic advance as percent of mean was moderate (16.91).

13. Thousand kernel weight (TKW)

The ranged from maximum 50.59 (RIL 91) to minimum 23.73 (RIL 168), the mean value was 37.13. The distribution was negatively skewed. Moderate genotypic (10.93) and phenotypic (14.07) coefficient of variability was noted. Heritability was high at 60.36 and genetic advance as percent of mean was medium (17.52).

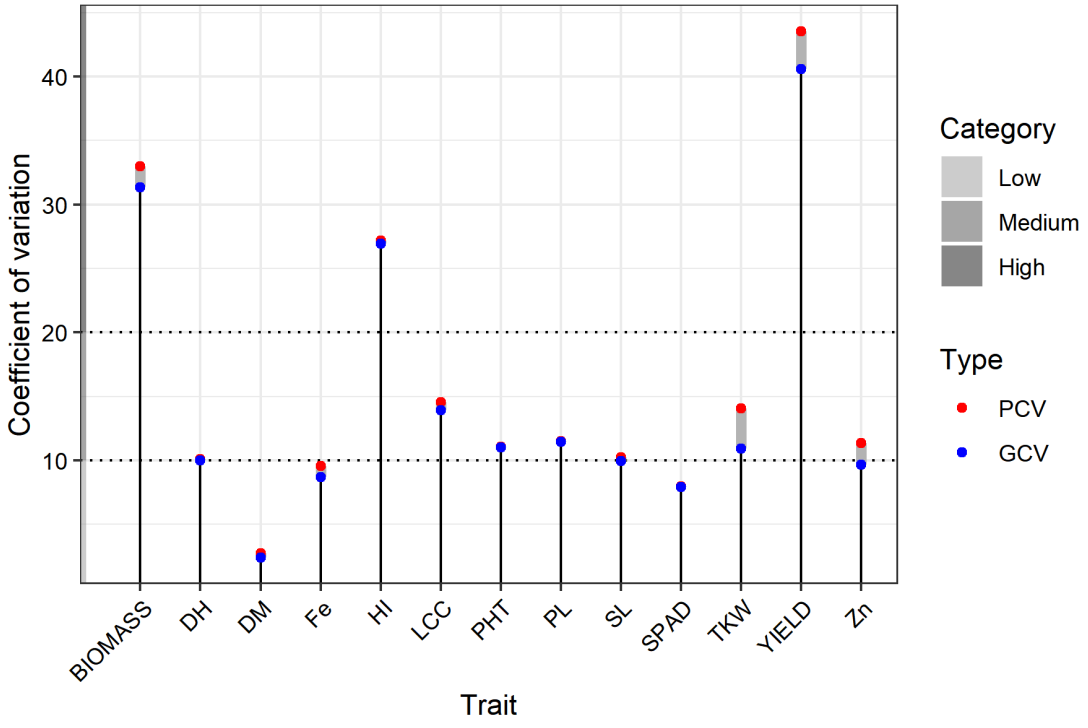


Figure 4.2: Coefficient of variation of given 13 traits

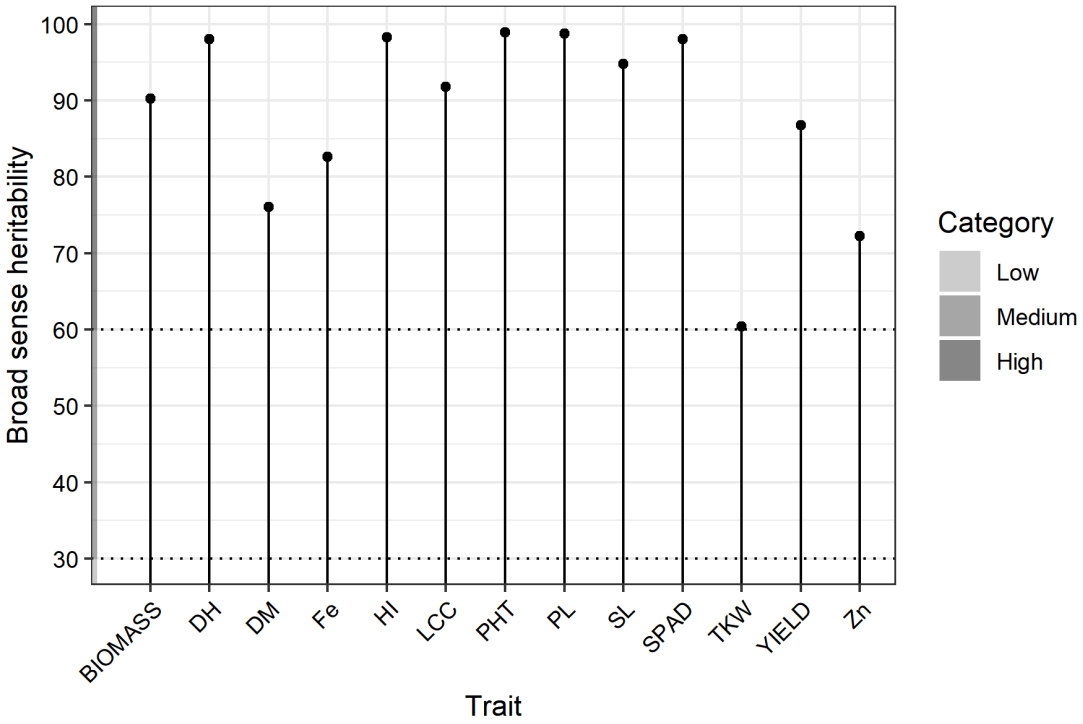


Figure 4.3: Broad sense heritability of given 13 traits

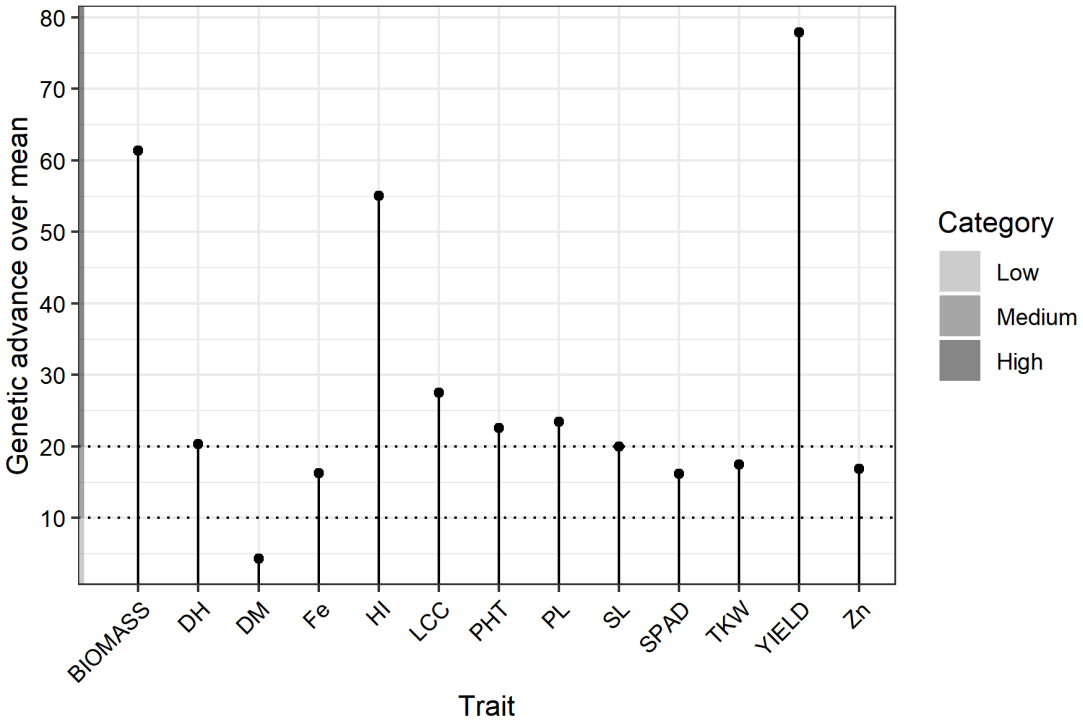


Figure 4.4: Genetic advance over mean for 13 traits

4.4 Correlation analysis

Yield is a complex, polygenic trait that is influenced by multiple interactions among different characters. For the 17 traits under examination, correlation coefficient analysis was used to determine the degree and direction of association between the yield, yield contributing traits and morphological traits like plant growth habit (PGH), Foliage color (FC) and Flag leaf angle (FLA), as well as the interrelationship between them. Correlation coefficient analysis identifies the component character on which selection can be based for genetic yield improvement by measuring the mutual interaction between several traits.

Correlation between different traits

1. Leaf color chart (LCC)

Flag leaf color showed significant, positive, low correlation with no of days to physiological maturity (0.148^{*}), foliage colour (0.162^{*}), spike length (0.172^{**}) and lodging percentage (0.22^{**}). Whereas, it showed significant moderate correlation with SPAD chlorophyll reading (0.428^{**}). However, it showed significant negative correlation with peduncle length (-0.156^{*}) and thousand kernel weight (-0.247^{***}).

2. Days to 50% heading (DH)

50% flowering days showed significant high positive correlation with no of days to physiological maturity (0.711^{***}) and plant growth habit (0.575^{**}). It had significant but low correlation with biomass (0.161^{*}), spike length (0.271^{***}), plant height (0.226^{***}). However, the relation with peduncle length (-0.236^{***}), LP (-0.155^{*}), flag leaf angle (-0.258^{**}) and TKW (-0.267^{***}) was significantly negative.

3. Days to Physiological maturity (DM)

Physiological maturity showed significant, positive correlation with biomass (0.134^{*}), foliage color (0.166*), SPAD chlorophyll content (0.239^{***}), spike length (0.259^{***}), plant height (0.226^{***}) and plant growth habit (0.561^{**}). The correlation was negative, significant and moderately weak with, peduncle length (-0.388^{***}), TKW (-0.267^{***}) and flag leaf angle (-0.35^{**}).

4. Yield

The correlation of yield was significantly positive and high with biomass (0.825^{***}) and harvest index (0.633^{***}) whereas, it was moderate with TKW (0.264^{***}). It also showed significantly negative relation with lodging percent (-0.489^{***}) Zinc content (-0.255^{***}).

5. Biomass

The correlation between biomass and spike length (0.164^{*}), plant height (0.255^{***}), iron (0.192^{**}) was weak but significantly positive. Lodging had negative correlation (-0.223^{***}) with biomass.

6. Harvest Index

Harvest index showed moderate and significantly positive correlation with thousand kernel weight (0.341^{***}) and low significant positive correlation with plant growth habit. The correlation was negative and significant with lodging percent (-0.6^{***}), chlorophyll content (-0.144^{*}), spike length (-0.279^{***}), peduncle length (-0.201^{**}), plant height (-0.243^{***}) and zinc content (-0.407^{***}).

7. SPAD chlorophyll reading

The chlorophyll content showed significant and positive correlation with leaf color chart (0.428^{***}), lodging percent (0.303^{***}) and low positive correlation with spike length (0.226^{***}). The correlation with peduncle length (-0.178^{**}) and TKW (-0.254^{***}) was negative and significant but weak.

8. Spike length

The correlation of spike length was positive and significant with plant height (0.313^{***}), Zinc content (0.169^{*}), 50% flowering (0.271^{***}), physiological maturity (0.259^{***}), biomass (0.164^{*}) and lodging (0.308^{***}). The relation was significant negative with thousand kernel weight ($-0.135^{*)}$.

9. Peduncle length

It was seen that peduncle length had strong, positive correlation with plant height (0.660^{***}) that was significant. The correlation was also significant positive with

iron content (0.144*), TKW (0.269***) and FLA (0.15*). The relation was significant and negative with plant growth habit (-0.452***) and foliage color (-0.275***)

10. Plant height

Plant height revealed significantly positive and strong correlation with peduncle length (0.660***), the relation was observed weaker with 50% heading date (0.226***), spike length (0.313***), iron content (0.209*), zinc content (0.148*) and TKW (0.204**). It had negative correlation with harvest index (-0.243*) and plant growth habit (-0.147*).

11. Iron content

Iron content exhibited significantly positive, moderate correlation with zinc content (0.360***) whereas, with plant height (0.209**) biomass (0.192**) and peduncle length (0.161*) it was relatively weak.

12. Zinc content

Zinc content had significant positive correlation with iron content (0.360***), spike length (0.169*), plant height (0.148*) and lodging (0.273***). Zinc content showed negative correlation with harvest index (-0.407***), yield (-0.255***) and thousand kernel weight (-0.258***).

13. Thousand kernel weight

TKW showed significantly positive correlation with yield (0.264***), flag leaf angle (0.21**) plant height (0.204**) and peduncle length (0.269***). It showed significant negative correlation with LCC (-0.247***), chlorophyll content (-0.254***), 50% heading date (-0.267***), physiological maturity (-0.500***), zinc content (0.360***), lodging (-0.383***) and plant growth habit (-0.279***).

14. lodging percent

Lodging percent had significantly positive correlation with LCC (0.22**), SPAD (0.303***), spike length (0.308***) and zinc content (0.273***). It showed negative correlation with days to heading (-.0155*), yield (-0.489***), biomass (-0.223***), harvest index (-0.6***), and plant growth habit (-0.196**).

15. Plant growth habit (PGH)

For using PGH for correlation analysis, pre-defined scale scoring was given to all the categories. The values increased from erect growth type (scale = 1) to prostrate (scale= 9), with intermediate growth type (scale= 5) between them. Plant growth habit showed positive significant correlation with days to heading (0.575^{***}), days to maturity (0.561^{***}), harvest index (0.15^{*}), and foliage color (0.211^{**}). It showed negative correlation with peduncle length (-0.452^{***}), plant height (-0.147^{*}), TKW (-0.279^{***}), lodging (-0.196^{**}) and flag leaf angle (-0.242^{***}).

16. Foliage color

The scale for foliage color denotes higher number for dark green (scale= 8) and lowest number to pale green (scale= 1). Foliage color showed significant positive correlation with LCC (0.162^{*}), days to maturity (0.166^{*}), and PGH (0.211^{**}). It showed negative correlation with flag leaf angle (-0.159^{*}) and peduncle length (-0.275^{***}).

17. Flag leaf angle

For flag leaf angle number scoring was done, and higher number was given to the flag leaves which were pendant (scale= 3) and lowest number to erect flag leaves (scale= 1). Flag leaf angle showed significant positive correlation with peduncle length (0.15^{**}) and TKW (0.21^{**}). It had negative correlation with days to heading (-0.258^{***}), days to maturity (-0.35^{***}) and lodging percent (-0.242^{***}).

Table 4.5: Phenotypic correlation among 13 traits

	LCC	***DH***	***DM***	***Yield***	***Biomass***	***HI***	***SPAD***	***SL***	***PL***	***PHT***	***Fe***	***Zn***	***TKW***	***LP***	***PGH***	***FC***	***FLA***
LCC																	
DH	0.014																
DM	**0.148***	**0.711*****															
Yield	0.018	0.083	0.065														
Biomass	0.110	**0.161***	**0.134***	**0.825*****													
HI	-0.106	-0.044	-0.066	**0.633*****	0.121												
SPAD	**0.428*****	0.053	**0.239*****	0.007	**0.131***	**-0.144***											
SL	**0.172****	**0.271*****	**0.259*****	-0.026	**0.164***	**-0.279*****	**0.226*****										
PL	**-0.156***	**-0.236*****	**-0.388*****	-0.039	0.104	**-0.201****	**-0.178****	0.121									
PHT	-0.079	**0.226*****	-0.104	0.037	**0.255*****	**-0.243*****	-0.070	**0.313*****	**0.660*****								
Fe	-0.127	0.054	0.012	0.087	**0.192****	-0.128	0.045	**0.144***	**0.161***	**0.209****							
Zn	0.005	0.091	0.111	**-0.255*****	-0.052	**-0.407*****	0.025	**0.169***	0.107	**0.148***	**0.360****						
TKW	**-0.247*****	**-0.267*****	**-0.500*****	**0.264*****	0.112	**0.341*****	**-0.254*****	**-0.135***	**0.269*****	**0.204****	0.073	**-0.258*****					
LP	**0.220*****	**-0.155***	-0.051	**-0.489*****	**-0.223*****	**-0.600*****	**0.303*****	**0.308*****	0.111	0.010	0.042	**0.273*****	**-0.383*****				
PGH	-0.039	**0.575*****	**0.561*****	0.128	0.059	**0.150***	0.003	0.026	**-0.452*****	**-0.147***	0.021	0.004	**-0.279*****	**-0.196****			
FC	**0.162***	0.055	**0.166***	0.073	0.020	0.116	0.105	0.019	**-0.275*****	-0.102	0.087	0.058	-0.009	-0.082	**0.211****		
FLA	-0.087	**-0.258*****	**-0.350*****	-0.018	-0.046	0.034	-0.044	-0.052	**0.150***	-0.057	0.037	-0.054	**0.210****	0.123	**-0.242****	**-0.159***	

Significant at * P <= 0.05; ** P <= 0.01, ***P <= 0.001

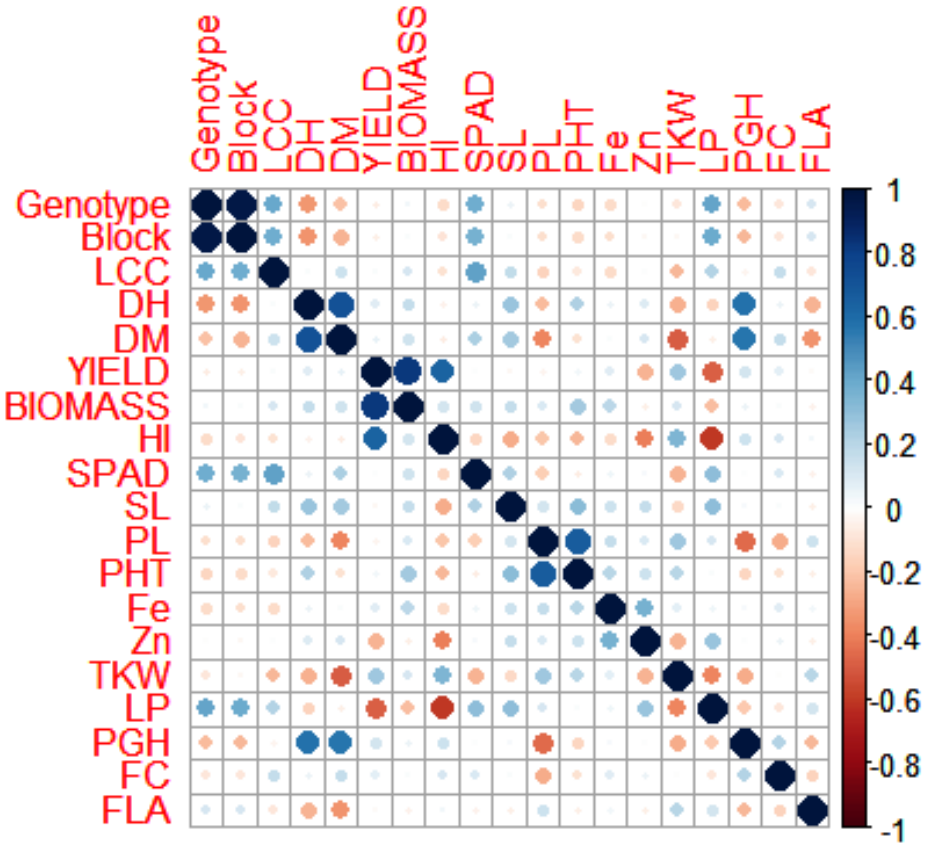


Figure 4.5: Correlation heat map

4.5 Principal Component Analysis (PCA)

4.5.1 Principal components and variation covered

PCA was performed in Origin Pro for 13 traits which were subjected to ANOVA. Out of 13 principal components (PC), 5 had eigen values greater than 1 covering 75.17% of variation.

The PC1, PC2 and PC3 covered 20.82% (eigen value 2.7) 17.98% (Eigen value 2.3) and PC3 17.39% (Eigen value 2.2) respectively and same were considered for biplot analysis. The cumulative variance represented by these three principal components was 56.20%.

As per the extracted eigenvectors presented in Table 4.7, The PC1 was mostly influenced by days to 50% heading, days to physiological maturity, chlorophyll content and spike length. Further, harvest index, grain yield highly contributed to PC2, and PC3 is much influenced by plant height, biomass and grain yield, as they contributed highly to PC3.

4.5.2 Genotype score plot

The score plot shows the clustering of the genotypes according to principal components based on their measured traits. In the present analysis, most of the RIL lines were clustered together around the origin. However, lines 58, 55 and 59 were grouped separately, while 33, 53,36,42,48,213 and 52 grouped separately. Lines 60 and 98 spotted separately in opposite direction.

4.5.3 Traits loading plot

PCA loading plot depicts the association of variables analyzed and their contribution to the each PC's. From the trait loading plot, biomass and yield highly contributes PC3 component variation (Table 4.7), followed by plant height (PHT), peduncle length (PL), grain Fe and TKW. However, DH, DM, SPAD and LCC highly contributed to PC1 variation (Table 4.7 and Fig). The greater the correlation between two traits, smaller is the angle between them. Least angle is observed between a. Biomass, harvest index and yield, b. leaf color chart (LCC), SPAD and days to maturity (DM), c. yield, iron content and spike length, d. peduncle length (PL) and thousand kernel weight (TKW).

4.5.4 PCA biplot (Genotypes and Traits)

Biplot analysis was carried out on three principal components (PC1, PC2, and PC3) which covered 55.79% of total variability. Genotype score plot and trait loading plot were merged in the form of PCA biplot for better visualization and information extraction. The location of the genotype or line in plot corresponds with the trait or variable vector and its length. RILs 55, 58 (highest biomass: 806 grams), 59 spotted at the end of the biomass and yield vector, which supposed to have higher biomass and yield. Similarly, RIL 52 and 36 need to have higher spike length. RILs 213, 42 and 46 spotted at the end of DH vector, which showed higher days to heading. Further RIL 38 which associated with Fe vector showed high grain Fe content.

Table 4.6: Eigenvalues of the correlation matrix

	Eigenvalue	**Percentage of Variance**	**Cumulative**
1	2.7066	20.82%	20.82%
2	2.33802	17.98%	38.80%
3	2.26076	17.39%	56.20%
4	1.36649	10.51%	66.71%
5	1.09978	8.46%	75.17%
6	0.72676	5.59%	80.76%
7	0.59306	4.56%	85.32%
8	0.56581	4.35%	89.67%
9	0.46515	3.58%	93.25%
10	0.44492	3.42%	96.67%
11	0.26511	2.04%	98.71%
12	0.15195	1.17%	99.88%
13	0.0156	0.12%	100.00%

Table 4.7: Extracted Eigenvectors for observed 13 traits

	Coefficients of PC1	**Coefficients of PC2**	**Coefficients of PC3**
Leaf Color Chart	0.24003	0.14029	-9.33E-04
Days to 50% Heading	0.34945	0.15602	0.2268
Days to Physiological Maturity	0.44826	0.25925	0.09945
Grain Yield	-0.20122	0.41687	0.42919
Biomass	-0.03328	0.23174	0.53372
Harvest Index	-0.31757	0.43201	0.0454
SPAD	0.28925	0.12619	0.04992
Spike length	0.2849	-0.12428	0.29
Peduncle length	-0.2005	-0.43262	0.27684
Plant height	-0.01932	-0.34497	0.43927
Fe content	0.05043	-0.18843	0.28821
Zn content	0.2528	-0.31593	0.06758
Thousand Kernel weight	-0.4528	-0.04252	0.15406

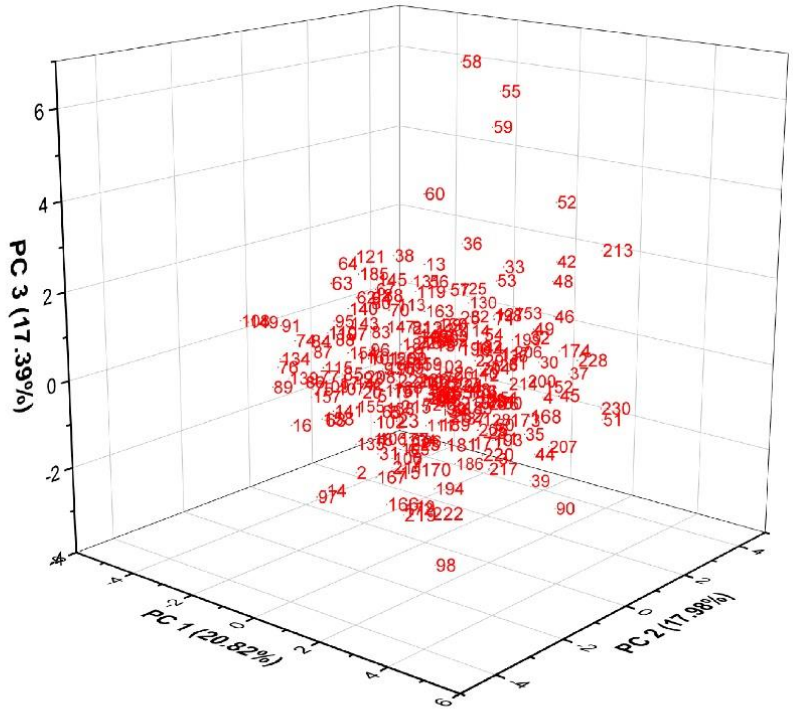


Figure 4.6: PCA Genotypen score plot representing 232 RILs

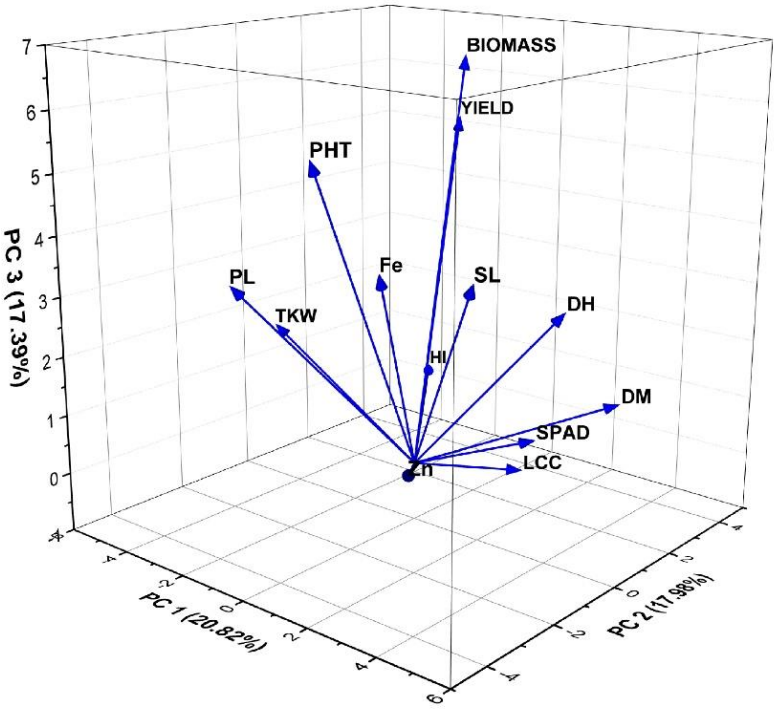


Figure 4.7: PCA traits plot representing 13 traits

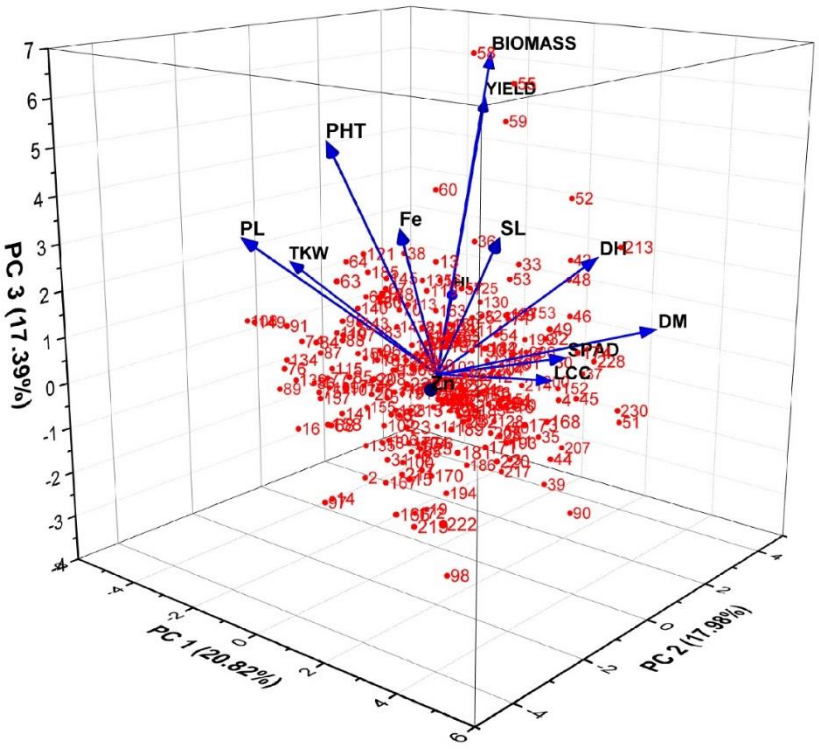


Figure 4.8: PCA Biplot representing both RILs and traits

LCC= Leaf color chart**, SPAD** = SPAD Chlorophyll reading **SL** = Spike length**, PL** = Peduncle length**, PHT** = Plant height**, DH** = Days to heading**, DM** = Days to physiological maturity**, HI** = Harvest index**, Fe** = Iron content**, Zn** = Zinc content**, TKW** = Thousand kernel weight

Chapter V

DISCUSSION

Wheat is one of the most important food source for majority of world population. It serves as a staple food for more than 30% of total population (Lobell *et al*., 2011). Projected climate variations predicted to have much negative impact on global wheat production and productivity. Bio prospecting for novel germplasm and deciphering the genetic loci governing important yield, agronomic and quality traits is very important and incentive. However, for any genetic mapping studies, a proper variation and normal distribution for the trait is pre-requisite.

Therefore, the present study entitled "Genetic variation for agronomic and grain quality traits in MNP-2 recombinant inbred line (RIL) population of bread wheat (Triticum aestivum)" was carried out amount of genetic variation and distribution pattern of agronomic and quality traits in the MNP-2 RIL and to check their association pattern.

The available information from the 232 RILs investigated for various aspects have been discussed under the following subheadings.

5.1 Analysis of variance (ANOVA)

5.2 Descriptive statistics

5.3 Variability, Heritability and genetic advance

5.4 Correlation Coefficient Analysis

5.5 Principal Component Analysis

5.1 Analysis of variance (ANOVA)

The ANOVA performed revealed a significant variation in RILs for traits studied i.e., leaf color chart, chlorophyll content, spike length, peduncle length, plant height, days to 50% heading, biomass, harvest index,. This variation among genotypes provides an opportunity for utilizing in different genetic mapping studies.

The RILs also showed significant difference from the check varieties HD2967 and TAW95 which proves that the variability present among RILs can be of significant importance. The two check varieties itself were also significantly different from each other for characters SPAD chlorophyll content, spike length, days to 50% heading, days to maturity, harvest index.

According to the findings, there is possibility for using these RILs for mapping other traits measured like SL, PL, biomass, harvest index, PHT, DH, LCC and SPAD, though these RILs originally developed for mapping spot blotch resistance. Nevertheless, there was no skewness was observed for LCC, PL, PHT, DH, Fe and TKW, which suggests normal distribution of data points for these variables. Thus further, this RIL can also be used for genetic mapping of Fe and TKW loci in addition above mentioned traits (Fig 4.1 and Table 4.1).

5.2 Descriptive statistics

Characterizing a distinguishable morphological marker helps in easy identification of germplasm and the observable morphological traits assists in phenotypic selection during any breeding program. Morphological features are commonly employed to assess genetic variability and characterize existing germplasm materials.

The mean performance of all genotypes was tabulated for the given 13 characters. The germination in all lines were uniform so the variation in results obtained is not due to difference in unequal germination. The leaf color chart showed that the line 160, 161 and 182 had highest score and the foliage was dark green in color. The result of LCC were in accord with SPAD reading, these lines had high chlorophyll content at 48, 42.87 and 46.57 respectively. The lowest score was of line 28, 68, 67,76 and 97 with score 2.5. Most of the lines were early flowering than the checks, the earliest flowering lines were 154, 155, 157 and 168. Heading was observed in 50% of the plant in these lines within 59 days of sowing. These lines matured early at around 110 to 113 days of sowing in comparison to check HD2967 (84,120) and TAW95 (79,114). Line 39 was the last one showing 50% heading on 93 days and maturing in 122 days. There was a huge difference in yield and biomass due to early lodging. Lodging soon after heading resulted in significant decline in grain filling. The grains

didn't form properly and it those which formed were light and shrivelled. The average yield was 112.83gm. Line 55, 58 and 59 performed well in terms of grain yield with 321.7gm, 311.69gm and 274.37gm respectively, while grain yield in checks were 238.5gm (C1) and 253.2gm (C2). Harvest index was highest in line 83 @ 60.854. spike length was longest in line 174 (14.2cm). The tallest plants were observed in line 73 (140. 6cm) and shortest in line 90 (82.8cm). 35 lines expressed a higher Fe content than mean of check C_2 (higher Fe and Zn content check). The highest value was recorded in line 94 (47.7ppm). Similarly, 49 lines exhibited higher Zn concentration than check C_2, highest being recorded in line 222 (35.2ppm). 19 lines for Fe and 22 lines for Zn reported higher content than maximum readings in the checks. 7 lines performed well as for both Fe and Zn content (line 94, 97, 134, 168, 174, 208, 222). TKW was significantly reduced in plants that underwent lodging soon after heading since the grain filling was not proper.

5.3 Variability, Heritability and Genetic Advance

The availability of sufficient genetic variability is essential for any selection program and its examination in combination with heritability, can help in determining the relative contributions of genetic and non-genetic factors to overall phenotypic variation. Thirteen characters were evaluated in 232 RILs along with two checks, to estimate genetic variability. There was a significant degree of variation among the genotypes. For all of the traits, the phenotypic coefficient of variation (PCV) was found to be slightly larger than the genotypic coefficient of variation (GCV). This shows that the environment has a lower influence than genotype since phenotypic variance is the summation of genotypic and environmental variance.

High GCV was observed for grain yield (40.55), biomass (31.33) and harvest index (26.93). LCC, PL, PHT, Zn content, TKW exhibited moderate GCV and PCV. DH and spike length showed medium PCV but low GCV. Chlorophyll content, days to maturity and Fe content had low GCV and PCV. Heritability (broad sense) was recorded high for all the traits and it ranged from 60.36 (TKW) to 98.92 (plant height). Peduncle length also reported a significantly high heritability (98.75), followed by harvest index (98.23), Days to heading (98.03) and chlorophyll content (98.02). Genetic advance as percent of mean (GAM) was recorded high for traits LCC (27.54), spike

length (20.03), Peduncle length (23.45), plant height (22.56), days to 50% heading (20.38), grain yield (77.94), biomass (61.39) and harvest index (55.07). high heritability with low genetic advance (4.32) was observed for days to physiological maturity whereas high heritability with medium GAM was recorded for chlorophyll content (98.02, 16.15), Fe content (89.64, 17.98), Zn content (76.41, 18.2) and TKW (61.57, 18.06).

Heritability, phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), and genetic advance estimations are helpful in defining the strategy of selection to improve a population for a certain trait. High genetic advance along with high heritability is considered the best condition for selection of a particular trait. They are more reliable and offers more sustainable crop improvement through selection of that trait.

5.4 Correlation analysis

Phenotypic correlation among favorable combination of morphological characters help increasing efficiency and ease of selection. Correlation arises primarily due to genetic linkages, pleitrophy or physiological and developmental relations. The correlation study is an important aspect for breeders during any selection program. It assists the breeder to make selection for yield or any other quality traits based on observable correlated morphological characters. It is possible to conduct indirect selection of desired trait with low heritability by selecting for number of correlated parameters with higher heritability.

Correlation coefficient revealed that biomass (biological yield), harvest index, thousand kernel weight (TKW) had a significant positive correlation with grain yield whereas lodging of plants significantly reduced yield, biomass and grain Zn content was negatively correlated with grain yield. Further, the lines with prostate like growth habit were less likely to be lodging, which is supported by -0.196 ** correlation value.

Days to 50% heading showed significantly high positive correlation with days to physiological maturity which were further correlated biomass and spike length. Thus, early heading varieties also mature early, whereas late maturing varieties have higher biomass, longer spikes and more height due higher vegetative growth. Peduncle length

and TKW had significant negative correlation with heading. Increased days to heading, showed to increase spike length, which in turn reduces the TKW, due to higher number of spikelets per spike and higher source sink partitioning.

Lodging % revealed significant positive correlation with chlorophyll content, spike length, peduncle length and Zinc content. The basal intermodal length and higher weight of spike usually causes stem lodging in wheat. Due to lodging there was significant reduction in the yield and harvest index. The higher SPAD and LCC values of lodging plants shows the earlier heading and chlorophyll maturation.

Grain Iron and Zinc content in grain showed moderate significant positive association with each other, whereas plant height and peduncle length had low but significant positive correlation with both micronutrients content. Harvest index negatively correlated with grain Fe and Zn, but association was high and significant in case of Zn and HI. Further, grain Zn significantly negatively correlated with yield, suggesting some yield penalty for selecting high zinc genotypes. Both these micronutrients positively correlated with spike length and plant height. Increasing in spike length tend to decrease TKW ($r = -0.135^{*}$), which further negatively correlated with grain Zn content, suggesting dilution effect in the grain might decreases grain zinc content. McDonald *et al*. (2008) suggested that grain yield increases as a result of more number of grain set on distant florets having lower micronutrient concentration, which may be due to sharing of relatively fixed and low concentration of Zn in the vegetative tissue i.e., the dilution effect. Similar results illustrating dilution effect of grain yield and kernel weight on grain zinc content were reported by Pleijel *et al*. (2009), Liu *et al.* (2014) and Murphy *et al.* (2008).

Plant growth habit (PGH) positively correlated with days to heading (DH) and days to maturity (DM), suggesting those with semi-prostrate to prostrate growth habit tend to flower and mature late and were of short stature with lower peduncle length, while those with erect or semi erect growth habit tend to mature early. Pendant type of flag leaves bearing genotypes showed flower and mature early and positively associated with TKW.

5.5 Principal Component Analysis (PCA)

Based on the principal component analysis (PCA) the 5 principal components (PC) had eigen value more than 1 and these 5 PCs accounted for 75.17% of total variation. The biplot analysis presented in figure 4.8 considered principal components PC1, PC2 and PC3 which represented 56.20% of total variation. Days to physiological maturity, grain yield, biomass showed highest contribution to PC1, PC2 and PC3 respectively.

The PCA shows the association pattern between the traits measured. There were grouping of traits, like yield, biomass and HI contributed for yield trait, whereas Peduncle Length (PL) and TKW grouped together suggesting increase in PL could increase TKW. Further SPAD, LCC and DM were clustered together. The longer projection of biomass and yield in traits loading plot (figure 4.7) signified higher variation for these traits.

The PCA score plot of genotypes (figure 4.6) showed more genotypes clustered together around the origin and few genotypes clustered around the trait vectors like biomass and yield. The genotypes present near the origin showed low variance for the traits whereas genotypes present on the extreme end represent high value for the given traits. Genotypes 55, 58, 59 cluster around biomass and yield, and the same have been recorded as highest yielding lines. In addition, these lines were also recorded to have highest concentration of iron signifying the correlation between iron content and yield. RIL 213 present at the outlier of DH, flowered latest at 126 days.

Similar results were reported by Škrbić *et al*. (2005), Beheshtizadeh *et al.* (2013) and Rymuza *et al.* (2012).

Chapter VI

SUMMARY AND CONCLUSION

The present investigation entitled "Genetic variation and principal component analysis for agronomic and grain quality traits in MNP-2 recombinant inbred line (RIL) population of bread wheat (*Triticum aestivum*)" was conducted at Agriculture research farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi during rabi season 2021-2022. The experimental material comprised of 242 recombinant inbred lines developed from cross between SOKOLL//W15.92/WBLL1 and CIANO T 79 which were evaluated in augmented block design along with two checks HD2967 AND TAW95. The treatments were divided into 5 blocks, each block consisted 48 RILs with checks replicated in each block.

Observations were made for different traits leaf color chart, chlorophyll content, spike length, peduncle length, plant height, days to 50% heading, days to physiological maturity, yield, biomass, harvest index, lodging, iron content, zinc content and thousand kernel weight.

The ANOVA table showed that there was significant variation among RIL lines for Biomass, DH, HI, LCC, PHT, PL, SL and SPAD. This study discloses that the RILs exhibit wide range of variability for other traits also. Further, there was no skewness observed for LCC, PL, PHT, DH, Fe and TKW, which suggests normal distribution of data points for these variables. Thus, this RIL can also be used for genetic mapping of Fe and TKW loci in addition above mentioned traits

A glance over the descriptive statistics revealed that RIL55,58 and 59 had highest grain yield along with highest Fe content (more than checks for both traits) however, Zn content was only average. High Fe and Zn content together were recorded in lines 94, 208 and 222. Most of the lines were early heading and subsequently matured earlier than checks. Lodging was observed in several lines, mostly ones with longer peduncle length. It caused severe reduction in grain yield.

Genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) differed based on traits. PCV was only slightly higher than GCV

suggesting that effect of environment was not very high. Heritability (H^2) was high for all the characters. High genetic advance as percent of mean (GAM) with high heritability was noted for 8 traits leaf color, spike length, peduncle length, plant height, days to 50% heading, grain yield, biomass and harvest index. It indicates that additive gene action is higher for these traits and selection may be effective. GAM for both iron and zinc content was medium.

Correlation analysis revealed that yield, biomass and HI were strongly correlated to each other and selecting for anyone of this trait can increase other. However, TKW correlated with yield, HI, PL and PHT and negatively associated with grain Zn content. Thus, selecting for high yield and TKW could have penalty on grain Zn content, which could be due to dilution effect. More lodging was observed in lines with high SPAD and SL, and negatively associated with HI and yield. Lines with semi-prostrate to prostrate plant growth habit, found to have early flowering and early maturing. Further, simultaneous selection for both grain Zn and Fe could be done, as they are positively associated.

Further PCA analysis showed the association of yield contributing traits like HI, biomass and grain yield. Grain Zn and Fe clustered with SL and PHT, suggesting their interrelationship. PCA biplot showed there was big cluster formation with most of the RIL lines around the center of the origin, which may be due to normal distribution of the most of the traits.

Overall, the present MNP-2 RIL can be used for genetic mapping of Biomass, DH, HI, LCC, PHT, PL, SL and SPAD. Further there was normal distribution for SPAD, SL, DM, yield, biomass, HI and Zn, thus this could also be evaluated further in replication trials to confirm variation among them to use this RIL for mapping grain Zn loci.

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APPENDIX

Table 1: Mean values of the all the genotypes including RILs, parents and checks

Genotype	Block	LCC	DH	DM	Yield	Biomass	HI	SPAD	SL	PL	PHT	Fe	Zn	TKW	PGH	FC	FLA	LP
SOKOLL//W15.92/WBLL1	P1	3.8	81	116	153.1	300	51.02	43.76	11.6	39.2	95	28.45	19.7	39.6	3	9	1	0
CIANO T 79	P2	3.5	80	116	153.1	428	35.78	42.18	9.6	50.6	122.6	37.65	23.35	41.5	1	5	3	0
C1	1	3.1	84	120	236.3	784	30.14	43.26	10.84	31.4	97.6	33.03	26.8	37.2	--	--	--	--
C2	1	3.2	79	114	241.0	680	35.44	44.16	10.08	31.4	93.2	38.83	28.26	49.2	--	--	--	--
RIL 1	1	4	71	113	100.9	266	37.94	45.90	10	53	113.4	34.25	23.05	42.9	1	5	3	0
2	1	3	72	115	53.4	210	25.42	41.72	9	52.8	97.8	37.95	27.45	35.2	3	1	3	90
3	1	3	84	118	78.3	148	52.93	45.10	9.4	34.6	94	33.4	21	36.3	5	9	3	0
4	1	4.1	91	122	81.6	328	24.89	46.66	9	39.2	127.8	35.9	26.65	29.7	5	9	1	0
5	1	2.8	76	114	102.7	238	43.15	45.62	10.4	51	111.6	31.6	24.65	41.2	1	5	3	0
6	1	3.5	82	118	46.1	216	21.34	41.18	10.6	50.2	109.6	35.1	30.45	37.1	1	5	3	90
7	1	3.8	81	117	47.5	164	28.99	39.50	11.2	53.6	116.2	34.3	31.75	27.9	3	5	3	95
8	1	3.5	78	114	72.7	165	43.88	48.36	10.4	50.4	120.4	35.35	27.15	39.5	1	5	3	0
9	1	3	80	115	131.6	308	42.64	42.02	11	49.6	117.6	34.75	22.7	34.0	3	5	3	0
10	1	4.5	83	116	65.8	180	36.53	50.18	11	54.4	122	38.35	26.4	36.2	3	5	3	0
11	1	3.5	83	118	90.4	232	38.98	44.12	11	38.8	103.6	30.65	28	34.1	5	9	3	0
13	1	4	83	118	142.9	442	32.33	48.92	11	60.4	139.6	36.4	28.45	35.4	3	5	2	0
14	1	4	68	113	21.1	142	14.86	41.82	10.8	55.4	116.8	35.88	25.54	35.2	1	5	3	90
15	1	3.8	74	118	29.2	134	21.81	44.24	11.2	52.8	115	35.5	25.8	34.6	1	5	3	95
16	1	2.8	63	112	61.7	188	32.81	45.80	10	57.8	119.8	38.1	21.6	37.8	1	5	3	90

17	1	4.2	84	113	47.9	250	19.16	41.96	10.6	54.2	136.2	38.45	26.35	41.9	3	9	3	30
18	1	4	66	113	83.0	202	41.11	44.42	10	53.4	109.2	35.2	26.4	33.6	1	5	3	20
19	1	2.8	68	117	67.4	165	40.69	37.18	9.2	42.2	84.8	33.35	24.7	31.9	3	5	3	0
20	1	3	68	113	120.9	346	34.95	41.58	9.4	48.8	105	38.1	28.1	36.2	1	5	2	0
21	1	3.5	77	115	126.8	314	40.38	48.84	9	47.4	124.4	38.85	28.9	39.5	3	9	3	0
22	1	4.5	71	117	121.4	336	36.12	45.10	11	55.2	124.6	37.75	27.3	34.3	1	5	3	0
23	1	3	85	118	34.7	198	17.53	47.50	11.4	54.2	130	38.6	31.75	33.1	5	9	3	60
24	1	3	80	120	68.1	194	35.08	49.00	11.2	53.8	109	40.2	24.5	35.1	3	5	3	90
25	1	4	88	120	93.6	304	30.78	50.14	11	51.6	135.8	40.15	25.65	39.0	1	5	3	40
26	1	3	83	120	88.7	292	30.37	49.18	10.6	49.8	120.8	34.35	23.2	37.2	1	9	3	0
27	1	3.2	75	120	81.5	228	35.74	48.58	11.4	50.6	112.6	36.3	25.35	36.8	1	9	3	10
28	1	2.5	76	120	93.6	234	39.99	49.54	10.6	44	96.2	33.4	22.75	32.2	3	1	3	0
29	1	3.5	75	118	135.1	370	36.52	48.70	11.6	50.6	118.4	33.7	22.25	40.3	1	1	2	0
30	1	4	84	120	147.0	290	50.71	47.60	11	40.6	104.8	32.05	21.35	36.2	5	5	2	0
31	1	3.5	77	118	38.1	148	25.76	40.80	10.6	55.2	122.4	35.8	26	32.2	3	1	3	40
32	1	4.5	88	121	97.6	374	26.11	49.14	10.4	49.6	129.4	41.83	25	25.7	5	9	1	60
33	1	4.5	88	121	135.1	427	31.60	50.00	12	54.2	130.8	38.1	25.8	36.3	3	1	3	20
34	1	4	77	122	35.7	313	11.38	52.82	11	62.6	112.6	34.8	24.7	34.9	1	5	3	90
35	1	4	78	122	86.4	245	35.28	51.64	10	47	96.4	32.5	23.7	34.3	3	5	3	20
36	1	3	86	119	207.9	586	35.44	45.86	9	54.8	121.2	35.88	25.3	32.8	5	5	2	0
37	1	3.8	83	120	178.4	392	45.52	44.60	10	35.8	83.2	35.05	25.8	31.0	5	1	3	0
38	1	3	77	115	182.8	557.5	32.79	42.68	10.6	55	116.2	40.16	25.96	35.8	3	1	3	0
39	1	4	93	122	57.4	160	35.87	39.94	9	39	98.6	31.65	26.1	33.0	3	1	3	0
40	1	3	77	120	142.5	322	44.21	43.84	10.4	43.2	90.4	36.8	23.1	38.0	3	1	3	0
41	1	3.2	88	118	81.2	217	37.28	41.74	10.4	48.2	128.2	35.4	23.85	34.3	3	1	3	0

42	1	3.5	85	123	226.0	498	45.38	48.54	10	41	104	39.65	26.15	37.0	7	5	3	0
43	1	2.8	81	122	109.9	235	46.64	49.30	9.4	53.8	110.8	35.4	26	30.5	3	5	3	0
44	1	4	89	124	57.7	157	36.72	48.22	9.2	49.6	101.2	38.45	28.25	28.2	3	5	3	0
45	1	4	91	125	79.3	252	31.48	48.02	11.2	49.6	117.8	33.9	25.8	27.4	3	9	1	0
46	1	4	92	124	137.5	305	44.99	48.32	11.8	49.6	118.8	35.35	24.4	32.6	3	5	1	0
48	1	4.2	91	123	165.5	440	37.60	45.68	12	50.2	122.8	33.5	27.25	30.6	3	5	3	0
49	1	4.2	88	122	122.6	331	37.00	47.98	12.6	50.8	123.2	33.65	28.35	31.1	3	9	3	0
50	1	3.5	78	122	90.0	260	34.62	45.58	10	47.8	109	32.55	24.2	29.4	3	5	3	0
51	1	4	88	126	114.9	300	38.31	42.84	10.4	40.8	95.4	32.9	28.8	24.6	3	5	3	0
52	1	4.8	80	123	237.6	580	40.97	47.92	12	52.4	121	37.75	24.65	31.4	3	5	2	0
53	1	4.2	77	122	181.9	381	47.71	48.26	10.6	51	120.2	39.9	24.1	35.4	3	5	3	0
54	1	3	85	121	126.7	357.5	35.45	50.04	10	44.4	97.8	43.63	26.66	43.3	3	9	3	0
55	1	3	84	121	321.7	662	48.53	48.92	12	52.6	119.4	47.93	30.06	37.5	3	9	3	0
56	1	3.8	76	114	172.1	353	48.72	49.12	12	48	115.6	41.65	23.4	42.3	1	9	3	0
57	1	4	73	119	145.3	435	33.35	49.94	11.4	51.2	118.4	39.65	24.35	41.3	1	9	3	0
58	1	4	78	119	311.7	806	38.64	50.30	11	59.4	130.6	45.05	26.9	40.8	1	9	3	0
C1	2	3	84	118	248.0	760	32.63	45.36	10.32	32.2	98.4	38.23	27.4	38.9	--	--	--	--
C2	2	2.9	80	112	290.0	744	38.98	47.32	9	33	97.4	41.1	29.82	41.4	--	--	--	--
59	2	4	84	118	274.4	706	38.83	49.86	11.4	52	129.4	40	24.6	40.1	1	9	3	0
60	2	4.2	78	115	217.7	530	41.08	47.78	12.4	58.6	129.2	38.7	23.5	39.2	1	5	3	0
61	2	3.5	78	118	161.5	290	55.70	50.70	9.4	40.8	97.6	37.25	20.15	36.1	1	9	2	0
62	2	2.8	76	113	184.3	435	42.36	40.56	9	53.6	119	38	24.95	37.6	3	1	3	0
63	2	3.8	78	115	92.9	450	20.64	44.72	11.6	63	131.8	42.95	32.05	39.9	3	5	3	20
64	2	2.8	76	114	153.1	380	40.29	47.86	11	56.8	129.6	41	24.2	46.1	1	5	3	0
65	2	2.8	75	115	82.9	202	40.87	48.72	9.4	46.6	111.2	40.3	29.75	41.3	1	5	3	0

66	2	4	76	118	135.5	257.5	52.62	44.64	11	47.4	119.2	34	23.05	43.6	1	5	2	0
67	2	3	78	114	178.5	377.5	47.28	44.50	10.4	51.8	122.8	35.5	26.75	43.7	3	5	3	0
68	2	2.5	76	116	26.1	266	9.78	47.04	11	56.6	128	33.5	25.15	41.7	1	5	3	0
69	2	3	80	118	75.3	215	35.03	48.50	10.8	44.4	118.4	42.8	24.5	47.7	3	5	3	0
70	2	3	73	117	142.9	352.5	40.52	47.14	11.6	57	123.8	35.45	25.3	38.2	1	5	3	0
71	2	3.5	76	125	134.6	366	36.77	48.08	10.6	48	134.2	37.25	30.05	33.1	5	9	1	0
72	2	3.5	74	117	98.0	262	37.42	46.52	9.6	50	111.8	35.8	25.1	35.2	1	5	3	0
73	2	3.5	87	119	113.5	332	34.20	42.28	10	48.8	140.6	31.75	25	37.3	3	5	1	0
74	2	2.5	79	114	103.1	275	37.50	41.72	9.8	62.6	138.8	33.95	24.05	39.0	1	1	3	0
75	2	4	67	113	90.1	224	40.23	44.00	10.4	61.4	120	38.85	32.45	38.1	1	5	3	0
76	2	2.5	70	113	98.0	248	39.51	42.04	9.6	56.6	132.8	36.15	27	43.0	1	5	3	0
77	2	2.8	68	113	118.1	282	41.86	43.14	9	55.2	124	36.35	27.3	37.1	1	9	3	0
78	2	4	76	115	143.9	328	43.88	46.90	10.4	49	123	31.65	23.35	42.3	1	5	2	0
79	2	4	87	119	98.6	240	41.10	49.12	8	44.4	123.8	35.1	28.65	34.0	3	9	3	0
80	2	4.2	72	114	137.5	384	35.80	48.00	11	56.2	134	34.05	26.35	43.4	1	5	3	0
81	2	4	81	115	134.6	312	43.14	45.74	10.6	52.6	132.8	33.65	26.55	38.0	3	5	2	0
82	2	4	69	115	212.4	446	47.62	49.52	10	46.6	108.6	31.7	24.7	35.6	1	5	3	0
83	2	3.2	77	115	125.4	206	60.85	44.20	11.2	52.8	118.6	38.53	24.73	44.3	3	5	3	0
84	2	3	77	114	75.0	228	32.90	45.78	11.4	57.2	134	42.36	25.8	43.6	3	5	3	50
85	2	3.5	77	115	88.5	276	32.05	46.44	9.4	59.4	128.2	39.4	34.93	36.5	1	5	3	20
86	2	3	69	112	127.3	292	43.61	36.66	9	53.2	116.8	31.5	25.4	43.2	1	5	3	0
87	2	3	70	112	134.6	272	49.50	42.72	9.8	59.2	116.8	37.25	27.65	39.5	1	5	3	0
88	2	3.8	83	115	95.6	284	33.65	41.30	10	59.4	133	33.7	24.35	45.8	3	5	3	0
89	2	2.8	69	113	95.3	216	44.14	38.66	10	61.2	119.4	33.05	25.95	43.7	1	5	3	0
90	2	4	90	122	45.1	125	35.91	43.82	9.2	35.2	82.8	36.65	27.95	32.9	5	9	2	0

91	2	2.8	74	114	104.7	284	36.87	43.04	10.2	57.4	127.8	40.03	28.16	50.6	3	1	3	0
92	2	3	67	115	92.0	338	27.22	45.38	10	46.6	126	37.55	24	39.9	3	9	3	0
93	2	3.5	74	115	113.3	282	40.18	42.46	11	52.4	120	33.95	25.45	37.8	3	9	3	40
94	2	3.5	76	114	117.8	338	34.84	47.44	11.8	53.8	127.4	47.7	30	37.2	1	5	3	20
95	2	3	76	113	118.2	276	42.81	42.56	12	50.8	128.8	36.7	24.5	47.5	1	5	3	20
96	2	2.8	80	114	118.2	312	37.87	41.12	10	52.2	117.2	40.55	22.75	35.3	1	5	3	90
97	2	2.5	61	113	45.3	192	23.59	43.48	9	44.6	89.6	41.86	30.86	42.7	1	1	3	50
98	2	4	64	116	13.0	108	12.06	44.00	11.2	43.4	89	38.3	26.8	27.5	1	5	3	100
99	2	4.2	73	115	85.4	264	32.34	49.04	12	45.6	112.4	33.5	27.45	38.3	1	9	3	80
100	2	4	64	114	68.1	200	34.05	47.78	10	47.8	106.2	35.7	26.95	37.5	1	9	3	70
101	2	4	75	115	84.7	282	30.04	48.94	12	60.4	128.6	33.25	28.25	40.4	1	9	3	80
102	2	4	59	112	87.1	238	36.58	47.12	11	47.4	108.8	41.6	26.9	35.0	1	9	3	50
103	2	4	80	115	122.0	288	42.38	44.28	11	46.2	115.8	31.15	23.3	41.9	1	9	3	0
104	2	4	67	113	83.5	288	28.99	44.34	10.4	43.6	98.6	35.1	25.4	39.0	1	9	3	80
105	2	3	67	113	102.1	230	44.39	46.44	9.4	44	88.2	37	28.7	37.4	1	5	3	0
106	2	3.5	68	114	97.5	218	44.72	47.30	8	51.2	100	31.65	23	42.1	1	5	3	0
107	2	3.5	67	113	119.8	276	43.42	48.04	8	58.4	116	29.75	21.85	44.0	1	5	3	0
108	2	3.8	68	111	145.1	340	42.69	29.10	9.4	61.8	125.8	36.05	24.25	46.4	1	5	3	0
109	2	4	66	112	116.0	298	38.93	44.70	8	58.8	119.2	31.35	22.8	43.4	1	9	3	0
110	2	3	67	113	152.0	362	41.98	43.08	9.8	56.6	112.8	36.25	24.25	42.8	3	5	3	0
111	2	4	67	113	138.3	294	47.05	45.06	8.4	44.8	88.8	34.5	26.6	36.7	3	5	3	0
112	2	4	84	119	112.6	272	41.40	44.80	10	46.8	113.4	35	25.95	38.2	5	9	3	10
113	2	3	77	115	200.7	334	60.10	43.66	9.4	54.2	125.2	30.95	24.1	36.7	1	1	3	0
114	2	3.5	84	118	140.4	332	42.29	48.30	10	49.8	114.6	36.95	22.15	37.9	5	9	3	0
115	2	3	68	112	110.7	244	45.39	46.82	10	58.2	121.4	36.25	21.2	39.3	1	1	3	0

116	2	3.5	68	115	127.6	266	47.99	46.44	9.8	53.2	124.4	31.6	24.7	46.3	1	5	3	0
C1	3	3.5	83	119	198.0	660	30.00	43.80	9.6	32.8	103.4	35.7	23.9	39.4	--	--	--	--
C2	3	3.8	76	114	200.0	609	32.84	46.16	9.1	35	102	36.8	28.2	48.7	--	--	--	--
117	3	3.5	85	119	135.4	284	47.69	48.56	10.2	42	109.4	38.85	25.05	36.8	5	9	2	0
118	3	3.8	84	118	78.3	194	40.38	44.96	10	49.4	130	34.5	29.05	35.5	3	9	3	20
119	3	3.5	80	115	169.2	442	38.27	44.60	11	55.2	127.8	31.55	24.95	35.0	3	1	3	30
120	3	4	72	111	130.0	314	41.40	48.04	9	53.6	110	31.9	21.3	36.1	1	5	3	0
121	3	4	78	112	144.7	514	28.15	47.28	10.6	58.6	139.4	36.4	26.55	41.4	1	1	3	0
125	3	3.5	74	117	251.5	530	47.46	39.82	9	43.8	92.4	33.4	24.15	41.9	1	5	3	0
126	3	4	73	120	167.7	330	50.81	44.56	9.4	46	93.6	34.5	21.3	37.0	3	5	3	0
127	3	4.5	67	117	194.3	476	40.82	50.24	10	43.6	111	36.85	26.7	34.4	3	5	3	0
128	3	5	74	117	108.1	226	47.84	46.58	10	44.4	95.2	33.55	24.4	40.1	3	9	3	0
130	3	3.5	73	114	227.9	528	43.15	46.56	10	44	93	32.9	23.05	38.8	3	5	3	0
131	3	3.2	71	115	199.8	474	42.16	45.08	10.4	47.6	115.2	37.15	24.5	40.6	3	5	3	0
132	3	3.5	75	116	125.3	256	48.94	46.12	9	45.8	95.8	32.1	24.05	40.6	1	5	3	0
133	3	4.5	76	118	134.1	386	34.74	45.88	9.8	51.6	122.8	32.15	20.5	43.1	1	5	3	10
134	3	3	71	112	104.2	240	43.41	39.46	9.8	50.4	120.4	42.85	31.4	49.4	1	5	3	0
135	3	3.5	60	111	108.7	248	43.81	47.60	7.4	48.8	95	38.85	26.2	37.9	1	9	3	20
136	3	3.5	82	119	141.6	330	42.91	48.76	10.6	42.6	109.6	32.55	24.35	38.6	5	9	2	0
137	3	4.2	78	117	91.0	328	27.75	45.80	13.2	55	129.4	34	24.75	33.6	1	5	3	30
138	3	3.2	69	112	111.9	256	43.69	49.02	9.6	49.8	104.2	35.9	25.1	37.0	1	9	2	70
139	3	3.8	64	113	89.9	242	37.15	39.40	10.4	56.8	114.4	39.2	23.8	47.6	1	9	3	20
140	3	3.5	65	113	167.1	382	43.73	43.36	10.4	53.4	122.6	38.3	25.15	40.1	1	5	3	10
141	3	3.5	70	114	89.7	254	35.32	41.40	8.8	56	117.8	35.1	26.15	37.8	1	5	3	20
142	3	4	68	114	105.0	220	47.73	41.96	10.2	55.2	126.8	33.55	23.2	37.5	1	9	3	30

143	3	4.5	65	113	139.9	380	36.81	43.12	9	52	126	41.85	22.8	42.1	1	9	3	0
144	3	4.2	77	117	143.2	336	42.62	46.62	10	43.2	107.6	39.6	21.45	38.2	3	9	3	0
145	3	3.5	79	115	133.4	434	30.73	44.06	11	53.2	136	37.55	21.85	41.6	1	5	3	30
146	3	3.5	77	117	117.6	332	35.42	46.22	12	46.2	108.4	36.8	24.3	49.5	3	9	3	0
147	3	3.5	76	115	133.0	266	50.02	39.28	12.4	46.4	110.2	36.2	17.85	50.0	3	5	3	0
148	3	3.2	76	116	131.1	282	46.49	40.96	10	44.2	98.8	32.45	23	40.3	1	5	3	0
149	3	2.8	63	112	136.9	354	38.68	43.08	9.8	66	130.4	32.75	26.05	45.0	1	1	3	0
150	3	4	77	118	109.1	220	49.58	52.12	11	46.4	99	34.5	27.8	37.5	1	9	2	0
151	3	4.2	77	120	114.2	250	45.68	47.64	10.4	48	99.4	34.05	26.25	37.5	3	5	2	0
152	3	4	87	121	96.8	234	41.36	48.42	12.4	43	104	34.25	21.8	35.4	5	9	2	0
153	3	4.5	81	116	168.6	442	38.15	50.62	11.4	41.8	110.2	31.65	21.9	41.5	1	5	3	0
154	3	3.5	59	112	150.3	336	44.72	45.56	11	53.8	108.6	35.2	24.4	41.0	1	5	3	0
155	3	4	59	110	132.5	252	52.56	41.92	9.2	49.8	107.4	35.06	20	35.4	1	5	3	0
156	3	4	76	113	115.8	280	41.35	48.18	10.6	52.4	118.2	31.45	21.55	46.3	1	1	2	0
157	3	3.8	59	112	98.3	222	44.26	44.12	10	55	109.8	38.65	23.45	44.2	1	9	3	0
158	3	3	81	114	19.8	246	8.05	45.88	10.2	53.2	131.8	40.2	26.45	40.8	1	1	3	0
159	3	3.8	82	115	109.7	294	37.33	46.98	9	47.2	131	35.95	27.6	39.2	3	1	3	0
160	3	5	73	111	159.5	372	42.87	53.40	10	45.6	119.6	37.65	26.8	42.4	1	9	3	0
161	3	5	65	114	125.4	278	45.11	47.92	11	53.8	115.8	32.95	23.15	42.2	1	9	3	20
162	3	3.8	75	115	131.7	304	43.32	50.30	12	49.6	118.8	33.1	21.6	39.6	1	5	3	20
163	3	4.5	75	115	147.9	378	39.13	47.40	11	52.2	120.4	35.25	21.75	41.9	1	1	3	0
164	3	4.2	74	114	115.2	308	37.42	52.08	12	53.2	123.2	33.5	22.5	40.2	1	1	3	60
165	3	4	73	117	82.3	286	28.76	50.00	12.4	52.6	119.8	33	28.45	34.1	1	5	3	90
166	3	4.1	71	114	19.8	152	13.06	51.08	10.2	52.8	115.2	36.9	31	32.4	1	1	3	95
167	3	4	65	113	52.1	208	25.06	47.14	10.6	53	107.8	30.9	26	35.2	1	1	3	100

168	3	3.5	84	120	64.7	242	26.74	50.82	12.8	47	108	41.25	29.9	23.8	5	9	3	100
169	3	4	74	117	72.5	344	21.08	50.00	12.4	48.6	119.6	35.1	26.2	31.6	1	5	3	100
170	3	4.2	71	115	44.8	164	27.29	50.24	10.8	55.2	113.4	34.65	24.35	28.6	1	5	3	100
171	3	4	74	118	68.8	230	29.92	50.38	10.8	53.8	107.6	34.55	23.5	26.1	1	1	3	90
172	3	4	62	113	50.9	200	25.46	50.98	9.2	48.8	100.8	28.5	23.05	35.0	1	5	3	100
173	3	3.8	82	119	62.0	292	21.24	53.04	11.2	44.8	127	33	29.6	28.9	1	5	3	90
174	3	4	84	120	98.1	332	29.56	52.12	14.2	44.4	112	41.35	31.05	27.5	3	5	3	100
C1	4	3.3	85	123	260.0	790	32.91	43.30	10.84	30.4	97.6	37.6	26.9	36.9	--	--	--	--
C2	4	3.1	78	113	241.0	680	35.44	44.20	10.08	31.4	93.2	39.7	26	40.0	--	--	--	--
175	4	4.1	59	113	131.4	326	40.29	48.30	10	51.2	103.2	34.35	25.85	40.2	1	5	3	0
176	4	4.3	73	117	119.5	294	40.66	48.98	9.4	43.2	106	38.76	27.33	37.5	1	5	3	0
177	4	4.5	63	115	73.3	246	29.80	50.66	10.2	53.2	111	32.7	26.8	38.6	1	5	3	80
178	4	4	65	117	123.7	324	38.19	47.88	10.6	49.4	104.2	36	28.15	39.1	1	9	3	80
179	4	4	65	115	133.6	302	44.24	50.30	10.8	47.2	103	37.1	26.65	38.6	1	5	3	90
180	4	4.3	63	115	110.5	294	37.59	53.60	10.2	50.8	113.4	35.3	27.05	35.9	1	5	3	60
181	4	4.3	81	116	26.9	216	12.43	52.64	10.6	47.8	129.4	40.1	29.7	32.1	3	5	3	100
182	4	5	82	119	86.0	270	31.86	51.62	11.6	50.8	127.2	39.2	28.7	41.1	3	5	3	100
183	4	4	73	117	93.9	306	30.69	49.98	11.4	48.6	108.8	28.85	23.7	37.3	1	5	3	90
184	4	4	71	115	113.7	412	27.59	51.34	10	54.4	121.8	33.85	24.1	40.8	1	5	3	100
185	4	3.5	72	113	163.5	372	43.94	49.30	11.6	57.4	124	39.46	25.86	43.9	1	1	3	70
186	4	3.5	64	115	95.7	194	49.35	47.70	9.4	38	90.4	36.85	22.6	33.6	3	5	3	0
187	4	4	68	115	109.1	206	52.97	51.94	10	41.8	98	36.4	24.15	40.1	3	5	3	0
188	4	3.5	73	115	129.8	384	33.79	51.44	10.6	56.2	135.8	38.63	25.26	41.0	1	1	3	90
189	4	4	78	118	59.5	186	31.97	45.44	11.4	53.2	118	38.55	26.8	28.9	1	5	3	100
190	4	4	78	117	87.0	312	27.88	53.96	12.4	53.8	119.4	41.3	25.55	33.3	1	1	3	60

191	4	4.2	68	114	106.4	232	45.88	49.48	9.6	54.2	114.8	36.6	24.1	37.6	1	1	3	0
192	4	4	68	114	122.0	312	39.10	52.92	9	51.8	112.8	37.25	21.55	34.2	1	5	3	0
193	4	3.8	81	118	80.6	220	36.62	47.78	9.4	45	109	36.25	23.45	27.3	3	1	3	0
194	4	4	64	115	36.7	320	11.46	50.80	9	42.6	94.8	35.2	21.2	37.0	1	5	3	0
195	4	4	68	115	114.4	342	33.45	45.30	10	55.4	111.4	38.75	24.55	35.8	1	5	3	100
196	4	3.5	68	113	86.7	224	38.70	50.60	9	45.2	101	37	20.9	36.9	1	9	3	100
197	4	3.2	79	115	76.6	294	26.05	46.58	11.4	52.8	136.6	38.85	27.6	47.5	1	1	3	90
198	4	4	76	118	179.7	366	49.09	51.00	11	42.4	108.4	32	20.4	45.4	3	9	3	0
199	4	4.2	80	120	172.6	354	48.77	48.50	10	45	105.6	30.95	24.75	40.2	3	5	3	0
200	4	4.5	82	120	132.2	272	48.61	47.12	10	43	100	34.15	21.1	36.8	3	5	3	0
201	4	4	71	114	139.7	302	46.26	46.54	9	46.8	96.6	32.3	24	40.3	1	1	3	0
202	4	4.5	71	114	119.3	246	48.49	47.08	9.4	49.8	105	35.35	21.65	37.6	1	5	3	0
203	4	4.8	70	113	163.4	400	40.86	50.90	9	50.4	106	39.83	23.7	37.5	1	5	3	0
204	4	4	73	118	132.1	352	37.52	53.92	9	47.4	111.4	37	24.75	34.8	1	5	3	90
205	4	4.2	76	118	77.7	236	32.92	52.76	10	50.6	109.2	32.8	22.8	33.4	3	5	3	100
206	4	4.2	76	115	168.4	386	43.62	49.64	11	41	105	32	20.1	33.3	5	5	3	20
207	4	4	76	119	112.4	236	47.62	50.24	10	40.2	92.4	29.6	20.4	28.2	5	5	3	80
208	4	4	63	114	109.1	296	36.84	49.36	9.6	55	116	43.05	30	35.6	1	5	3	90
209	4	4.5	66	115	108.8	344	31.62	48.34	11	55.6	124	37.6	27.9	34.7	1	5	3	100
210	4	4	78	115	90.8	342	26.54	46.48	10	53.2	118.2	32.9	21.65	36.7	1	1	3	100
211	4	3.5	65	116	37.1	210	17.69	51.00	11	50.4	118.2	34.3	26.4	34.9	1	5	3	100
212	4	4	71	115	109.6	416	26.34	50.48	12.4	54	119.8	35.95	26.85	39.3	1	1	3	90
213	4	4	88	126	180.7	594	30.42	45.74	11.4	43	117.4	40.9	31.73	27.0	3	9	2	30
214	4	4.8	76	116	132.4	290	45.66	55.46	10.8	46.6	90	36.75	29.9	39.7	1	5	3	50
215	4	3.5	72	115	84.8	270	31.40	46.96	11	54.4	124.2	35	28.65	28.1	1	1	3	100

216	4	4	80	117	84.2	342	24.63	46.88	12.4	48.4	108.6	32.45	27.9	29.8	1	5	3	100
217	4	4.5	67	114	97.8	278	35.17	53.16	10	44.2	87.8	28.75	25.6	33.3	3	9	3	90
218	4	4.5	72	114	106.3	412	25.80	48.02	10	45.4	101.2	34.65	27.75	34.4	1	5	3	90
219	4	4.1	65	115	14.3	164	8.73	46.76	12.6	52.8	114.8	35	27.9	24.9	1	5	1	100
220	4	4	83	118	33.4	294	11.35	47.22	11	43.8	119.8	32.65	28.35	31.2	1	9	3	100
221	4	4	67	115	80.3	322	24.93	50.48	12	54	127.6	37.75	31.3	32.5	1	9	3	100
222	4	4.2	66	118	25.0	206	12.16	48.32	10.4	47	93.4	40.85	35.15	34.1	1	5	1	70
223	4	4.1	66	114	82.8	284	29.15	47.00	11.2	48.8	105.2	30.1	31.5	37.1	1	5	3	30
224	4	4	80	115	115.2	324	35.54	44.64	11	39	108.2	35.05	30.3	32.4	3	5	3	30
225	4	4	66	117	118.2	322	36.71	50.40	11	44.8	106	35.15	27.45	32.0	1	9	3	0
226	4	4.5	80	114	71.2	312	22.83	50.74	10.4	54	120.6	41.65	29	34.5	1	5	3	90
227	4	4.5	67	115	113.5	344	32.99	51.18	10.6	50.4	109.6	36.9	24	32.8	1	5	3	90
228	4	4	85	121	122.5	356	34.40	54.36	12	42.8	112	33	25.5	30.3	7	9	3	80
229	4	4	70	116	167.5	432	38.77	51.68	9	48	111.6	28.1	22.75	33.9	1	5	2	0
230	4	4.5	83	122	120.6	342	35.26	51.76	9.6	38.2	98.8	36.85	28.6	26.3	5	9	3	60
231	4	4.2	81	118	56.0	300	18.67	51.14	11.6	56	125.4	37.55	25.85	31.2	1	1	3	90
232	4	4	78	118	49.3	334	14.75	47.80	11.4	55.6	129.4	33.5	28.35	24.2	1	1	3	90
## RIL lines 12, 47, 122, 123, 124, and 126 did not germinate due to unknowing																		