GENETIC DIVERSITY STUDIES IN ADVANCED BREEDING LINES OF WHEAT (*Triticum aestivum* L.em Thell)

by

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(J-21-M-824)

A Thesis submitted to
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Genetics and Plant Breeding

Division of Genetics and Plant Breeding
Sher-e-Kashmir University of Agricultural Sciences & Technology of Jammu
Main Campus, Chatha, Jammu - 180009
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CERTIFICATE – I

This is to certify that the thesis entitled **"Genetic Diversity Studies in Advanced Breeding Lines of Wheat (*Triticum aestivum* L.em Thell)"** submitted in partial fulfillment of the requirements for the degree of **Master of Science in Agriculture (Genetics and Plant Breeding)** to the Faculty of Agriculture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, is original work and has similarities with the published work not more than minor similarities as per UGC norms of 2018 adopted by the university. Further the level of minor similarities has been declared after checking the manuscript with **URKUND** plagiarism detection software provided by the university.

The work has been carried out by **Ms. Mahpara Bashir,** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma. It is further certified that help and assistance received during the course of investigation have been duly acknowledged.

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CERTIFICATE – II

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ABSTRACT

Title of thesis	:	GENETIC DIVERSITY STUDIES IN ADVANCED BREEDING LINES OF WHEAT (*Triticum aestivum* L.em Thell)
Name of the student	:	Mahpara Bashir
Registration No.	:	J-21-M-824
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The present research entitled **"Genetic Diversity Studies in Advanced Breeding Lines of Wheat (*Triticum aestivum* L. em Thell)"** was carried out during *rabi* 2022 to study genetic diversity for yield contributing traits and yellow rust resistance in wheat. The material comprised of 96 advanced wheat breeding lines obtained from CIMMYT and ICARDA with four checks namely Agra local, DBW187, HD2967 and HD3043. These genotypes were evaluated in Augmented Block Design with six blocks, 16 test entries and four checks at Research farm of division of Plant Breeding and Genetics at main campus SKUAST-J, Chatha. The analysis of variance revealed that mean sum of squares for treatments (eliminating blocks) and treatments (ignoring blocks) were significant except chlorophyll content indicating that the test entries were significantly different from checks. The trait grain yield per plot showed high heritability coupled with high genetic advance suggesting additive gene action and thus selection is effective. Cluster analysis categorised wheat genotype into four clusters. Highest cluster mean value was exhibited by Cluster II for number of spikelets per spike, number of grains per spike, grain yield per plot, test weight and harvest index, whereas, the lowest value was observed in days to 50% flowering. Significant difference between wheat lines was observed for rust resistance in field. 40 promising genotypes showing rust resistance on the basis of field rust score were used for molecular characterization using 12 linked SSR markers. Cluster analysis of molecular data grouped wheat genotypes into two clusters. Cluster I consisted 25 genotypes and cluster II with 15 genotypes. The value of Polymorphism Information Content (PIC) ranged from 0.43 to 0.68. The highest PIC value was shown by marker Barc349 (0.68) and lowest by marker S19M93 (0.43). These findings provide valuable insights for the selection of diverse genotypes for conservation and further use in crop improvement.

Keywords: Wheat, Diversity, Clusters, Yellow rust, SSRs

Signature of Major Advisor

Signature of the Student

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LIST OF SYMBOLS AND ABBREVIATIONS

%	:	Percentage
°C	:	Degree Celcius
S. No.	:	Serial number
ANOVA	:	Analysis of variance
cm	:	Centimeter
g	:	Gram
kg	:	Kilo gram
et al.	: s	Et alia meaning ‘and others’
&	:	And
CV	:	Coefficient of variation
Df	:	Degree of freedom
ml	:	Milli litre
µl	:	Micro litre
min	:	Minute
GA	:	Genetic advance
GAM	:	Genetic advance as percent of mean
bs	:	Broad sense
PCV	:	Phenotypic coefficient of variation
GCV	:	Genotypic coefficient of variation
bp	:	Base Pairs
CD	:	Critical difference
CV	:	Coefficient of variation
CTAB	:	Cetyl trimethyl ammonium bromide
dNTPs	:	Deoxy Nucleotide Triphosphates
DNA	:	Deoxyribose Nucleic acid
pH	:	Potential of Hydrogen
e.g.,	:	Exampli Gratia (for Example)
EtBr	:	Ethidium bromide
Fig.	:	Figure

G	:	Gram
ha	:	Hectare
hr	:	Hour
i.e.,	:	In reference to; that is
mt	:	Metric tones
M	:	Molarity
No.	:	Number
PCR	:	Polymerase Chain Reaction
PIC	:	Polymorphic Information Content
rpm	:	Revolution per minute
RNase	:	Ribonuclease
RCBD	:	Randomized complete block design
RNA	:	Ribonucleic acid
SSRs	:	Simple Sequence Repeats
SE	:	Standard Error
TE	:	Tris- EDTA
viz.	:	Namely
ppm	:	Parts per million

Chapter-1

INTRODUCTION

Wheat is an edible grain, one of the oldest and most important of the cereal crops. It is a self-pollinated annual and belongs to the family poaceae with three cultivated species, viz., hexaploid bread wheat (*Triticum aestivum* L. 2n = 6x = 42), tetraploid durum wheat (*T. turgidum* subsp. *durum*, 2n = 4x = 28) and diploid dicoccum wheat (*T. dicoccum* 2n = 2x = 14). Wheat (*Triticum* species) is a crop of global significance where winters are mild, therefore, today wheat is grown almost all over the world, with different varieties developed and grown according to the various climates (Simmonds, 1976). About one-sixth of the total arable land in the world is under wheat cultivation. Winter and spring wheats are the two major types of this crop, with the severity of the winter determining whether a winter or spring type is cultivated. Bread wheat accounts for 95 percent of the wheat production at the global level and hence termed as the "King of cereals" (Kalender, 2021). Though grown under a wide range of climates and soils, wheat is best adapted to temperate and sub-tropical regions with seasonal rainfall between 30 and 90 cm. At world level wheat occupies an area of 221.82 million hectares with a production of 775.8 million tonnes and productivity of 3.50 million tonnes per hectare (Anonymous, 2021). India produced 107.9 million tonnes and ranked next to the China in global wheat production during 2020-21 with an acreage of 31.36 million hectares and average yield of 32.42 quintals per hectare. In Jammu and Kashmir, it forms an important component of the rice-wheat-maize based diet and is been grown in 288 thousand hectares with production of 504 thousand tonnes and a productivity of 17.5 quintals per hectare (Anonymous 2019).

The greatest portion of the wheat flour produced is used for bread making. Among the cultivated wheat the hard type tetraploid wheat (*T.turgidum* L.) produces the flour best suited for cakes, crackers, cookies, pastries and bread making. Its grain contains 55% carbohydrate, 2.1% fat and about 14.70% protein (Kumar *et al*., 2011) but lacks in certain important dietary components especially micronutrients. The wheat of humid areas is often softer, with protein content of about 8-10 percent and weak gluten.

The wheat grown in dry climates is generally hard type, having protein content of 11-15 percent and strong gluten (elastic protein). It is a staple food of millions of people. It supplies about 20 per cent of the food calories for the world's growing population.Wheat is cultivated for grain purposes, used whole or ground.

In plant breeding, genetic diversity is vital for exploiting heterosis or generating productive recombinants. In a breeding programme, choosing the right parents is crucial. As a result, understanding the genetic diversity and relativeness in available germplasm is must for any crop improvement programme. Thus, precise information on the nature and degree of genetic variation contained in wheat collections from the main agricultural areas will help in the selection of parents for developing superior varieties. Diverse genotypes from existing germplasm should be selected and used in future breeding programmes to improve the genetic base of this crop.

To fulfil the demands of the developing world in the next 40 years, there is an urgent need for a 40–60% increase in wheat production due to the constantly growing worlds population and demand for food production (Goutam *et al*., 2015). Although, the biggest obstacles for achieving the objective include both biotic and abiotic stresses, the most difficult problem that wheat breeders face today is to increase grain production with enhanced grain quality. These two factors must apply appropriate methods to increase resistance to biotic and abiotic stresses (Todorovska *et al*., 2009; Kamal *et al*., 2010). The most significant disease affecting wheat crop in the globe, causing losses in both quantity and quality of wheat, is rust. The three rust species that affect wheat crop are; yellow (stripe) rust, leaf (brown) rust, and stem (black) rust species. Rust infections have long been a significant issue for breeders, farmers, and seed companies (Marsalis and Goldberg, 2006).

Yellow streaks of small, bright yellow, elongated uredial pustules grouped in rows on the leaves, leaf sheaths, glumes, and awns are the disease's initial symptoms. When mature pustules rupture, yellowish-orange masses of urediospores are released. Bright yellow to orange, single-celled uredospores with a diameter of 20 to 30 m are produced by the fungus. Urediospores that are carried by the wind can cause primary infection. Rapid racial evolution of the fungus is facilitated by mutation and somatic hybridization (Stubbs, 1985). Being air borne, local races can shift to new places and quickly cover regional and frequently global areas. The most harmful disease in cold

climates is stripe rust (Singh *et al*., 2001). It typically occurs in areas where the temperature is between 10 and 15 degrees Celsius, and it develops at lower temperatures.These penalties results from rust colonies in the leaf, draining carbohydrates from the plant and reducing green leaf area. Severe infections result in poor root growth and drought susceptibility (Martinez *et al.*, 2022). Depending on the cultivars' level of resistance, it may result in yield losses of up to 100%. (Jindal and Sharma, 2010). A serious rust infection results in grain shrivelling, which lowers test weight and lowers net yield (Ahmad *et al*., 2010). Both early as well as late stripe rust epidemics has an impact on yield. In susceptible cultivars, long season epidemics had an impact on all of the yield parameters (Ash and Brown, 1990). Due to yield losses and the expense on chemicals needed to control the disease, stripe rust has a significant negative impact on the economy (Pretorius *et al*., 2001).

For the development of genotypes resistant to rusts, we need to screen large population against diseases and the tolerant and resistant lines can be further used for breeding desirable genotypes for a particular region or area. Molecular markers are considered to be the best and very precise tool for determining genetic diversity, as they are unlimited in number and can show high polymorphism and are also independent of environmental interaction i.e., highly heritable. Molecular markers, including RAPDs, RFLPs, SSRs, and RGAPs, are useful tool for gene mapping in wheat. In particular, Simple Sequence Repeats (SSR) loci are used much more frequently than other markers due to their advantages with a higher level of polymorphism with known map location, accuracy, repeatability and PCR-based amplification. Based on SSR markers, several molecular linkage maps have been developed for wheat, which are being extensively used to locate genes and QTLs responsible not only for disease resistance, but also for numerous other agronomic characteristics (Roder *et al*., 1995; Pestsova *et al*., 2000; Varshney *et al*., 2000; Gupta *et al*., 2002). SSRs are valuable as genetic markers because they detect high levels of allelic diversity, co-dominant, easily and economically assayed by PCR and easily automated. The technical efficiency and multiplex potential of SSRs makes them preferable for many forms of high throughput mapping, genetic analysis and marker assisted plant improvement strategies. Closely linked SSR markers can provide a powerful tool for pyramiding yellow rust resistance genes and marker assisted selection in breeding programmes (Roders *et al.*, 1995 and Somers *et al.*, 2004)

With aforesaid points in view, the present investigation entitled, “Genetic diversity studies in advanced breeding lines of wheat (*Triticum aestivum* L.em Thell)”, has been devised with the following objectives:-

1. To evaluate the advanced breeding lines of wheat for yield and yellow rust.
2. To analyse the genetic diversity by using molecular markers.

Chapter-2

REVIEW OF LITERATURE

Wheat is the most important cereal crop and plays a vital role in food and nutritional security of the country. The high nutritional value and very good storage properties made wheat indispensable for world nutrition. To fulfil the demands of the developing world in the next 40 years, there is an urgent need for a 40–60% increase in wheat production to meet up the demand for food the constantly growing world's population. (Goutam *et al*., 2015). In the present study, we aimed to find out the superior most diverse genotypes those can be utilized in further breeding programs.Thus, this chapter deals with brief account of work carried out by different workers over the years and which are relevant to the objectives of the present investigation entitled, **Genetic diversity studies in advanced breeding lines of wheat *(Triticum astivum* L.em Thell) ,** is reviewed under following headings:

2.1 Genetic variability for diversity studies

2.2 Molecular characterization for yellow rust resistance.

2.1. Genetic variability for diversity studies

Genetic diversity is a broad term encompassing all the variability occurring among different genotypes with respect to total genetic make-up of genotypes related to single species or between species. Genetic diversity can be measured by assess the number of different genes in a gene pool. Swingland (2013), defined genetic diversity as, the variation of heritable characteristics present in a population of the same species. The variation in heritable characters may express itself in the form of altered morphology, anatomy, physiological behaviour or biochemical features. Genetic diversity has received the greatest attention in plant breeding. Genetic diversity and genetic characterization are the prerequisite for any crop improvement program. Genetic advance and heritability are the essential genetic parameters for the selection of promising genotypes for further crop improvement.

Korkut *et al*. (2001) reported that the results of data analysis showed the phenotypic coefficients of variability (PCV) in bread wheat varieties were high for plant height and thousand kernel weights whereas the highest genotypic coefficient of variability (GCV) was obtained for grain yield per hectare. The genotypic coefficients

of variability were the lowest than the phenotypic variability coefficients for spike length and the number of spikelets per spike indicates the environmental effect.

Khodadadi *et al*. (2011) carried an experiment comprising 36 winter wheat genotypes and their study revealed that all traits, except emergence time and heading time were statistically significant among different genotypes. Cluster analysis based on squared Euclidean distance and Ward's method, categorized the cultivars into seven groups. The differences in genetic component of traits studied in the manuscript can be applied as a new source of variation in other breeding programs and crossing nurseries for wheat improvement.

Ullah *et al.* (2011) conducted studies on 41 genotypes of wheat for yield and yield attributing traits. They showed that the estimates of genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) were highly significant for spike length, days to 50% flowering and plant height. High heritability values were found in plant height, days to 50% flowering, days to heading, spikelets per spike and spike length whereas, the chracters like plant height, days to physiological maturity and spikelets per spike also have high genetic advance. Based on Euclidean cluster analysis 41 genotypes of wheat were divided into four groups and nine different clusters.

Kallimullah *et al*. (2012) evaluated 41 bread wheat genotypes for variability parameters and correlation studies.They observed that the genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) for grain yield per plant, flag leaf area, and number of tillers per plant were high. The PCV and GCV estimations for the remaining characteristics ranged from moderate to low. Grain yield per plant showed highly significant positive correlation with the number of tillers per plant and number of grains per spike. Cluster analysis grouped 41 wheat genotypes into eight different clusters.

Talebi *et al*. (2012) conducted an experiment on 24 Iranian bread wheat cultivars under two constructing water regimes to estimate genetic parameters and relationship among morpho-agronomic traits. They found that the GCV values were highest for plant height, number of seeds per spike, and plant grain yield while the harvest index had the lowest (<2) values. The weight of 1000 seeds, plant biomass

and the number of seeds per square metre had comparatively low GCV values (3.8-8.8%) than other characters.

Bhushan *et al*. (2013) evaluated 40 wheat cultivars to estimate the genetic variability, correlation and path coefficient analysis of yield and contributing traits. They reported that the higher levels of GCV and PCV were observed for grain yield, biological yield, productive tillers per plant, and plant height. The least genotypic and phenotypic variability were seen in the number of spikelets per spike, days to heading, test weight, harvest index, grain filling duration, and days to maturity. The correlation coefficient estimate showed high direct genotypic and phenotypic correlations for the test weight, biological yield, spikelets per spike and grain filling period.

Kaddem *et al*. (2014) conducted an experiment on 26 wheat genotypes to assess the magnitude of genetic variability parameters. They observed that test weight had lowest genotypic coefficient of variation (GCV), followed by tillers per plant and spike length. On the other hand, grain yield had the highest phenotypic coefficient of variation (PCV), while harvest index, biological yield, the number of tillers per plant, and test weight had intermediate PCV values. Positive and highly significant correlation as observed for harvest index, plant height, biological yield and test weight with grain yield at both genotypic and phenotypic levels.

Desheva *et al*. (2014) conducted an experiment on 16 common bread wheat genotypes to evaluate variability, heritability and genetic advance. They observed phenotypic variance ranged from 0.12 (grain weight per spike) to 237.07 (plant height). Between 0.05 (number of productive tillers per plant) to 225.82 (plant height), were the genotypic variance values. Grain weight per spike environmental variations ranged from 0.06 to 24.63. In contrast to PCV, which ranged from 8.38% for the number of spikelets per spike to 28.24% for grain production per plant, GCV ranged from 6.22% for the number of spikelets per spike to 19.55% for spike length. Characteristics like plant height, spike length and number of grains per spike showed high heritability coupled with high genetic advance ,indicates additive gene action.

Awan *et al.* (2014) studied six wheat genotypes and their nine crosses to examine genetic variability, heritability and correlation. They found that the genotypic variance was largest for peduncle length, followed by plant height and grain yield per plant, but it was least for the number of tillers per plant. Flag leaf area showed the

highest genotypic coefficient of variability, but the trait days to 50% heading showed the lowest value of GCV. Plant height and peduncle length revealed high phenotypic variance, however, the number of tillers per plant showed the lowest value for this parameter. Flag leaf area had the highest phenotypic coefficient of variation, followed by grain output per plant, and the lowest by days to 50% heading. In comparison to genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV) values were greater for all the traits under consideration.

Verma *et al*. (2014) assessed the genetic diversity for yield and yield variables in 108 bread wheat accessions from India and Australia. In cluster analysis they found that the distribution of these genotypes into eleven clusters and greatest number of genotypes grouped into cluster IV (26) followed by cluster VI (22) and cluster II (12) were also notable. Most of the time, the inter-cluster distance was greater than the intra-cluster distance, showing greater genetic diversity among the accessions from various groups. Cluster VIII and IX had the greatest inter-cluster distance, measuring 113.94, followed by cluster VIII and X, measuring 97.72, demonstrating the great variation across the groups. Cluster X had the highest intra-cluster distance (13.96) while cluster VII had the smallest (00.00). Grain yield, harvest index, and spike weight all had highest mean values in cluster X. Perenjori, KRL 261 and KRL 283, from cluster X, and Gutha, from cluster IX, are the genotypes in these clusters that may be utilized as prospective donors for a hybridization programme to create genotypes with high grain yields.

Maurya *et al.* (2014) conducted an experiment comprising 40 bread wheat genotypes to assess the genetic variability among tested material and found high PCV and GCV for yield per plant. Greater heritability were found for grain yield per plant, grain weight, grains per spike, and plant height, whereas, moderate heritability was found for spike length, days to maturity, and days to heading. For grain yield per plant, number of grains per spike, and grain weight expressed as a percentage of the mean, high genetic advance was assessed.

Choudhary *et al.* (2015) characterised 18 genotypes of wheat for four factors that affect yield. The PCV and GCV were found to be high for spikelet per spike and number of grains per spike while moderate for number of effective tillers per plant and spikelet per spike. The heritability for all the characters was found to be high.

Dargicho *et al.* (2015) examined sixty-eight lines of bread wheat and observed that the phenotypic coefficients of variation values were higher than the values of genotypic coefficients of variation. High PCV and GCV were recorded for productive tiller, spike length, kernels per spike,1000 grain weight, biomass yield, harvest index and grain yield.

Dutamo *et al.* (2015) evaluated 68 germplasm lines of bread wheat and observed that the high PCV and GCV values were found for number of productive tillers, spike length, kernels per spike, 1000-grain weight, biomass yield, harvest index, and grain yield. All the characters under study showed high heritabilitiy. The traits, such as spikelets per spike, spike length, grain yield per plant, harvest index, and test weight, whereas all traits showed low values of genetic advance as percentage of mean.

Fikre *et al.* (2015) evaluated 64 bread wheat genotypes and found the moderate PCV and GCV for 1000-kernel weight, grain yield per plant, and harvest index. The PCV and GCV of the remaining characters were low. In contrast to biomass yield, the traits harvest index and grain yield per plant showed moderate heritability. Moderate genetic advance expressed as percentage of mean was reported for harvest index, grain yield per plant, days to 50% heading, number of grains per plant and 1000 grain weight. Grain yield per plant showed moderate heritability and high genetic advance, whereas days to 50% heading, 1000 kernel weight, plant height, and other traits showed high heritability and moderate genetic advance.

Yadawad *et al*. (2015) investigated genetic variation in wheat genotypes for grain yield per plant and its component attributes, and leaf rust resistance in the F2 population of the cross DWR162 X PBW343. All the characters, except days to 50% flowering, spike length, and number of spikelets per spike, showed high PCV and GCV. With the exception of days to 50% flowering, spike length, and number of spikelets per spike, all the traits had high heritability and genetic advance. High heritability, genetic advance, and a high genetic coefficient of variation were for the incidence of leaf rust.

Rehman *et al.* (2015) conducted an experiment on 24 spring wheat genotypes to estimate genetic divergence and principle component analysis. Their findings

showed that the 24 genotypes were grouped into three clusters in which genotypes of cluster II and cluster III exhibited maximum divergence as their inter cluster distance was highest followed between cluster I and cluster II. So, genotypes from these groups could be used as parents in hybridization program.

Khan and Hassan (2017) studied a set of 27 genotypes of wheat for the estimation of heritability, genetic advance and association of yield and yield related traits. Studies revealed that mean squares showed the presence of significant variation ($p \leq 0.01$) among the genotypes. Heritability estimates were observed as high ($h2 > 0.60$) for all the traits. These results suggested that all the traits showing significant correlation with grain yield and needs better attention in future wheat breeding programs for increasing yield.

Devesh *et al*. (2018) conducted studieson genetic variability, heritability and genetic advance for fifteen traits of wheat genotypes and revealed that the phenotypic coefficient of variation (PCV) was higher in magnitude than that of genotypic coefficient of variation (GCV) for all the traits study. This indicates that these traits may be under the control of additive gene action that can be utilized for further breeding programs and crossing of wheat improvement.

Thakur *et al.* (2018) evaluated fourteen different wheat genotypes including three standard check varieties and found enough variability for all the parameters studied. Characters like grain yield per plot showed the highest genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV). Whereas, the phenotypic coefficient of variation (PCV) ranged between 5.03% (days to maturity) and 20.85% (grain yield hectare1), the genotypic coefficient of variation (GCV) ranged from 4.59 (days to maturity) to 13.76% (grain yield per hectare). Heritability in the broad sense and genetic advance as percent of mean (GAM) varied from 33.33% (for tiller per plant) to 84.67% (for a peduncle length) and 8.66% (for days to maturity) to 18.74% (for grain yield per hectare), respectively.

Rajshree and Singh (2018) carried out an experiment on 33 genotypes of bread wheat for yield and contributing traits. Findings showed that the variability indicated high to moderate phenotypic and genotypic coefficient of variation accompanied by high heritability and genetic advance as per cent of mean for traits like, plant height, number of tillers per plant, flag leaf area, chlorophyll content, canopy temperature,

spike length, grains per spike, grain yield per plot and harvest index indicating their importance in selection for yield improvement. The thirty-three genotypes of bread wheat were grouped into six clusters using Tocher's method. The genotypes in cluster III and cluster VI, exhibited high degree of genetic diversity.

Singh *et al.* (2018) evaluated 35 diverse wheat genotypes to assess the genetic diversity for various morphological and quality traits. Their findings revealed that the traits like days to 50% flowering, days to maturity, productive tillers, plant height, spike length, spikelets per spike, grains per spikelet, biological yield, harvest index, 1000 grain weight, grain yield and gluten content showed highly significant differences for all genotypes. Based on multivariate analysis, 35 genotypes were grouped into 6 clusters.

Patel *et al.* (2019) carried out an experiment to study the genetic variability, heritability and genetic advance for eight traits in 40 advance breeding lines of wheat. Their findings revealed that all genotypes exhibited wide range of variation for all the traits except for number of tillers per meter indicating the presence of enough genetic variability in the experimental material. PCV values were higher than GCV values for all the traits which reflect the influence of environment on the expression of traits. However, very high values of heritability were observed for days to heading (92.78%), spike length (90.76%) and days to maturity (89.45%). High heritability coupled with high genetic advance as percent of mean was observed for spike length and number of grains per spike suggesting selection for these traits would give better response. Thus, these characters may be effective as selection indices during breeding programme for improving grain yield.

Choudhary *et al*. (2020) conducted an experiment in 60 pre-released promising genotypes of wheat for yield and yield contributing traits. They found that the high degree of genotypic and phenotypic coefficient of variation were observed for ear weight, grain yield/plant, biological yield/plant, peduncle length, sedimentation value, number of effective tillers/plants, number of ear/plant and ear length. Hence, improvement of grain yield can be achieved by improving these traits. It indicated that these characters had a high association with grain yield/plant and selection for these traits would lead to increase in yield.

Singh *et al.* (2020) conducted diversity studies on 39 bread wheat genotypes for traits viz., days to fifty per cent flowering, days to maturity, plant height, spike length, flag leaf area, relative water content (RWC), chlorophyll content, canopy temperature, number of tillers per plant, number of grains per spike, 1000 grain weight, harvest index and grain. The genotypes of bread wheat were grouped into seven clusters using Tocher method. Therefore, the selection of parents from this cluster for these traits would be effective. Under stress, the genotypes viz., GW 2007-80, WR 1872, NAIW-1607, NAIW-1342 and WR 1743 were found promising as indicated by low drought susceptibility index.

Elahi *et al.* (2020) studied in 36 promising wheat genotypes and found enough variability for all the traits. The highest genotypic and phenotypic coefficients of variation were found in ear length and number of ear per plants. The weight of thousand grains and the length of ear both have shown strong heritability and high genetic advance at <1% level of significance) among all the genotypes studied. Path coefficient analysis revealed that the magnitude of positive direct effect on grain yield per plant was highest through harvest index.

Brawed *et al*. (2022) carried out an investigation on 48 genotypes of wheat to assess the genetic diversity under two environment conditions. They reported that the genetic diversity analysis led to the formation of seven clusters in both environments. Based on the genetic distance, it was concluded that crossing of genotypes from cluster V with clusters I and IV to get a broad spectrum of variation for both environments. Cluster VII for timely sown and cluster V for late sowing conditions contained genotypes with high mean performance for grain yield and other traits and therefore these genotypes can be utilized for yield improvement.

Kumar and Singh (2022) carried out an experiment in 20 advanced breeding lines of wheat to study genetic variability. Findings indicated that the genotypes exhibited wide range of variation for all the traits except for days to maturity, 1000-seed weight, biological yield per plant indicating the presence of enough genetic variability in the study material. Phenotypic coefficients of variation were higher than the genotypic one. Traits like plant height, effective tillers per plant, ear length, spike weight, grains per spike, 1000-seed weight, biological yield per plant, and harvest index are those character which have high x medium or medium x high heritability

and genetic advance, respectively, indicates that this character can also be improved through selection.

Kumar *et al*. (2022) conducted an experiment on 65 genotypes of bread wheat to assess the genetic diversity for yield and physiological traits under normal conditions. The observations were recorded on 15 morphological and two physiological traits. After analysis of genetic diversity, it was observed that maximum percent contribution towards genetic divergence in 65 wheat genotypes was from 1000 grain weight, grain yield per plant and biological yield and minimum from number of effective tillers per plant, spikelet number per spike and spike density.

2.2 Molecular characterization for yellow rust

Molecular characterization is an important tool in providing an understanding the nature of the genetic makeup and genetic modification in plants Rust disease response is used to assess the resistance status of breeding lines, which is required to be tested across location and complemented with molecular markers.

Huang *et al.* (2002) examined the genetic diversity of 998 accessions of hexaploid bread wheat (*Triticum aestivum* L.) using a set of 24 wheat microsatellite markers. A total of 470 alleles were found, with 18.1 alleles on average per locus. The B genome has the most 19.9 alleles per locus, compared to 17.4 and 16.5 for the A and D genomes, respectively. The number of alleles were associated with gene diversity. For the 26 microsatellite loci, Nei's genetic diversity estimates of gene diversity ranged from 0.43 to 0.94, with an average of 0.77.

Naghavi *et al.* (2004) conducted an experiment on bread wheat to assess genetic diversity by using two different DNA-based techniques viz., Randomly Amplified Polymorphic DNA (RAPD) and Simple Sequence Repeats (SSR) markers. A total of 188 clear and repeatable bands were amplified from 17 selected RAPD primers and 35 SSR primer pairings resulted in the detection of a total of 101 fragments.

Luo *et al.* (2009) identified several stripe rust resistance genes in bread wheat, including *Yr5, Yr7, Yr27, Yr31, YrV23, YrSp, YrQz, YrTp1,* and *YrCN19* on chromosome 2B. They included *YrV23,* which was discovered to be associated to the microsatellite marker Xwmc356, *YrQz,* which was linked to Xgwm526 and

Xgwm388, and *YrCN19*, which co-segregated with Xgwm410 and might be utilised as a diagnostic marker.

Mclntosh *et al.* (2010) identified several stripe rust resistance genes in bread wheat using molecular markers Xcfb3530, Xbarc75, Xwms501, XSTS7/8, Xbarc6 and Xgpw2181 . Yellow rust resistance genes *Yr4, Yr38, Yr40, Yr43*, *Yr44, Yr45, Yr46, Yr47* and *Yr48* were identified.

Tabassum. S. (2011) evaluated 135 advanced wheat lines for examining slow yellow rusting used Resistant Gene Analog Polymorphism (RGAP), Simple Sequence Repeat (SSR), and Sequence Tagged Site (STS) markers to ascertain the presence and lack of some significant yellow rust resistance genes in wheat genotypes, including *Yr5, Yr8, Yr9, Yr15*, and *Yr18*.

Cabuk *et al*. (2011) examined repeat motifs and numbers to assess the DNA sequence differences among wheat cultivars using molecular markers for Yr genes (*Yr7, Yr9, Yr15, Yr18, Yr26,* and *YrH52*) on chromosomes 1B, 2B, and 7D of wheat.The most conserved sections among the resistant (Izgi 2001, Sonmez 2001, PI178383) and susceptible (Aytin98, ES14, Harnankaya99) cultivars to yellow rust were found in the molecular markers (Xgwm526) Yr7 and (Xgwm273) *YrH52* genes, while *Yr 15* (Xgwm413) had the least conserved portions.

Zhang *et al.* (2011) reported that *YrShan 515* resistance was caused by a single dominant gene, and it was consequently given the temporary name *YrShan515* in bread wheat. On wheat chromosome 7BL, twelve SSR markers (Xwmc335, Xwmc696, Xwmc476, Xbarc267, Xgwm333, Xwmc653, Xwmc396, Xgwm213, Xgwm112, Xgwm274, Xcfd22, and Xwme517) were identified.

Drikvand *et al*. (2012) carried out a study to understand the genetic diversity of bread wheat and to evaluate polymorphism information content (PIC) of some wheat SSR primers. Cluster analysis using the UPGMA method based on Jaccard similarity coefficient was performed. Based on cluster analysis, 92 wheat cultivars were grouped in six clusters. Results indicated that Iranian grown wheat cultivars had high genetic diversity which could be exploited in wheat breeding programs.

Pourkhorshid *et al.* (2014) used YrSTS7/8, Xpsp3000, and Xwmc44 markers to screen the race-specific seedling genes *Yr5* and *Yr10* and the race-non-specific APR gene *Yr29* among 40 Iranian genotypes. Twelve genotypes were found to

contain *Yr10*, while thirteen genotypes had *Yr29*. They came to the conclusion that these genes were effective for long-lasting resistance.

Singh *et al.* (2015) indicated that by applying the gene matching technique, three yellow rust resistance genes viz., *Yr2*, *Yr9* and *Yr18* were postulated. Adult plant resistance was assessed through host response and epidemiological parameters *i.e.*, final rust severity, relative area under rust progress curve, coefficient of infection and infection rate. All these promising yellow rust resistant cultivars at adult plant stage were susceptible at seedling stage to one or more pathotype(s) of yellow rust, which indicated the presence of adult plant resistance. At seedling stage, adult plant resistance gene *Yr18* was characterized in eight cultivars. All these wheat cultivars exhibited effective adult plant resistance.

Mukhtar *et al*. (2015) evaluated breeding lines of wheat for stripe rust resistance. Findings confirmed that the existence of the resistant *Yr5*, *Yr10*, *Yr15*, and *Yr18* genes in 14 entries, 29 entries, 25 entries and 9 entries including reference lines, while susceptible control did not amplify in the study.

Yaniv *et al.* (2015) evaluated that the introduction of the *Yr15* gene into the 34 isogenic lines and 23 sensitive wheat types using Xbarc and Xgwm413 markers. They hypothesised that MAS would be effective in introducing *Yr15* into different tetraploid and hexaploid wheat accessions.

Rani *et al.* (2019) evaluated 68 wheat genotypes to identify the distribution of 35 Yr genes using 70 molecular marker. Out of 35 Yr genes, 25 genes amplified the loci associated with Yr genes. Of the 35, 18 were All-stage resistance (ASR) genes and 7 were Adult- plant resistance genes (APR). In the field tests, evaluation for stripe rust was carried under artificial inoculation of Puccinia striformis, 53 wheat genotypes were found resistant to yellow rust among all genotypes.

Ahmad *et al*. (2020) study revealed that the total 2308 alleles of 302 markers were observed with average density of 7.6 alleles per marker. The mean values of gene diversity were identified in B-genome (0.78) followed by D-(0.77) and A-genome (0.71), which indicated the maximum variation in B-genome and graded the three genomes as B>D>A-genome for genomic variation. The experiment indicates the reliability of SSR markers that would be beneficial and robust resource for future

analyses of genetic diversity and molecular marker-assisted breeding in wheat for selection of promising genotypes.

Haider *et al*. (2023) evaluated thirteen known Yr gene-associated markers pertaining to genes (*Yr5, Yr10, Yr15, Yr24\Yr26*), and found that *Yr5* was detected in sixteen lines with the help of two markers Xwmc175 and Xgwm120. *Yr10* was detected in ten lines with the marker Xpsp3000 and *Yr15* was detected in fourteen lines with two linked markers; Xgwm413 and Xgwm273.*Yr24\26* was detected in 15 lines with two linked markers; Xbarc181 and Xbarc187. Frequencies of *Yr5, Yr15*, and *Yr26\Yr24* was high among test wheat germplasm in comparison to *Yr10.*

Chapter-3

Materials and Methods

The present investigation entitled **"Genetic diversity studies in advanced breeding lines of wheat (*Triticum aestivum* L.em Thell)"** was carried out during *Rabi* 2022-23. The details of the experimental material and methods used in the present study are described in this chapter under the following heading:-

3.1 Experimental site and weather condition

The present experiment was carried out at Research Farm of Division of Plant Breeding and Genetics main campus SKUAST-J, Chatha and Molecular laboratory, Division of Plant Breeding and Genetics, Faculty of Agriculture, SKUAST-J, Chatha, Jammu during *Rabi* season 2022-23

3.1.1 Experimental material

The experimental material comprised of 100 lines of wheat including 4 checks. The advanced breeding lines of wheat were obtained from selections of nurseries of ICARDA and CIMMYT. The details of experimental materials are given in Table 3.1.

Table 3.1 List of experimental material of wheat genotypes

S.No.	Entries	Origin	S. No	Entries	Origin
1	Agra Local	Released variety	51	CM-118	CIMMYT's 54th IBWSN
2	HD-2967	Released variety	52	CM-119	CIMMYT's 54th IBWSN
3	DBW-187	Released variety	53	CM-121	CIMMYT's 54th IBWSN
4	HD-3043	Released variety	54	CM-122	CIMMYT's 54th IBWSN
5	CM-2	CIMMYT's 54th IBWSN	55	CM-123	CIMMYT's 54th IBWSN
6	CM-4	CIMMYT's 54th IBWSN	56	CM-125	CIMMYT's 54th IBWSN
7	CM-6	CIMMYT's 54th IBWSN	57	CM-133	CIMMYT's 54th IBWSN
8	CM-7	CIMMYT's 54th IBWSN	58	CM-139	CIMMYT's 54th IBWSN
9	CM-11	CIMMYT's 54th IBWSN	59	CM-175	CIMMYT's 54th IBWSN
10	CM-12	CIMMYT's 54th	60	CM-199	CIMMYT's 54th IBWSN

		IBWSN			
11	CM-15	CIMMYT's 54th IBWSN	61	CM-214	CIMMYT's 54th IBWSN
12	CM-16	CIMMYT's 54th IBWSN	62	CM-221	CIMMYT's 54th IBWSN
13	CM-17	CIMMYT's 54th IBWSN	63	CM-229	CIMMYT's 54th IBWSN
14	CM-21	CIMMYT's 54th IBWSN	64	IC-12	ICARDA's 22nd HT&DTSBWON
15	CM-24	CIMMYT's 54th IBWSN	65	IC-13	ICARDA's 22nd HT&DTSBWON
16	CM-25	CIMMYT's 54th IBWSN	66	IC-17	ICARDA's 22nd HT&DTSBWON
17	CM-31	CIMMYT's 54th IBWSN	67	IC-18	ICARDA's 22nd HT&DTSBWON
18	CM-35	CIMMYT's 54th IBWSN	68	IC-19	ICARDA's 22nd HT&DTSBWON
19	CM-38	CIMMYT's 54th IBWSN	69	IC-30	ICARDA's 22nd HT&DTSBWON
20	CM-40	CIMMYT's 54th IBWSN	70	IC-41	ICARDA's 22nd HT&DTSBWON
21	CM-42	CIMMYT's 54th IBWSN	71	IC-56	ICARDA's 22nd HT&DTSBWON
22	CM-44	CIMMYT's 54th IBWSN	72	IC-62	ICARDA's 22nd HT&DTSBWON
23	CM-49	CIMMYT's 54th IBWSN	73	IC-88	ICARDA's 22nd HT&DTSBWON
24	CM-52	CIMMYT's 54th IBWSN	74	IC-101	ICARDA's 22nd HT&DTSBWON
25	CM-53	CIMMYT's 54th IBWSN	75	IC-110	ICARDA's 22nd HT&DTSBWON
26	CM-54	CIMMYT's 54th IBWSN	76	IC-112	ICARDA's 22nd HT&DTSBWON
27	CM-55	CIMMYT's 54th IBWSN	77	IC-124	ICARDA's 22nd HT&DTSBWON
28	CM-57	CIMMYT's 54th IBWSN	78	IC-133	ICARDA's 22nd HT&DTSBWON
29	CM-58	CIMMYT's 54th IBWSN	79	IC-141	ICARDA's 22nd HT&DTSBWON
30	CM-60	CIMMYT's 54th IBWSN	80	IC-142	ICARDA's 22nd HT&DTSBWON
31	CM-64	CIMMYT's 54th IBWSN	81	IC-149	ICARDA's 22nd HT&DTSBWON

32	CM-65	CIMMYT's 54th IBWSN	82	IC-156	ICARDA's 22nd HT&DTSBWON
33	CM-69	CIMMYT's 54th IBWSN	83	IC-166	ICARDA's 22nd HT&DTSBWON
34	CM-72	CIMMYT's 54th IBWSN	84	IC-168	ICARDA's 22nd HT&DTSBWON
35	CM-79	CIMMYT's 54th IBWSN	85	IC-169	ICARDA's 22nd HT&DTSBWON
36	CM-84	CIMMYT's 54th IBWSN	86	IC-175	ICARDA's 22nd HT&DTSBWON
37	CM-86	CIMMYT's 54th IBWSN	87	IC-178	ICARDA's 22nd HT&DTSBWON
38	CM-89	CIMMYT's 54th IBWSN	88	IC-189	ICARDA's 22nd HT&DTSBWON
39	CM-90	CIMMYT's 54th IBWSN	89	IC-218	ICARDA's 22nd HT&DTSBWON
40	CM-91	CIMMYT's 54th IBWSN	90	IC-232	ICARDA's 22nd HT&DTSBWON
41	CM-92	CIMMYT's 54th IBWSN	91	IC-239	ICARDA's 22nd HT&DTSBWON
42	CM-93	CIMMYT's 54th IBWSN	92	HP-14	CIMMYT's 12th HZWYT
43	CM-94	CIMMYT's 54th IBWSN	93	HP-15	CIMMYT's 12th HZWYT
44	CM-95	CIMMYT's 54th IBWSN	94	HP-24	CIMMYT's 12th HZWYT
45	CM-98	CIMMYT's 54th IBWSN	95	HP-25	CIMMYT's 12th HZWYT
46	CM-106	CIMMYT's 54th IBWSN	96	HP-31	CIMMYT's 12th HZWYT
47	CM-109	CIMMYT's 54th IBWSN	97	HP-32	CIMMYT's 12th HZWYT
48	CM-111	CIMMYT's 54th IBWSN	98	HP-33	CIMMYT's 12th HZWYT
49	CM-113	CIMMYT's 54th IBWSN	99	HP-35	CIMMYT's 12th HZWYT
50	CM-116	CIMMYT's 54th IBWSN	100	HP-37	CIMMYT's 12th HZWYT

3.1.2 Experimental layout

One hundred genotypes of wheat were sown in Augmented Block Design with plot size of each genotype 1.6 m^2 *i.e.*, 4 rows of 2 m length with row to row spacing of 20 cm. All recommended agronomic practices were performed during the season for proper plant growth.

3.2 Experimental observations

The details of the methods used during the course of investigation are described below:

3.2.1 Morphological observations recorded

3.2.1.1 Quantitative traits

Quantitative traits are the traits controlled by several genes, with small additive, dominant or epistatic effects and in interaction with the environment. Quantitative traits have strong link with genetics as it helps in identifying regions on crop genomes that are associated with observed variation in phenotypic trait. Data was recorded for different traits on randomly selected five plants from each plot of hundred wheat genotypes. The details of traits and their methods recording individual are given below:

3.2.1.1.1 Days to 50 % flowering

It was calculated as total number of days from date of sowing to the stage when panicles have emerged from main tiller in 50 percent population. The average number of days for 50% flowering was calculated for each genotype.

3.2.1.1.2 Plant height (cm)

Plant height was measured in centimeter from the point of base to the top of the main stem of randomly selected five plants at the time of physiological maturity.

3.2.1.1.3 Number of tillers per plant

The total number of tillers arising from the base of the stem were counted at maturity stage. Data was recorded on five randomly selected plants.

3.2.1.1.4 Chlorophyll content of leaf (µM\m^2)

SPAD chlorophyll meter monitors the absorbances of the leaf in the red and infra-red areas. The device determines a numerical value that is proportionate to the amount of chlorophyll present in the leaf. The SPAD value was calculated for all the randomly five plants in a row and average value was computed. Reading was taken after 60 days after sowing.

3.2.1.1.5 Number of spikelets per spike

The total number of spikelets was counted from base of spike to top of spike at the time of maturity.

3.2.1.1.6 Number of grains per spike

The number of grains of a spike in each selected plant was counted after harvesting of individual plant.

3.2.1.1.7 Flag leaf area (cm^2)

The length and breadth of flag leaf were multiplied and later multiplied with correction factor (K=0.75).

3.2.1.1.8 Grain yield per plot (g)

After harvesting and threshing the grain yield of each plot was recorded in grams.

3.2.1.1.9 Test weight (g)

The character test weight was recorded as the weight of 1000 seeds taken from the threshed grains of each line randomly.

3.2.1.1.10 Harvest index (%)

Harvest index was computed using the following formula:

$$\text{Harvest index (\%)} = \frac{\text{Economic Yield (g)}}{\text{Biological yield (g)}} \times 100$$

Where, Economic yield = Grain yield

Biological yield = Total Biomass (grain + straw)

3.2.1.2 Qualitative traits

Qualitative traits are controlled by one or few genes and show discontinuous variation. The environment has a very little or no influence on the phenotype of these traits. These traits have profound effect on plant value and utilization. Data was recorded for different traits. The details of traits are given below:

3.2.1.2.1 Foliage colour:

Foliage colour was observed at the leaf sheath stage and based on colour intensity it can be classified into following three categories:

- Pale green
- Green
- Dark green

3.2.1.2.2 Flag leaf attitude:

Flag leaf attitude was observed at flag leaf stage and is categorized into following three groups:

- Erect
- Semi-erect
- Drooping

3.2.1.2.3 Spike attitude: It was observed at maturity stage and can be classified into three following three categories:

- Straight
- Bent
- Crooked

3.2.1.2.4 Ear colour: The observations on ear colour were taken at maturity stage and can be classified into three groups:

- White
- Light brown
- Dark brown

3.2.1.2.5 Ear shape: It was observed at maturity stage and can be classified into following three groups

- Tapering
- Club shaped
- Fusiform

3.2.1.2.6 Grain colour: Observations were recorded after threshing and can be classified into following three categories;

- White
- Amber
- Red

3.2.2 Pathological studies

3.2.2.1 Procedure for creating artificial epiphytotic condition

The leaves of susceptible wheat varieties with heavy sporulation were taken and soaked in water until they turned yellow. Tween 20 was then added to the sporulated water before spraying in order to increase the stickiness of spores on uninfected leaves, creating artificial epiphytotic condition in the field. Then, either the early morning or late at night, spraying was done with a fine sprayer. The process was repeated three times to get the stripe rust.

3.2.2.2 Disease Scoring for Yellow Rust Resistance

Disease severity for yellow rust was done according to Modified Cobb's Scale (Peterson *et al.* 1948) mentioned in Table 3.2 . Severity was recorded as percent of infection based on the percentage scale mentioned in Table 3.3. Below 5 percent severity, the intervals used were trace to 2. Usually 5 percent intervals were used between 5 to 20 percent severity and 10 percent intervals for higher reading.

Table 3.2 Modified Cobb's scale for screening of yellow rust in wheat

Reaction types	Response value	Category	Visible symptoms
O	0.0	Immune	No visible infection on plant
R	0.2	Resistant	Necrotic areas with or without minute uredia present
MR	0.4	Moderately resistant	Small uredia present surrounded by necrotic areas
MS	0.8	Moderately susceptible	Medium uredia with no necrosis but possibly some distinct chlorosis
S	1.0	Susceptible	Variable sized uredia, susceptible

Table 3.3 Severity scale and type of infection of yellow rust in wheat

Scale	Description
TR	Trace severity of a resistant type of infection
5MS	5 percent severity of a moderately susceptible type of infection
10MR	10 percent severity of a moderately resistant type of infection
30S or 100S	30 percent or 100 percent severity of a susceptible type of infection

3.2.2.3 Coefficient of Infection (CI)

Coefficient of Infection (CI) was estimated by using data on disease severity and host reaction by multiplying the severity value by a value of 0.10, 0.4, 0.8 or 1.00 for host response rating of R, MR, MS or S respectively (Pathan and Park, 2006).

3.2.3 Statistical methods used

The recorded data was then analysed using various statistical methods that are given below with subheadings.

3.2.3.1 Analysis of variance

The field data was evaluated using Augmented Block Design given by Federer, 1956. Augmented designs consist of two kinds of treatments, the checks or the standard treatments and new or augmented treatments (Federer, 1956). The design presumes checks as fixed effects whereas, the new entries as random effects. The new entries are usually not replicated especially when dealing with large germplasm sets. However, the checks are replicated to act as points of reference. Both the checks and new entries are randomly distributed among the blocks. Federer (1956) provided for statistical analysis of such designs, wherein, the random effects of new entries are used to account for various sources of variation. Augmented Block Design (ABD) accommodates both replicated as well as un- replicated entries and is highly useful in early testing of a huge number of test entries/accessions, when the replication is not practically possible, amount of seed is very limited as well as in case of unequal plot sizes. It saves time and money without compromising on the precision of critical comparisons among treatments. However, a major bottleneck in ABD is that loss of an un-replicated treatment means loss of entire information about that treatment. Augmented Block Design is a method of choice to undertake initial evaluation of a large set of germplasm accessions to select genotypes suitable for different aspects of crop breeding. This is more important in cases where initial seed is limited in quantity to undertake replicated experiments as well as our failure to ensure comparably homogenous experimental units which is a basic requirement of standard field designs. The design makes use of a procedure wherein, a large number of test entries to be evaluated are evaluated along with standard checks, with the checks being replicated randomly in all blocks. The data from checks is used to adjust mean values of test entries to make them comparable and also provide an estimate of experimental error.

The augmented completely randomized block design is a special case of partially balanced incomplete block design (Cochran and Cox, 1957). The replication of one or more checks in each block ensures that the design stays connected. The basic model for augmented block design is:

$$Y_{ij} = \mu_j + Cj + \beta_i + \varepsilon_{ij},$$

Where,

Y_{ij} = overall mean

μ_j = effect of j^{th} block

C_j = effect of checks in j^{th} block

β_i = effect of ith treatment

ε_{ij} = random variation of i^{th} treatment in j^{th} block

In augmented designs, the checks to be used are usually the varieties which have been under cultivation for a large time and as such serve to monitor the progress. Other genotypes may also be used as checks for acting as base lines for diverse purposes. However, for yield a new variety could be the best standard. The checks used in the design are assumed as being treatments of Randomized block Design for estimation of experimental error. There are certain basic prerequisites for efficient use of augmented designs such as:

1. The minimum number of degrees of freedom for error in the ANOVA should be 10, with the error degree of freedom calculated as (R-1)(C-1), where R is the number of blocks and C is the number of checks in each block.
2. The minimum number of blocks in augmented block design should be $r \geq [10 / (C-1)] +1$. Therefore, with four checks, minimum number of blocks is $[10 / 2]+ 1= 6$.
3. Each block in augmented block design should have at least C+1plots.

The augmented design also provides estimates of standard errors of difference for four different comparisons to compute least significant increase. However, the most useful comparison is the difference between adjusted means of test entries and a check mean designated as Least Significant Increase (LSI) (Table 3.4).

Table 3.4: Formulae for calculating standard errors of mean for comparison

Standard Error	**Formula**
Difference between two check varieties (Sc)	2MSE/R
Difference between adjusted means of two Test entries in the same block (Sb)	2MSE
Difference between adjusted means of two test entries in different blocks (Sv)	2 (C + 1) MSE/ C
Difference between adjusted test entry and check mean (Svc)	{(R +1) (C + 1) MSE}/ R.C
Least Significant increase (LSI)	tα. Svc

Where,

MSE, is the error mean square estimated by the design at (R-1)(C-1) degrees of freedom,

R, is the number of blocks or the replicates of a check,

C, is the number of checks used and

t , is the value of "t" at (R-1)(C-1) degrees of freedom.

In the present study, the material was evaluated in Augmented Block Design (Federer, 1956), consisted of 6 blocks containing 20 genotypes in each with 16 test entries and four check entries. The plants were space planted with a plot size of $1.6m^2$ for optimal expression of traits. Data was collected from five randomly selected competitive plants on various physiomorphological traits. In each block the checks were allotted randomly.

3.2.3.2 Assessment of variability

The various variability parameters comprised range, mean, standard deviation and coefficient of variation are detailed as below:-

3.2.3.2.1Range

Range is calculated by computing difference between highest and lowest value of the character, which can be expressed as follows:

Range = highest value – lowest value

3.2.3.2.2 Mean

The average of recorded observations was calculated as follows:

$$X = \sum \frac{Xi}{n}$$

Where,

Σxi = Summation of all the observations

n = Total number of observations

3.2.3.2.3 Standard Deviation

It is defined as the positive square-root of the arithmetic mean of the square of the deviations of the given observation from their arithmetic mean and calculated as:

$$\text{S.D} = \sqrt{\frac{\sum(xi-\bar{x})^2}{n-1}}$$

Where,

S.D. (σ) = Standard deviation of sample

Xi = An observation/variate value

$\bar{X}$ = Mean of sample

n = number of given observations

3.2.3.2.4 Standard error

The standard deviation of the sampling distribution of a statistic (estimate) is known as the standard error of that statistics and calculated as:

$$\text{S.E} = \frac{\sigma}{\sqrt{n}}$$

Where,

S.E. = Standard Error

σ = Standard deviation of Sample

n = Number of given observations

3.2.3.2.5 Coefficient of variation

The coefficient of variation is obtained by dividing the standard deviation by the mean and expressed in percentage and calculated as:

$$CV = \frac{\sigma}{\bar{x}} \text{ X } 100\ \%$$

Where,

CV= coefficient of variation

σ= Standard deviation of Sample

$\bar{X}$ = Mean of sample

3.2.3.3 Estimation of components of variance

Components of variation (σ^2_g and σ^2_p) were evaluated by the formula proposed by Syukur *et. al*. (2012).

$\sigma^2_g = [(M_g) – (M_e)] / r$ $\sigma^2_p = [\sigma^2_g + (\sigma^2_e/r)]$

Where,

M_g = Mean sum of square of genotypes

M_e= Error mean sum of square

σ^2_p= Phenotypic variance

σ^2_g= Genotypic variance

σ^2_e= Environmental variance (error mean square from ANOVA)

r = Number of replications

3.2.3.4 Coefficient of variation

It is the statistical technique used to assess the variability that arises during the course of this experiment. It is expressed as a percentage of the ratio of standard deviation to mean of the sample.

$$GCV = [(\sigma^2 g)^{½} / \text{Mean}] \times 100$$

$$PCV = [(\sigma^2 p)^{½} / \text{Mean}] \times 100$$

Where,

σ^2_g= Genotypic variance

σ^2_p= Phenotypic variance

3.2.3.5 Heritability

Heritability in Broad sense (h^2_{bs}) was calculated as the ratio of genotypic variance to phenotypic variance (Allard, 1960). Where σ^2_p and σ^2_g are the phenotypic and genotypic variance of the character, respectively.

$$h^2_{bs} = [(\sigma^2_g) / (\sigma^2_p)] \times 100$$

Where,

h^2_{bs}= heritability in broad sense

σ^2_g = genotypic variance

σ^2p = phenotypic variance

$(\sigma^2 g) + (\sigma^2 e)$ σ^2_e= environmental variance

3.2.3.6 Genetic advance (GA)

Genetic advance is defined as an increase in the mean genotypic value of selected plants over the parental population. It is a measure of genetic gain as a result of selection (Johnson *et al*., 1955).

$$\text{Genetic advance} = h^2_{bs} \times \sigma_p \times K$$

Where,

K = Selection differential (K = 2.06 at 5% selection intensity)

σ^2p = Phenotypic variance

h^2_{bs}= Broad sense heritability

3.2.3.7 Genetic advance as percent of mean (GAM)

It was calculated as per the method given by Johnson *et al.* (1955) as follows:

$$\textbf{GA (\%)} = \frac{\text{Genetic advance}}{\text{Grand Mean}} \times 100$$

3.2.3.8 Evaluation of genetic divergence using Euclidean cluster method

The genetic diversity of wheat genotypes was estimated by using Euclidean cluster analysis.

This method was given by Beale (1969). Cluster analysis was done in "R software"

3.2.4 Molecular characterization

Chemicals used for DNA extraction

- **1M Tris-HCL**

To prepare 100 ml of 1X Tris-HCL, about 12.11 g of Tris-base was measured in a beaker to which 80 ml of distilled water was added. To maintain a pH of 8, few drops of concentrated HCL was added and checked by litmus paper test. Finally, volume of 100 ml was made by adding distilled water.

- **0.5M EDTA**

To prepare 100 ml of 1X EDTA (Hi-Media) about 18.60 g was weighed and dissolved in 80 ml distilled water. In order to adjust the pH upto 8.0 NaOH pellets were added. The final volume of the solution was made upto 100 ml by adding distilled water.

- **5M NaCl**

To make 1000 ml of 5M NaCl, 292.20 g of NaCl was weighed and dissolved in 700 ml of distilled water then after thorough mixing the total volume was made 100 ml by adding more distilled water.

- **10X TBE Buffer**

To make 1000 ml of 10X TBE Buffer about 108 g of Tris-base (Hi-Media) was added to 900 ml of distilled water in addition to it 55 g of boric acid (Hi-Media) and 40 ml of EDTA (Hi-Media) were also added then the final volume of 1000ml was made by putting more distilled water.

- **70% Ethanol**

In order to make 100 ml of 70 per cent Ethanol, Absolute ethanol (99%) was measured upto 70 ml in a measuring cylinder then 30 ml of distilled water was added to make upto 100 ml of 70 per cent Ethanol.

- **10X TE Buffer**

For making1000 ml of 10X TE buffer 100 ml of 1M Tris-HCl (pH=8) and 20 ml of 0.5M EDTA were mixed and then to make final volume upto 1000 ml distilled water was added.

- **CTAB DNA Extraction buffer**

To make 1000 ml of CTAB buffer 100 ml of 1M Tris-HCL (pH=8), 280 ml of 5M NaCl, 40 ml of 0.5M EDTA, 20 g of CTAB were mixed and the remaining volume of 1000 ml was made up by adding distilled water.

3.2.4.1 DNA Isolation methodology

Genomic DNA was isolated using the protocol given by Doyle and Doyle, 1987 and is given below:

0.5-1 g of fresh leaf tissue material was crushed by using liquid nitrogen and grounded to a fine powder using a sterilized pestle and mortar.

- The fine powder (0.5g) was poured into 2 ml of micro-centrifuge tube and 1 ml of preheated CTAB extraction solution buffer at 60°C was added and thoroughly mixed.
- Eppendorf tubes containing samples were incubated in a water bath at 65°C for 1 hour followed by mixing every 15 minutes.
- Equal volume (1ml) of Chloroform : Isoamyl alcohol (24:1) solution was added, vortex well for 1 min. Then the tubes with loaded samples were centrifuged at 10,000 rpm for 10 min at 4°C in centrifuge.
- After that, the upper aqueous phase was carefully transferred to a new Eppendorf tube and equal amount (1ml) of Phenol: Chloroform: Isoamyl alcohol (PCI) 25:24:1 solution was added and mixed gently for 10 min. The tubes were centrifuged as per the previous step.
- The supernatant was taken out and carefully transferred to a new 1.5 ml microcentrifuge tube and chilled Isopropanol (500µl) was added. The tubes were mixed gently by inversion and kept at -20°C for 30 minutes.
- The tubes were taken out and kept at room temperature for 5 min and later centrifuged at 8000 rpm for 10 min at 4°C.
- The supernatant was discarded gently and DNA pellet was kept.

- 70% ethanol (300 µl) was added in the tubes and centrifuged at 4000 rpm for 4 min at 4°C.
- The DNA pellet was air dried and 200µl of TE buffer was added and further stored at -20°C.

3.2.4.2 DNA Purification

- To the isolated DNA, 3 µl of RNase (10 mg/ml) was added and was incubated at 37°C for 60 min.
- After that, 1 ml of Phenol: Chloroform: Isoamyl alcohol (25:24:1) solution was added and gently mixed by inversion. The tubes were then centrifuged for 10 min at 4°C at 10000 rpm in a high speed refrigerated centrifuge. The aqueous phase was placed in a new 2 ml centrifuge tube.
- About 300 µl 3M Sodium acetate and 500 µl isopropanol was added and kept at -20°C for overnight.
- Later the tubes were centrifuged at 8000 rpm for 10 min at 4°C and the supernatant was gently discarded. The DNA pellet was air dried.
- Purified DNA was dissolved in 100 µl TE buffer.

3.2.4.3 Quantification and Quality Check of DNA

The DNA samples quality was assessed by running them through 0.8 % agarose gel and observed for signs of fragmentation or DNA degradation and impurities.

3.2.4.4 Primers Dilution

Each primer was dissolved in ddH_2O (100 µM) and diluted to a working concentration of 10µM. (10 µl primer + 90 µl ddH2O). The primers were designed by Manimekalai *et al*., 2018 that were used for diversity studies.

3.2.4.5 PCR amplification of DNA by SSR primer

DNA amplification of the genotypes was carried out using 200µl PCR tubes containing 15µl reaction mixture. The components used for PCR reaction were buffer, dNTPs, $MgCl_2$, *Taq* DNA polymerase, template DNA, forward and reverse primers. List of SSR markers and the quantity of the components is given in the Table 3.5 and Table 3.6, respectively.

Table 3.5: List of SSR markers used for molecular characterisation of yellow rust.

S.No.	Marker	Yr genes	Forward Primer	Reverse Primer	Ta ^{0}C	Refrences
1.	Barc349	Yr5	CGAATAGCCGCTGCACAAG	TATGCATGCCTTTCTTTACAAT	58	Murphy et al. (2009
2	Xgwm501	Yr5	ACTTACATGAATTATCTTTCTTGGTCC	CGTATTCAAATAATCTTTCATCAGTC	55	Sun et al. (2002)
3	S19M93	Yr5	TAATTGGGACCGAGAGACG	TTCTTGCAGCTCCAAAACCT	58	Smith et al. (2007)
4	Xgwm526	Yr7	CAATAGTTCTGTGAGAGCTGCG	CCAACCCAAATACACATTCTCA	55	Yao et al. (2006)
5	Xpsp3000	Yr10	GCAGACCTGTGTCATTGGTC	GATATAGTGGCAGCAGGATACG	52	Bariana et al.(2002)
6	Xgwm582	Yr9	AAGCACTACGAAAATATGAC	TCTTAAGGGGTGTTATCATA	45	Cabuk et al.(2011)
7	IB-267	Yr9	GCAAGTAAGCAGCTTGATTTAGC	AATGGATGTCCCGGTGAGTGG	56	Mago et al.(2005)
8	Xbarc8	Yr15	GCGGGAATCATGCATAGGAAAACAGAA	GCGGGGGCGAAACATACACATAAAAACA	56	Murphy et al.(2009)
9	Xgwm273	Yr15	ATTGGACGGACAGATGCTTT	AGCAGTGAGGAAGGGGAT	58	Revathi et al.(2010)
10	Cssfr2	Yr18	TTGATGAAACCAGTTTTTTTTCTA	TATGCCATTTAACATAATCATGAA	55	Lagudah et al.(2009)
11	Cssfr1	Yr18	TTGATGAAACCAGTTTTTTTTCTA	GCCATTTAACATAATCATGATGGA	56	Lagudah et al.(2009)
12	Barc181	Yr24	CGCTGGAGGGGGTAAGTCATCAC	CGCAAATCAAGAACACGGGAGAAAGAA	58	Wang et al.(2008)

Table 3.6: List of reagents, concentration and quantity of single PCR reaction used in molecular studies in wheat

S.no.	Reagents	Concentration	Quantity (µl)
1.	PCR Buffer	5 X	3.0
2.	DNTPs	0.25 mM	0.3
3.	$MgCl_2$	2.00 mM	1.2
4.	Forward primer	10 µM	0.3
5.	Reverse primer	10 µM	0.3
6.	*Taq* DNA Polymerase	0.75 U	0.15
7.	Template DNA	~50 ng/ µl	1.0
8.	Nuclease free water	-	8.75
		Total Volume	**15.0**

The 15µl reaction mixture was vortexed and spinned down. For amplification of DNA sequence Polymerase chain reaction (PCR) was performed using a thermal cycler. The thermal profile used for amplification is given in Table 3.7.

Table 3.7: Thermal profile used for PCR amplification

Steps	Cycles	Temperature (°C)	Duration
Initial Denaturation	1	94	5 minutes
Denaturation	35	94	30 seconds
Annealing		55-62	30 seconds
Extension		72	60 seconds
Final Extension	1	72	10 minutes
Storage		4	

3.2.4.6 Agarose gel electrophoresis

- Agarose (4.5 g) was added to conical flask containing 150 ml of 1X TBE buffer and was heated in microwave oven.
- The solution was cooled to about 50-55°C and 6 µl of ethidium bromide was added to it and swirled gently.
- In casting gel unit, the gel solution was poured and the combs were set on the assigned slots.
- At room temperature the gel was allowed to solidify.
- The gel was placed carefully in the electrophoresis unit in such a way that the ends of the well are in line with the cathode.
- The electrophoresis unit was filled with 1X TBE buffer in order to submerge the gel and comb was removed preventing entry of air bubbles.
- 6 µl of PCR products were loaded carefully in the wells of the gel.
- The unit was connected to power supply and electrophoresis carried at 120 volts for 1.5 hr.

The amplified PCR products were visualized under Gel documentation system.

CHAPTER 4

RESULTS

The present investigation entitled "**Genetic Diversity Studies in Advanced Breeding Lines of Wheat (*Triticum aestivum* L.em Thell)**" was aimed to study agro-morphological and molecular characterization of wheat genotypes to assess the genetic diversity among the genotypes. The results so obtained from the present investigation are described here under the following sub-headings:

4.1 Evaluation of wheat genotypes for yield and contributing traits

4.1.1 Mean performance of genotypes

Mean performance of wheat genotypes with respect to yield and contributing traits are given in table 4.1.

Table 4.1 Mean performance of wheat genotypes for ten yield contributing traits

S.no.	Entries	DFF	PH	CC	NT	FLA	SP	GP	GY	TW	HI
1	Agra Local ©	106	119.60	31.30	9.63	46.00	16.86	40.12	253.00	24.70	21.43
2	HD-2967©	109	86.00	34.70	10.00	42.80	16.20	43.60	312.00	29.84	32.54
3	DBW-187©	104	81.65	35.80	8.36	33.50	18.00	57.00	316.00	34.04	38.76
4	HD-3043©	103	96.00	40.70	9.00	44.70	16.00	39.61	295.00	31.10	26.74
5	CM-2	106	94.80	39.10	11.00	35.50	22.80	72.98	319.00	41.00	29.75
6	CM-4	111	102.30	41.40	8.30	45.40	20.40	51.76	490.00	37.10	35.90
7	CM-6	109	92.30	40.40	12.10	41.90	17.60	47.86	476.00	28.10	36.67
8	CM-7	112	100.60	36.90	11.00	53.80	17.60	48.95	390.00	29.60	33.63
9	CM-11	109	96.00	40.80	16.60	63.60	19.20	76.34	312.00	41.02	25.38
10	CM-12	112	92.30	36.90	9.00	40.60	20.80	69.30	456.00	27.13	33.30
11	CM-15	106	99.70	37.90	11.60	41.70	19.60	49.00	291.00	53.10	26.27
12	CM-16	109	96.80	36.50	15.60	41.30	17.60	65.00	251.00	50.00	26.20
13	CM-17	109	100.20	45.40	14.30	41.40	23.20	74.85	210.00	42.80	28.14
14	CM-21	111	109.00	38.80	9.00	45.10	19.20	68.96	482.00	29.40	38.53
15	CM-24	106	100.00	36.50	13.00	41.30	18.40	57.30	316.00	41.00	34.48
16	CM-25	106	84.60	38.10	10.00	36.80	22.80	40.60	210.00	43.19	27.29
17	CM-31	113	99.60	28.00	15.30	45.30	20.00	78.44	254.00	39.01	28.12
18	CM-35	106	113.60	39.40	16.30	45.20	19.20	58.94	214.00	37.19	27.09
19	CM-38	106	99.60	40.50	10.60	44.75	18.80	53.00	310.00	24.91	25.35
20	CM-40	106	94.10	41.20	13.30	45.70	19.60	58.36	281.00	32.01	26.54
21	CM-42	103	93.60	40.20	11.00	43.40	18.40	54.00	200.00	39.08	25.47
22	CM-44	111	105.00	31.90	14.30	33.90	17.20	47.21	242.00	29.80	25.66
23	CM-49	106	109.60	32.70	8.00	34.10	20.00	64.80	536.00	40.10	35.58

24	CM-52	109	102.60	36.10	12.30	32.30	18.80	54.32	543.10	33.02	33.30
25	CM-53	109	109.30	47.20	12.30	51.50	20.00	63.74	460.00	32.10	36.86
26	CM-54	109	112.00	42.40	11.60	49.20	20.20	64.87	296.00	38.01	26.21
27	CM-55	103	113.60	40.30	12.00	46.70	21.00	52.10	284.00	29.40	28.03
28	CM-57	104	110.00	40.00	14.00	39.00	20.80	69.12	244.00	32.42	26.49
29	CM-58	106	105.50	35.10	10.60	42.10	22.80	77.85	402.00	31.61	27.61
30	CM-60	109	99.60	39.10	8.65	41.90	23.20	65.33	264.00	41.01	26.04
31	CM-64	111	97.00	35.30	8.30	48.60	17.20	44.53	312.00	37.21	26.79
32	CM-65	109	106.60	40.20	15.20	39.20	22.40	74.98	480.00	27.42	31.64
33	CM-69	111	89.00	36.50	9.00	56.20	21.20	64.20	410.00	39.82	35.78
34	CM-72	106	110.60	39.30	15.30	45.00	19.60	58.52	271.00	45.29	25.78
35	CM-79	113	98.00	38.30	11.00	42.40	22.80	73.87	216.00	46.10	25.35
36	CM-84	109	83.61	41.10	8.64	33.60	20.40	61.32	500.00	39.31	42.41
37	CM-86	109	106.60	37.40	12.30	47.50	21.60	69.32	336.00	29.82	26.12
38	CM-89	113	113.30	33.20	13.00	54.10	18.00	52.00	492.00	23.42	38.01
39	CM-90	111	108.60	46.30	12.00	59.00	18.80	79.86	196.00	42.40	23.91
40	CM-91	109	99.30	33.80	11.30	44.00	19.60	65.50	235.00	38.50	32.06
41	CM-92	111	109.00	40.30	8.00	35.30	18.80	55.00	330.00	51.40	37.63
42	CM-93	99	97.30	41.00	13.00	47.20	21.60	67.45	280.00	32.50	34.88
43	CM-94	106	98.60	42.40	12.10	50.40	18.40	39.60	200.00	42.40	29.67
44	CM-95	111	92.30	41.60	12.30	42.90	17.20	46.66	420.00	37.40	42.60
45	CM-98	106	112.00	34.10	9.00	50.70	20.40	59.70	205.00	40.10	29.73
46	CM-106	106	119.30	29.60	9.20	43.40	23.20	78.34	390.00	43.08	39.17
47	CM-109	113	105.00	41.80	9.00	44.80	16.80	44.90	460.00	48.42	30.83
48	CM-111	106	102.00	34.20	12.60	44.40	18.00	51.32	192.00	21.90	25.05
49	CM-113	98	96.80	34.50	11.00	47.30	19.20	56.00	270.00	40.00	28.89
50	CM-116	102	102.60	41.80	11.00	41.10	19.20	40.62	356.00	33.10	28.71
51	CM-118	109	108.60	40.30	9.00	36.70	20.40	65.88	412.00	52.08	37.52
52	CM-119	109	95.50	38.50	8.32	34.70	17.60	48.41	196.00	24.48	25.72
53	CM-121	98	111.30	36.00	10.20	45.60	19.60	66.75	370.00	39.04	31.36
54	CM-122	98	109.60	43.20	10.00	46.40	18.40	53.55	210.00	42.40	29.00
55	CM-123	106	110.60	48.40	9.30	50.60	21.60	62.10	336.00	54.02	29.44
56	CM-125	106	98.00	39.00	15.00	55.40	21.60	66.60	310.00	42.40	26.72
57	CM-133	106	100.00	40.40	10.60	44.20	19.60	59.76	332.00	37.42	25.46
58	CM-139	109	100.30	42.50	11.00	46.00	18.40	55.30	340.00	42.52	26.47
59	CM-175	104	92.00	36.00	8.46	37.60	16.80	47.53	366.00	23.18	31.60
60	CM-199	109	110.60	38.90	8.10	44.90	17.20	40.90	352.00	52.12	24.40
61	CM-214	109	93.00	42.00	10.30	54.90	19.18	57.60	240.00	44.71	26.22
62	CM-221	97	105.00	41.10	9.00	68.10	17.20	68.23	300.00	39.90	31.07
63	CM-229	106	91.60	33.00	11.00	43.40	18.40	53.42	410.00	51.40	39.80
64	IC-12	102	111.60	40.20	10.00	31.60	18.80	57.80	466.00	38.14	33.43
65	IC-13	106	105.60	39.20	9.12	45.60	18.80	54.43	436.00	41.23	33.90
66	IC-17	109	100.00	43.50	8.62	33.70	18.80	58.00	223.00	53.22	23.31
67	IC-18	109	108.30	39.10	13.60	32.00	21.30	51.99	424.00	43.10	33.95

68	IC-19	106	99.00	34.40	14.00	55.20	21.60	60.00	328.00	26.00	30.70
69	IC-30	111	104.00	42.30	9.00	37.10	19.60	62.60	352.00	39.86	34.05
70	IC-41	106	103.30	40.80	9.00	24.20	16.80	47.51	223.00	21.60	23.94
71	IC-56	99	104.20	31.10	9.60	48.10	15.20	58.23	320.00	35.90	32.07
72	IC-62	109	105.00	31.70	13.30	41.60	19.10	46.94	340.00	39.00	31.84
73	IC-88	109	98.60	35.90	13.20	40.30	22.80	75.00	264.00	31.02	28.29
74	IC-101	106	105.30	39.70	9.60	42.80	17.60	59.60	454.00	54.53	30.04
75	IC-110	106	104.00	39.10	10.70	41.30	19.20	57.84	240.00	49.10	29.00
76	IC-112	106	112.30	39.20	11.00	38.20	21.20	70.00	260.00	50.00	28.29
77	IC-124	106	105.60	41.10	8.10	38.00	16.80	47.25	430.00	43.20	32.41
78	IC-133	112	102.30	32.00	13.30	45.60	18.80	72.43	241.00	39.47	25.81
79	IC-141	109	101.60	39.80	12.00	39.60	20.00	47.65	516.00	48.29	33.51
80	IC-142	99	103.60	35.00	11.20	39.30	20.00	64.80	472.00	51.02	32.73
81	IC-149	106	104.60	31.70	10.00	55.00	23.10	76.10	512.00	43.02	35.19
82	IC-156	111	111.60	36.60	8.60	39.70	18.80	56.40	412.00	39.40	32.79
83	IC-166	106	105.60	34.10	9.00	38.00	18.00	48.92	481.00	42.04	29.14
84	IC-168	106	109.60	39.40	12.30	47.80	16.80	49.54	210.00	48.01	25.29
85	IC-169	111	107.60	46.50	10.10	45.40	19.60	58.76	510.00	39.97	30.83
86	IC-175	109	90.60	41.10	11.00	41.20	18.80	65.00	412.00	45.54	39.29
87	IC-178	109	102.60	37.40	11.60	39.50	22.40	78.65	390.00	21.00	33.26
88	IC-189	106	104.30	38.80	12.00	43.40	16.00	34.21	412.00	54.12	40.52
89	IC-218	113	100.60	37.30	8.14	45.80	18.40	53.40	324.00	39.30	29.09
90	IC-232	111	105.00	32.50	11.00	34.90	20.80	68.95	321.00	45.91	25.76
91	IC-239	106	116.60	43.40	10.30	40.20	18.40	53.45	214.00	51.10	25.69
92	HP-14	106	111.30	42.60	9.54	38.80	18.40	53.80	519.00	38.05	31.10
93	HP-15	109	95.60	38.80	10.60	32.00	22.80	75.66	500.00	40.10	32.85
94	HP-24	104	106.30	40.70	12.60	42.20	18.00	39.60	518.00	29.42	40.95
95	HP-25	109	108.60	36.80	13.21	63.50	19.60	63.21	472.00	44.10	28.07
96	HP-31	106	99.60	40.60	11.32	67.20	20.00	64.52	531.00	39.03	28.64
97	HP-32	106	103.00	43.10	8.00	52.20	23.60	79.00	542.00	32.41	33.85
98	HP-33	111	116.00	40.00	10.20	44.30	17.20	49.10	522.00	39.71	32.39
99	HP-35	109	106.30	41.00	12.00	77.20	21.60	58.00	525.00	38.90	34.48
100	HP-37	109	117.30	39.20	9.00	51.20	19.60	60.00	545.00	48.40	32.74
CV		2.25	8.80	9.80	6.78	11.82	6.01	10.70	9.80	10.51	11.38
Mean		10.738	102.91	38.68	10.98	44.25	19.55	59.01	355.88	39.30	40.70
Std. Er.		0.37	0.74	0.39	0.21	0.83	0.21	1.11	11.37	0.96	0.48

DFF=Days to 50% flowering, **PH=**Plant height, **CC**=Cholorophyll content, **NT**=Number of tillers per plant, **FLA**=Flag leaf area, **SP**=Number of spikelets per spike, **GP**=Number of grains per spike, **GY**=Grain yield per plot, **TW**=Test weight, **HI**=Harvest index

Table 4.2: Critical difference (5%)

Comparison	**DFF**	**PH**	**CC**	**NT**	**FLA**	**SP**	**GP**	**GY**	**TW**	**HI**
A test treatment and a control treatment	6.20	35.13	9.61	1.87	13.33	1.51	20.58	152.14	10.58	11.91
Control treatment means	2.97	16.80	4.60	0.89	6.38	0.72	9.84	72.74	5.06	5.69
Two test treatments (different blocks)	8.12	46.00	12.59	2.44	17.46	1.98	26.95	199.20	13.86	15.59
Two test treatments (same block)	7.26	41.14	11.26	2.18	15.62	1.77	24.11	178.17	12.39	13.94

DFF=Days to 50% flowering,**PH=**Plant height,**CC**=Cholorophyll content,**NT**=Number of tillers per plant,**FLA**=Flag leaf area,**SP**=Number of spikelets per spike,**GP**=Number of grains per spike,**GY**=Grain yield per plot,**TW**=Test weight,**HI**=Harvest index

4.1.1.1 Days to 50 % flowering

The variation in days to 50 % flowering ranged from 113 to 97 days. The general mean for days to 50 % flowering was observed as 107.38 days. Genotype CM-123 found to be earliest in flowering *i.e.*, 97 days followed by CM-122 (98 days), while, CM-109 was found to be as late in flowering (113 days).

4.1.1.2 Plant height (cm)

Plant height varied from 119.60 cm to 81.65 cm. The general mean of this trait was found as 102.91 cm. Agra-local recorded the highest value (119.60 cm) of plant height followed by HP-37 (117.30 cm) and HP-33 (116.60cm), whereas, DBW-187 recorded shortest (81.65 cm) plant height.

4.1.1.3 Chlorophyll content

The variation in chlorophyll content were ranged from 48.40 to 28.00. The general mean for chlorophyll content was observed as 38.63. The genotype CM-123 recorded the highest chlorophyll content value (48.40) followed by CM-53 (47.20) and

Plate 1: Recording of observations in field conditions

CM-90 (46.30) whereas, Genotype CM-31 recorded the lowest chlorophyll content value (28.00).

4.1.1.4 Number of tillers per plants

Number of productive tillers ranged from 8.00 to 16.60 with a general mean of 10.98. Maximum number of productive tillers per plant was recorded in genotype CM-11 (16.60) followed by CM-35 (16.30) and CM-16 (15.60), whereas, minimum number of productive tillers were recorded in genotype CM-49 (8.00).

4.1.1.5 Flag leaf area (cm^2)

The value of flag leaf area varied from 31.60 cm^2 to 68.10 cm^2. The general mean of flag leaf area was observed as 44.25 cm^2. The genotype CM-125 showed highest flag leaf area (68.10 cm^2) followed by HP-31 (67.20 cm^2) and CM-11 (63.60 cm^2) whereas, Genotype IC-12 showed the lowest value of flag leaf area (31.60 cm^2).

4.1.1.6 Number of spikelets per spike

The value of number of spikelets per spike varied from 16.00 to 23.60. The general mean for number of spikelets per spike was recorded as 18.78. The genotype HP-32 (23.6) observed the highest number of spikelets per spike followed by CM-109 (23.20) and IC-149(23.10) whereas, Genotype HD-3043 recorded the lowest number of spikelets per spike (16).

4.1.1.7 Number of grains per spike

Number of grains per spike ranged from 40.12 to 79.86 with a general mean of 59.01. Maximum number of grains per spike was recorded in genotype CM-90 (79.86) followed by HP-32 (79.00) and CM-31 (78.44), whereas, minimum number of grains per spike was recorded in genotype Agra-local (40.12).

4.1.1.8 Grain yield per plot (g)

Grain yield per plot was varied from 192 g to 545 g. The general mean of yield was recorded as 355.88 g. Genotype HP-37 (545 g) recorded the highest grain yield per plot followed by CM-52 (543.10 g) and HP-32 (542 g), while, CM-111 (192 g) recorded the lowest grain yield per plot.

4.1.1.9 Test weight (g)

Test weight varied from 21 g to 54.53 g. The general mean was recorded as 39.30 g. The genotype IC-101 (54.53 g) was recorded the highest value of test weight followed by IC-189 (54.12 g) and CM-123 (54.02 g). The genotype IC-178 (21 g) recorded the lowest test weight.

4.1.1.10 Harvest index (%)

The value of Harvest index was varied from 21.43 to 42.60.The general mean was recorded as 30.7. CM-95 (42.60) recorded the highest value of harvest index followed by CM-84 (42.41) and HP-24 (40.95), whereas, lowest value was recorded by the genotype Agra-local (21.43).

4.1.2 Categorization of qualitative traits

Different wheat genotypes were categorized for qualitative traits according to DUS standards has been presented in Table 4.3.

For foliage colour, 61 wheat genotypes showed dark green colour, 36 genotypes revealed green colour and 3 genotypes showed pale colour. The flag leaf attitude was categorised into three groups viz., erect (19), semi-erect (20) and drooping (61). The spike attitude was classified into three groups; straight (24), bent (38) and crooked (38). Ear colour was categorised into three groups viz., dark brown (27), light brown (58) and white (15). Ear shape was classified into four groups viz., tapering (29), parallel (18), fusiform (16) and clavate (37). Majority of wheat genotypes (95) showed amber grain colour whereas only 5 genotypes showed white grain colour.

Table 4.3: Categorization of the genotypes on the basis of qualitative characters

State	No.of genotypes	Genotypes
Flag leaf attitude		
Erect	19	CM-175,CM-6,CM-24,IC-133,IC-141,IC-142,IC-56,IC-189,IC-232,CM-35,CM-111,CM-44,CM-52,CM-86,CM-2,CM-42,CM-53,CM-116,HD-2967
Semi – erect	20	CM-7,CM-12,CM-229,IC-12,IC-30,IC-166,IC-101,IC-169,HP-15,CM-92,CM-125,CM-16,CM-25,CM-40,CM-79,CM-57,CM-72,CM-122,CM-121,CM-31

Drooping	61	CM-133,CM-109,CM-123,CM-139,CM-214,CM-84,CM-64,CM-49,CM-199,CM-69,IC-18,IC-17,IC-62,IC-175,IC-110,IC-156,IC-168,IC-88,IC-112,IC-124,IC-19,IC-218,IC-149,IC-13,IC-178,IC-239,IC-41,HP-33,HP-31,HP-32,HP-14,HP-24,HP-25,HP-35,HP-37,CM-4,CM-11,CM-15,CM-25,CM-38,CM-65,CM-93,CM-58,CM-89,CM-118,CM-94,CM-95,CM-106,CM-113,CM-119,CM-123,CM-60,CM-16,CM-54,CM-55,CM-90,CM-91,CM-98,HD-3043,DBW-187,Agra-local
Spike attitude		
Straight	24	CM-12,IC-18,IC-17,IC-62,IC-141,IC-110,IC-156,IC-168,IC-112,IC-124,IC-13,HP-15,HP-35,CM-44,CM-6,CM-86,CM-106,CM-109,CM-123,CM-53,HP-35,CM-4,11,Agra-local
Bent	38	CM-84,CM-7,CM-199,CM-229,CM-6,IC-12,IC-30,IC-88,IC-142,IC-19,IC-149,IC-56,IC-169,HP-33,HP-32,HP-14,HP-24.HP-25,HP-3793,CM-111,CM-58,CM-24,CM-79,CM-118,CM-57,CM-60,CM-2,CM-16,CM-42,CM-54,CM-90,CM-116,CM-121,CM-15,CM-38,CM-65,HD-2967,DBW-187
Crooked	38	CM-133,CM-109,CM-123,CM-139,CM-214,CM-64,CM-49,CM-175,CM-24,CM-69,IC-175,IC-166,IC-133,IC-101,IC-218,IC-178,IC-189,IC-232,IC-239,IC-41,HP-31,CM-35,CM-92,CM-52,CM-125,CM-40,CM-89,CM-72,CM-94,CM-95,CM-122,CM-55,CM-91,CM-98,CM-25,CM-31,HD-3043
Foliage colour		
Dark green	61	CM-109,CM-123,CM-84,CM-64,CM-7,CM-12,CM-49,CM-175,CM-199,CM-6,IC-18,IC-30,IC-62,IC-175,IC-166,IC-133,IC-141,IC-101,IC-156,IC-168,IC-88,IC-124,IC-218,IC-149,IC-13,IC-178,IC-169,IC-41,HP-33,HP-31,HP-32,HP-14,HP-24,HP-25,HP-35,H-37,CM-92,CM-111,CM-58,CM-125,CM-06,CM-86,CM-89,CM-118,CM-72,CM-94,CM-113,CM-122,CM-123,CM-2,CM-53,CM-54,CM-90,CM-91,CM-98,CM-116,CM-121,CM-31,CM-65,HD-2967,HD-3043
Green	36	CM-133,CM-139,CM-214,CM-24,CM-69,IC-12,IC-17,IC-110,IC-112,IC-142,IC-56,IC-189,IC-232,IC-239,HP-15,CM-35,CM-93,CM-52,CM-44,CM-24,CM-40,CM-79,CM-57,CM-95,CM-106,CM-109,CM-60,CM-16,CM-42,CM-55,CM-4,CM-11,CM-15,CM-25,CM-38,DBW-187
Pale	03	CM-229,IC-19,Agra-local
Ear shape		

Tapering	29	CM-214,CM-12,CM-49,CM-199,CM-24,IC-18,IC-62,IC-175,IC-166,IC-110,IC-156,IC-168,IC-19,IC-13,IC-56,IC-169,IC-232,HP-31,HP-15,CM-93,CM-125,CM-118,CM-94,CM-123,CM-90,CM-91,CM-4,Agra-local,DBW-187
Parallel	18	IC -30,IC-133,IC-149,CM-229,CM-64,CM-109,HP-24,HP-25,CM-2,CM-15,CM-42,CM-44,CM-53,CM-54,CM-92,CM-98, HD-3043
Fusiform	16	CM-84,IC-17,IC-101,IC-112,IC-124,IC-178,HP-32,HP-33,HP-35,HP-37,CM-35,CM-111,CM-40,CM-79,CM-72,CM-113,
Clavate	37	CM-133,CM-123,CM-139,CM-7,CM-175,CM-6,CM-69,IC-12,IC-141,IC-88,IC-142,IC-218,IC-189,IC-239,HP-14,CM-52,CM-58,CM-24,CM-89,CM-57,CM-95,CM-106,CM-109,CM-122,CM-60,CM-16,CM-55,CM-116,CM-121,CM-11,CM-15,CM-25,CM-31,CM-38,CM-65,HD-2967.
Ear colour		
Dark brown	27	CM-109,CM-84,CM-7,CM-199,CM-24,IC-18,IC-175,IC-166,IC-133,IC-156,IC-168,IC-142,IC-19,IC-218,IC-149,IC-56,IC-232,HP-33, HP-35,HP-37,CM-86,CM-118,CM-94,CM-95,CM-121,CM-11,DBW-187
Light brown	58	CM-123,139,CM-214,CM-64,CM-49,CM-175,CM-229,CM-69,IC-12,IC-17,IC-30,IC-141,IC-110,IC-88,IC-112,,IC-124,IC-13,IC-178,IC-169,IC-189,IC-239,HP-14, HP-15HP-24,HP-25,HP-31,HP-32,CM-35,CM-92,CM-93,CM-44,CM-16,CM-25,CM-40,CM-58,CM-125,CM-89,CM-57,CM-72,CM-113,CM-119,CM-122,CM-123,CM-60,CM-2,CM-16,CM-42,CM-90,CM-91,CM-98,CM-4,CM-15,CM-25,CM-38,CM-65,HD-2967,HD-3043,Agra-local
White	15	CM-133,CM-12,CM-6,IC-62,IC-101,IC-41,CM-111,CM-52,CM-79,CM-106,CM-53,CM-54,CM-55,CM-116,CM-31
Grain colour		
Amber	95	CM-133,CM-109,CM-123,CM-139,CM-214,CM-84,CM-64,CM-7,CM-49,CM-175,CM-199,CM-229,CM-6,CM-24,CM-69,IC-12,IC-18,IC-17,IC-30,IC-62,IC-175,IC-166,IC-133,IC-141,IC-101,IC-110,IC-156,IC-168,IC-112,IC-124,IC-142,IC-19,IC-218,IC-149,IC-13,IC-56,IC-178,IC-189,IC-169,IC-232,IC-239,IC-41,HP-33,HP-31,HP-32,HP-14,HP-15,HP-24,HP-25,HP-35,HP-37,CM-35,CM-92,CM-93,CM-111,CM-44,CM-52,CM-125,CM-6,CM-24,CM-40,CM-79,CM-86,CM-89,CM-118,CM-57,CM-94,CM-95,CM-106,CM-113,CM-119,CM-122,CM-125,CM-60,CM-2,CM-16,CM-42,CM-53,CM-54,CM-55,CM-

		90,CM-91,CM-116,CM-121,CM-4,CM-11,CM-15,CM-25,CM-31,CM-38,CM-65,HD-2967,HD-3043,DBW-187,Agra-local
Red	05	CM-12,IC-88,CM-58,CM-72,CM-98

Table 4.4: Analysis of variance for ten agro-morphological traits in wheat genotypes

Source	**DF**	**Days to 50% flowering**	**Plant height**	**Chlorophyll Content**	**Number of Tiller\plant**	**Flag leaf area**	**Number of Spikelets\spike**	**Number of Grains\spike**	**Grain yield\plot**	Test weight	Harvest index
Treatment (ignoring blocks)	99	12.78*	76.65*	17.01	5.01**	71.52*	0.95**	120.16*	11743.98**	63.38**	21.45*
Treatment (eliminating blocks)	99	12.34*	71.83*	17.02	4.44**	63.68**	0.92**	116.62**	10968.56**	69.51**	21.52*
Treatment: Check	03	16.38*	493.39	39.49*	0.93	62.62*	0.87	153.08*	2623.67*	81.52*	7.40**
Treatment: Test	95	12.35*	52.04**	14.45	4.52**	71.15*	0.96	117*	11976.80**	68.45**	22.09
Treatment: Test Vs Check	01	42.01*	1164.11*	192.53*	63.92*	133.78*	0.32*	321.07*	16982.49*	22.76*	2.90*
Block (eliminating treatments)	05	2.14	3.72	4.28	0.05	0.06	1.71**	152.34	2086.77	81.92**	9.97
Block (ignoring treatments)	05	10.79	98.76*	4.03	11.7**	155.26**	2.29**	222.40*	17439.11**	59.57*	8.71*
Residuals	15	5.81	186.31	13.95	0.53	26.84	0.34	63.95	3493.63	16.90	21.40

Plate 2 : Diversity in flag leaf attitude in wheat genotypes

Erect

Semi-erect

Drooping

Plate 3: Diversity in spike attitude in wheat genotypes

Straight

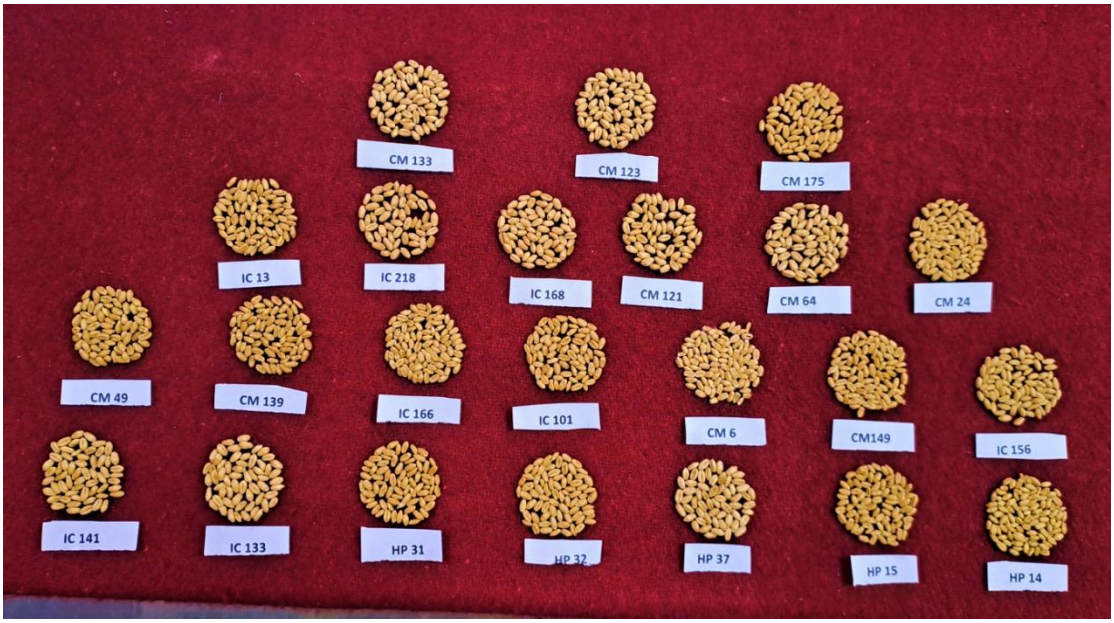
Bent

Crooked

Plate 4: Diversity in Ear colour of wheat genotypes

Brown **Light brown** **white**

Plate 5: Diversity in grain colour in wheat genotypes



4.1.3 Analysis of variance

Analysis of variance for various agro-morphological traits (Table 4.4) revealed that mean sum of squares for treatments ignoring blocks and treatments eliminating blocks showed significant difference for the traits like days to 50% flowering, plant height, number of tillers per plant, flag leaf area, number of spikelets per spike, number of grains per spike, grain yield per plot, test weight and harvest index except chlorophyll content. The mean sum of squares for blocks (eliminating treatments) showed non-significant results for the traits days to 50% flowering, plant height, number of tillers per plant, flag leaf area, number of grains per spike, grain yield per plot and harvest index except number of spikelets per spike and test weight, indicating homogeneity of evaluation blocks while as for blocks (ignoring treatments) revealed significant result for the traits plant height, number of tillers per plant, flag leaf area, number of spikelets per spike, number of grains per spike, grain yield per plot, test weight and harvest index except days to 50% flowering and chlorophyll content. The mean sum of squares was significant for all the traits for check vs entries indicating that the test entries were significantly different from checks. For checks, the mean sum of squares showed significant result for most of the traits like, days to 50% flowering, chlorophyll content, flag leaf area, number of grains per spike, grain yield per plot, test weight and harvest index except plant height, number of tiller per plant and number of spikelets per spike. For test entries, the mean sum of squares was significant for most of the traits except chlorophyll content, number of spikelets per spike and harvest index.

Table 4.5: Estimates of genetic parameters of ten agro-morphological traits in wheat

Characters	Genotypic coefficient of variance	Phenotypic coefficient of variance	Heritability (bs)	Genetic advance	Genetic advance as percentage of mean
Days to 50% flowering	2.38	3.27	52.98	3.84	3.58
Plant height	8.26	8.54	72.1	13.22	12.8
Chlorophyll content	1.83	9.84	79.5	6.09	15.7
No.of tillers\plant	18.20	19.36	88.37	3.87	35.29
Flag leaf area	15.04	19.06	62.28	10.84	24.49
Spikelets \spike	8.02	10.01	64.10	3.94	20.9
Grains \spike	12.34	18.33	45.34	10.12	17.15
Grain yield \plo	25.88	30.75	70.83	53.00	14.89
Test weight	18.27	21.05	75.31	12.85	32.71
Harvest index	2.71	15.31	3.13	5.91	19.2

4.1.4 Estimation of genetic parameters for agro-morphological traits

The estimates of genotypic coefficient of variation, phenotypic coefficient of variation, heritability, genetic advance, genetic advance as percent of mean is presented in Table 4.5 and are described as under: -

4.1.4.1 Genotypic coefficient of variation

For all the traits, a wide range of genotypic coefficients of variation (GCV) has been observed which ranged from 1.83% to 25.88%. Highest value of genotypic coefficient of variability was varied for grain yield per plot (25.88%). Moderate GCV was observed for test weight (18.27%) followed by number of tillers per plant (18.20%), flag leaf area (15.04%) and number of grains per spike (12.34%) whereas low GCV was

observed for plant height (8.26%) followed by number of spikelets per spike (8.02%), harvest index (2.71), days to 50%flowering (2.38) and chlorophyll content (1.83).

4.1.4.2 Phenotypic coefficient of variation

Phenotypic coefficient of variation ranged between 3.27% to 30.75%. High phenotypic coefficient of variation was observed for grain yield per plot (30.75%) and test weight (21.05%). Moderate PCV was observed for number of tillers per plant (19.36%) followed by flag leaf area (19.06%), number of grains per spike (18.33) and harvest index (15.31). Low PCV was observed for number of spikelets per spike (10.01) followed by chlorophyll content (9.84), plant height (7.01) and days to 50% flowering (3.27).

4.1.4.3 Heritability (bs)

Heritability in broad sense estimates for all traits ranged from 3.13 to 88.37 %. Maximum heritability was recorded for number of tillers per plant (88.37%) followed by chlorophyll content (79.5%), test weight (75.31%), plant height (72.1%), grain yield per plot (70.83%), number of spikelets per spike (64.10%) and flag leaf area (62.28). Moderate heritability was observed for days to 50% flowering (52.98%) and number of grains per spike (45.35%). Lowest heritability was observed for harvest index (3.13%).

4.1.4.4 Genetic advance

Perusal of result in Table 4.5 showed that genetic advance ranged from 3.84 to 53.00. Highest value of genetic advance was observed for grain yield per plot (58.00).Moderate value of genetic advance was observed for plant height (13.22) followed by test weight (12.85), flag leaf area (10.84) and number of grains per spike (10.12) whereas low value of genetic advance was observed for chlorophyll content (6.09) followed by harvest index (5.91), number of spikelets per spike (3.94), number of tillers per plant (3.87) and days to 50% flowering recorded lowest value (3.84) .

4.1.4.5 Genetic advance as per cent of mean

Perusal of result in Table 4.5 showed that genetic advance as percent of mean varied from 3.58% to 35.29%. Maximum genetic advance as percentage of mean was observed for number of tillers per plant (35.29%) followed by test weight (32.71%), flag leaf area (24.49%) and number of spikelets per spike (20.9%). Moderate genetic advance as percentage of mean was observed in harvest index (19.2%) followed by number of

grains per spike (17.15%), chlorophyll content (15.7%), grain yield per plot (14.89%) and plant height (12.8%) whereas lowest value of genetic advance as percentage of mean was observed in days to 50% flowering (3.58%).

Table 4.6: Allocation of wheat genotypes in various clusters based on Euclidean Cluster analysis

Cluster no.	Cluster size	Genotypes
Cluster I	27	CM-123,CM-49,IC-110,IC-149,IC-142,IC-178,IC-232,IC-239,HP-31,HP-32,HP-15,CM-4,CM-38,CM-93,CM-58,CM-125,CM-17,CM-79,CM-86,CM-57,CM-106,CM-113,CM-125,CM-16,CM-53,CM-54,HP-25.
Cluster II	25	HD-2967,CM-84,CM-12,CM-229,CM-6,IC-130,IC-18,IC-12,CM-69,IC-175,IC-166,IC-156,IC-101,IC-141,IC-124,IC-13,IC-189,HP-33,HP-14,CM-92,CM-52,CM-95,CM-118,HP-24,HP-37
Cluster III	17	CM-109,CM-175,IC-17,IC-133,IC-169,IC-88,IC-19 ,HP-35,CM-90,CM-121,CM-11,CM-31,CM-65,CM-2,CM-21,CM-35,CM-72
Cluster IV	31	Agra-local,HD-DBW-187,HD-3043,CM-133,CM-139,CM-214,CM-7,CM-64,CM-199,CM-24,IC-62,IC-168,IC-112,IC-218,IC-56,IC-239,CM-111,CM-44,CM-40,CM-89,CM-94,CM-119,CM-122,CM-60,CM-42,CM-55,CM-91,CM-98,CM-116,CM-15,CM-25

4.1.5 Estimation of genetic diversity

Genetic diversity have been measured using Euclidean cluster analysis, which is the most common type of hierarchical clustering used to group the genotypes in clusters based on their similarity. This is the essential tool for estimating the level of divergence at the genotypic level, which helps in the identification and selection of genetically diverse genotypes for further crop improvement programmes.

4.1.5.1 Clustering of genotypes

The 100 genotypes were categorized into four cluster groups from the Table 4.6 and Fig 1. It has been observed that among all the four clusters, cluster I consisted of highest (31) no.of genotypes followed by Cluster II which comprised of 27 genotypes, cluster III comprised 25 and cluster IV comprised of 17 genotypes.

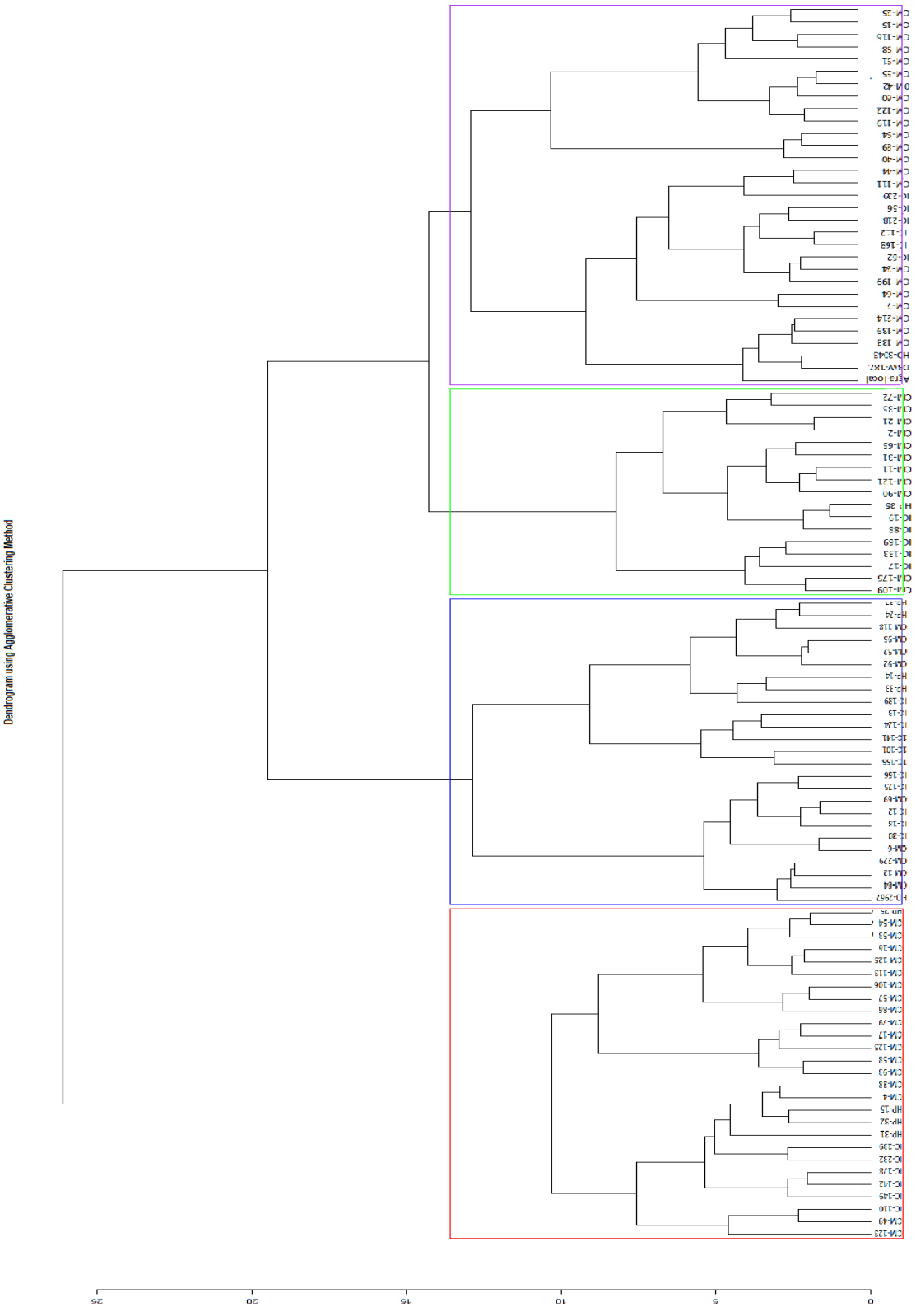


Fig 4.1: Cluster diagram of hundred genotypes of wheat.

Table 4.7: Estimates of Inter and Intra cluster distance based on Euclidean Cluster analysis

	Cluster I	Cluster II	Cluster III	Cluster IV
Cluster I	3.41	4.14	4.60	4.91
Cluster II		3.56	4.85	3.65
Cluster III			4.41	4.26
Cluster IV				4.12

4.1.5.2 Intra and Inter cluster distances

The perusal of result in Table 4.7 showed the average inter and intra-cluster values for all four clusters. The diagonal values in the table represent the intra-cluster relative genetic distance and all the remaining values in the table are inter-cluster genetic distances.

Highest intra-cluster distance was found in Cluster III (4.41) followed by Cluster IV (4.12), Cluster II (3.56) and Cluster I (3.41). Maximum inter-cluster distance was found between cluster I and cluster IV (4.91) , indicating higher genetic divergence between the genotypes present in the clusters followed by cluster II and cluster III (4.85), cluster I and cluster III (4.60), cluster III and cluster IV (4.26) and cluster I and cluster II (4.14). Minimum inter cluster was observed between cluster II and cluster IV (3.65).

Table 4.8: Cluster means of wheat genotypes for yield and contributing traits

Cluster no.	**DFF**	**PH**	**CC**	**NT**	**FLA**	**SP**	**GP**	**GY**	**TW**	HI
Cluster I	108.2	104.7	36.15	10.65	45.18	18.28	47.15	395.03	36.28	29.80
Cluster II	102.9	102.2	39.03	9.56	42.53	20.03	59.70	444.08	41.57	34.28
Cluster III	104.7	102.1	38.82	9.86	42.80	18.00	54.61	292.96	41.04	27.03
Cluster IV	106.82	103.1	41.22	13.49	48.43	17.32	47.93	279.06	40.27	26.55

DFF=Days to 50% flowering, **PH=**Plant height, **CC**=Cholorophyll content, **NT**=Number of tillers per plant, **FLA**=Flag leaf area, **SP**=Number of spikelets per spike, **GP**=Number of grains per spike,**GY**=Grain yield per plot,**TW**=Test weight,**HI**=Harvest index

4.1.5.3 Cluster means for agro-morphological traits

Persual of result given in Table 4.8 showed the estimated cluster means for all 10 traits. Significant differences has been observed between clusters for all the traits under study.

4.1.5.3.1 Days to 50% flowering

Cluster mean for days to 50% flowering was observed to be the maximum for cluster I (108.2) followed by cluster IV (106.82), cluster III (104.7) and cluster II (102.9).

4.1.5.3.2 Plant height (cm)

Maximum cluster mean for plant height was observed for cluster I (104.7) followed by cluster IV (103.1), cluster II (102.2) and cluster III (102.1).

4.1.5.3.3 Chlorophyll content

Maximum cluster mean for chlorophyll content was observed in cluster IV (41.22) followed by cluster II (39.03), cluster III (38.82) and cluster I (36.15).

4.1.5.3.4 Number of tillers per plant (no.)

Maximum cluster mean for number of tillers per plant was observed in cluster IV (13.49) followed by cluster I (10.65), cluster III (9.86) and cluster II (9.56).

4.1.5.3.5 Flag leaf area (cm^2)

Cluster mean for flag leaf area was observed to be as maximum for cluster IV (48.43) followed by cluster I (45.18), cluster III (42.80) and cluster II (42.53).

4.1.5.3.6 Number of spikelets per spike (no.)

Cluster mean of number of spikelets per spike was observed to be as asmaximum for cluster II (20.03) followed by cluster I (18.28), cluster III (18.00) and cluster IV (17.32).

4.1.5.3.7 Number of grains per spike (no.)

Cluster mean for number of grains per spike was observed to be as maximum for cluster II (59.70) followed by cluster III (54.61), cluster IV (47.93) and cluster I (47.15).

4.1.5.4.8 Grain yield per plot (g)

Cluster mean for grain yield per plot was observed to be as maximum for cluster II (444.08) followed by cluster I (395.03), cluster III (292.96) and cluster IV (279.06).

4.1.5.3.9 Test weight (g)

Cluster mean for test weight was observed to be as maximum for cluster II (41.57) followed by cluster III (41.04), cluster IV (40.27) and cluster I (36.28).

4.1.5.3.10 Harvest index (%)

Cluster mean for harvest index was observed to be as maximum for cluster II (34.28) followed by cluster I (29.80), cluster III (27.03) and cluster IV (26.55).

4.1.6 Phenotypic screening of yellow rust in wheat germplasm

One hundred genotypes of wheat including four checks were artificially inoculated in the field with the the mixture of races of *Puccinia striformis* to screen and acertain their response to infection and select resistant lines. The stripe rust severity was recorded as percent of the rust infection on the wheat plants according to the Modified Cobb's scale (Peterson *et al*.,1948).

4.1.6.1 Disease response and Coefficient of Infection (CI)

The data on disease reaction and the coefficient of infection was recorded during *Rabi* 2023 has been presented in the Table 4.9. Persual of data that revealed that disease response to stripe rust under field conditions ranged from 0-80S indicating disease response from immunity to susceptible reaction in wheat genotypes showed resistant disease reaction, 14 wheat genotypes showed moderately resistant reaction, 29 showed moderately susceptible and 19 showed susceptible reaction.

Table 4.9: Response of dfferent wheat genotypes to yellow rust diseases with coefficient of infection

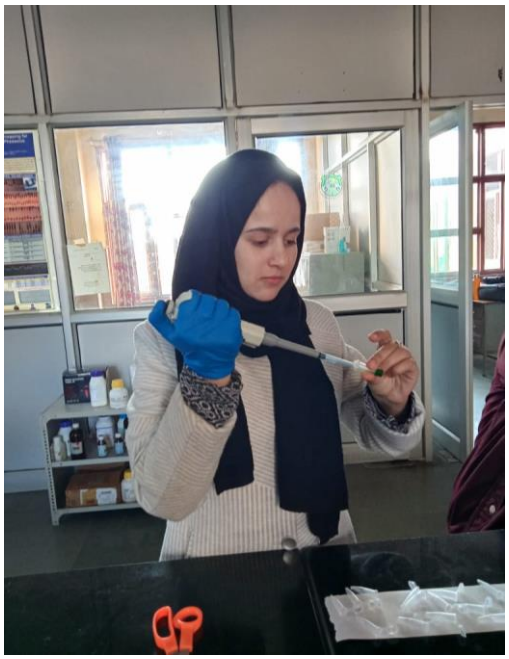
S.no	Genotype	Disease responce	CI	Disease reaction	S.no.	Genotype	Disease Response	CI	Disease Reaction
1	CM-2	20MS	16	MS	51	CM-123	10MS	8	MS
2	CM-4	20S	20	S	52	CM-125	R	0.2	R
3	CM-6	R	0.2	R	53	CM-133	R	0.2	R
4	CM-7	20MS	16	MS	54	CM-139	5R	1	R
5	CM-11	20S	20	S	55	CM-175	10MS	8	MS
6	CM-12	R	0.2	R	56	CM-199	10MS	8	MS
7	CM-15	20S	20	S	57	CM-214	R	0.2	R
8	CM-16	20MS	16	MS	58	CM-221	10MR	4	MR
9	CM-17	5MR	2	MR	59	CM-229	R	0.2	R
10	CM-21	20MS	16	MS	60	IC-12	R	0.2	R
11	CM-24	R	0.2	R	61	IC-13	20MS	16	MS
12	CM-25	20S	20	S	62	IC-17	10MS	8	MS
13	CM-31	20S	20	S	63	IC-18	10R	2	R
14	CM-35	R	0.2	R	64	IC-19	10S	10	S
15	CM-38	20S	20	S	65	IC-30	10MS	8	MS
16	CM-40	10MS	8	MS	66	IC-41	20S	20	S
17	CM-42	20MR	8	MR	67	IC-56	20MR	8	MR

18	CM-44	R	0.2	R	68	IC-62	10MR	4	MR
19	CM-49	20MS	16	MS	69	IC-88	5R	1	R
20	CM-52	R	0.2	R	70	IC-101	5S	5	S
21	CM-53	20MR	8	MR	71	IC-110	R	0.2	R
22	CM-54	20MS	16	MS	72	IC-112	10MS	8	MS
23	CM-55	20MS	16	MS	73	IC-124	10MS	8	MS
24	CM-57	10MR	4	MR	74	IC-133	R	0.2	R
25	CM-58	R	0.2	R	75	IC-141	R	0.2	R
26	CM-60	10S	10	S	76	IC-142	10MS	8	MS
27	CM-64	R	0.2	R	77	IC-149	10S	10	S
28	CM-65	20S	20	S	78	IC-156	R	0.2	R
29	CM-69	20MS	16	MS	79	IC-166	R	0.2	R
30	CM-72	10MR	4	MR	80	IC-168	5R	1	R
31	CM-79	5R	1	R	81	IC-169	20MR	8	MR
32	CM-84	R	0.2	R	82	IC-175	10MR	4	MR
33	CM-86	R	0.2	R	83	IC-178	10R	2	R
34	CM-89	R	0.2	R	84	IC-189	20MS	16	MS
35	CM-90	20MS	16	MS	85	IC-218	10S	10	S
36	CM-91	20MS	16	MS	86	IC-232	5R	1	R
37	CM-92	5R	1	R	87	IC-239	20MS	16	MS

38	CM-93	R	0.2	R	88	HP-14	20MS	16	MS
39	CM-94	5MS	4	MS	89	HP-15	20MR	8	MR
40	CM-95	5MR	2	MR	90	HP-24	20MS	16	MS
41	CM-98	20MS	16	MS	91	HP-25	20MS	16	MS
42	CM-106	R	0.2	R	92	HP-31	5R	1	R
43	CM-109	10R	2	R	93	HP-32	10S	10	S
44	CM-111	R	0.2	R	94	HP-33	5R	1	R
45	CM-113	5MS	4	MS	95	HP-35	20MS	16	MS
46	CM-116	R	0.2	R	96	HP-37	20MS	16	MS
47	CM-118	5MR	2	MR	97	Agra-local	60S	60	S
48	CM-119	5R	1	R	98	HD-2967	60S	60	S
49	CM-121	R	0.2	R	99	HD-3043	80S	80	S
50	CM-122	5R	1	R	100	DBW-187	10MS	6	MS

Where R= Resistant, MR= Moderately resistant, MS= Moderately susceptible, S= Susceptible

Plate 6: Molecular work on wheat for yellow rust resistance



Preparation of DNA samples

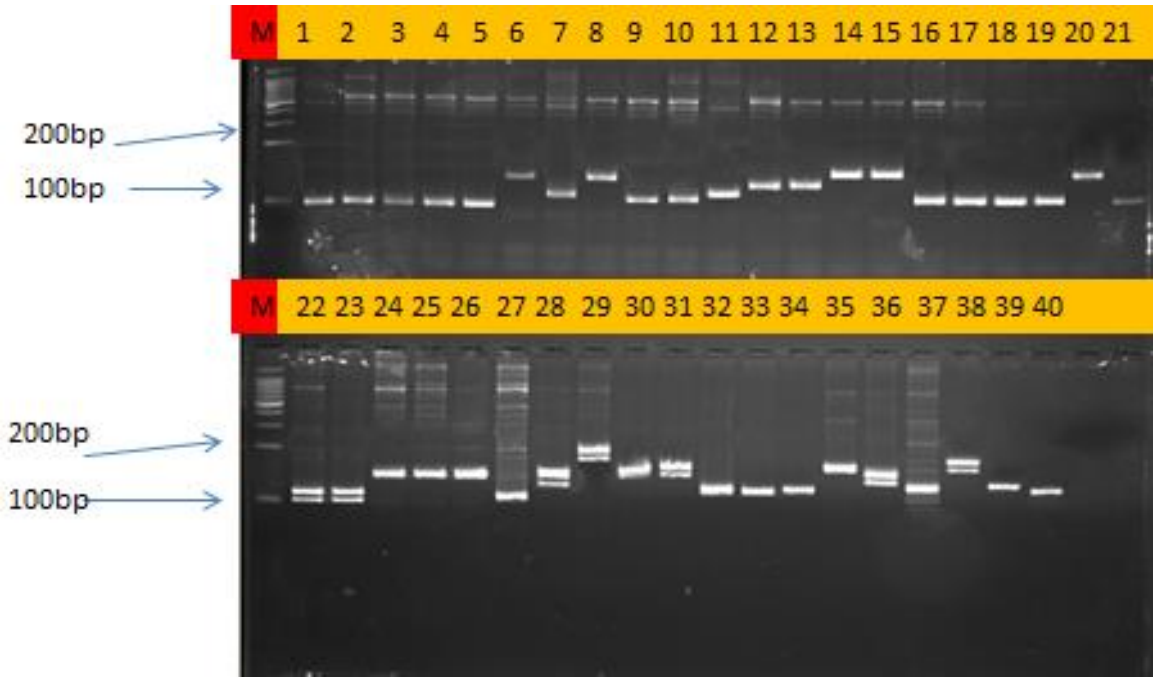


Plate 7: SSR profiling of 40 wheat genotypes using molecular marker Barc349 linked to Yr5, M=100bp DNA ladder

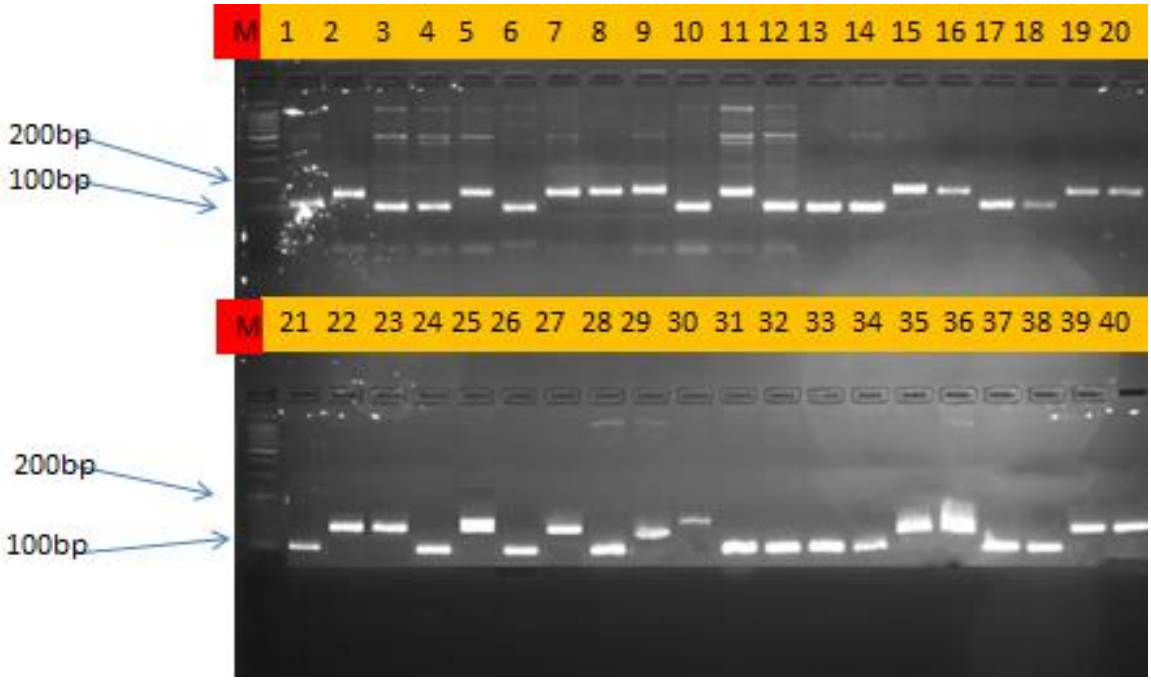


Plate 8: SSR profiling of 40 wheat genotypes using molecular marker S19M93 linked to Yr5, M= 100bp DNA ladder

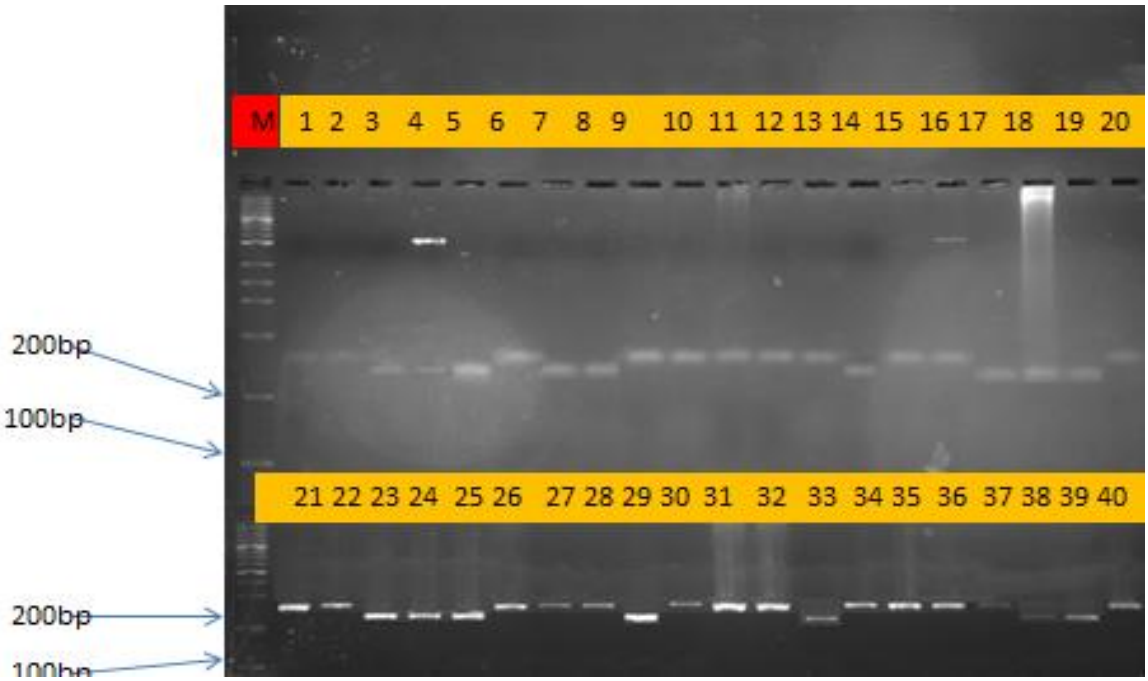


Plate 9: SSR profiling of 40 wheat genotypes using molecular marker Xbarc8 linked to Yr15, M= 100bp DNA ladder

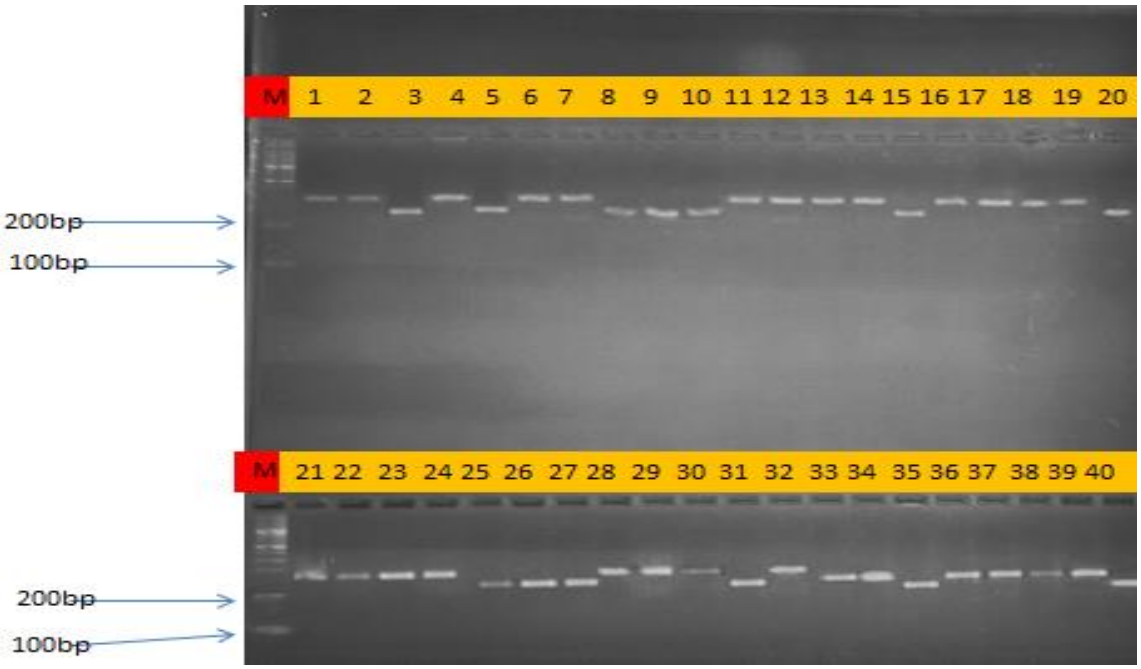


Plate 10: SSR profiling of 40 wheat genotypes using molecular marker Xpsp3000 linked to Yr10, M=100bp DNA ladder

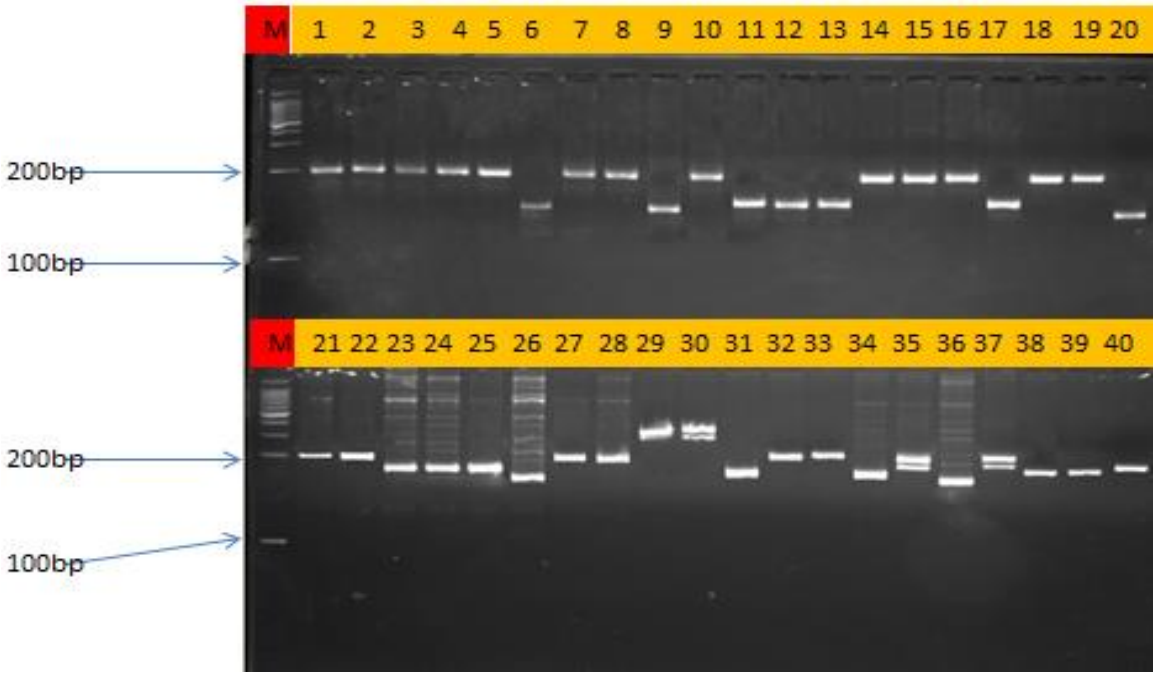


Plate 11: SSR profiling of 40 wheat genotypes using molecular marker Barc181 linked to Yr24, M=100bp DNA ladder

Table 4.10 : Profiling of genotypes using molecular markers linked to yellow rust resistance in wheat

S.No	Genotypes	Field score	Amplicon size of linked molecular markers				
			Barc349 (100bp,105bp,120bp,140bp),220bp)	**S19M93 (100bp,140bp)**	**Xsps3000 (240bp,260bp,280bp,285bp)**	**Xbarc8 (260bp, 280bp)**	**Barc181 (180bp, 200bp)**
1	IC-166	0.2	100	100	260	280	200
2	IC-12	0.2	100	140	260	280	200
3	IC-110	0.2	100	100	236	260	200
4	IC-156	0.2	100	100	260	260	200
5	IC-232	1.0	100	140	240	260	200
6	IC-141	0.2	140	100	260	280	160
7	IC-168	1.0	120	140	260	265	200
8	IC-214	0.2	140	140	240	265	200
9	IC-133	0.2	105	145	240	280	160
10	IC-88	1.0	105	100	240	280	200
11	CM-12	0.2	120	140	260	280	165
12	CM-84	0.2	125	100	260	280	165
13	CM-64	0.2	125	100	260	280	165
14	CM-229	0.2	140	100	260	265	200
15	CM-6	0.2	140	145	236	280	200
16	CM-109	1.0	100	140	260	280	200
17	CM-123	0.2	100	100	260	260	165
18	CM-139	1.0	100	100	260	260	200
19	CM-214	0.2	100	140	260	260	200
20	CM-133	0.2	135	140	240	280	160
21	HP-33	1.0	120	100	285	280	200
22	HP-31	1.0	100	140	285	280	200
23	CM-24	0.2	100	140	285	260	180

24	CM-35	0.2	140	100	290	260	180
25	CM-44	0.2	140	145	260	260	180
26	CM-52	0.2	140	100	260	280	175
27	CM-58	0.2	105	140	260	280	200
28	CM-79	2.0	125	100	290	280	200
29	Agra-local	60	150	135	290	265	305
30	HD-3043	80	135	145	290	285	305
31	CM-86	0.2	135	100	260	280	180
32	CM-89	0.2	120	100	290	280	200
33	CM-92	1.0	120	100	285	260	205
34	CM-93	0.2	120	100	285	280	180
35	CM-106	0.2	140	140	260	280	190
36	CM-109	2.0	135	140	285	280	175
37	CM-111	0.2	125	100	285	280	190
38	CM-121	0.2	140	100	285	260	180
39	CM-122	1.0	125	140	285	260	180
40	CM-125	0.2	120	140	260	280	190

4.2 Assessment of molecular diversity among yellow rust resistant wheat genotypes

In the present investigation, forty wheat genotypes including two checks Agra-local and HD-3043 were selected on the basis of disease reaction presented in Table 4.9 were subjected to SSR analysis using twelve markers. Out of 12 SSR markers 5 markers showed polymorphism. DNA amplification of 40 wheat genotypes at different base pairs in accordance to the product size of the marker used is presented in Table 4.10.

Table 4.11: List of molecular markers with product size, PIC value and no. of wheat genotypes showing their polymorphism

Marker	Product size	PIC	No.of Polymorphic genotypes
Barc349	100bp,105bp,120bp,140bp, 220bp	0.68	30
S19M93	100bp,140bp	0.43	36
Xbarc8	260bp,280bp	0.56	35
Xsps3000	240bp,260bp,280bp, 285bp	0.64	33
Barc181	180bp,200bp	0.66	29

4.2.1 Polymorphic Information Content (PIC)

Polymorphic information content (PIC) was used to analysize each marker and its discriminatory potential and is presented in table 4.11. PIC values were ranged from 0.43 to 0.68. Highest PIC values was shown by marker Barc349 (0.68) followed by Barc181 (0.66), Xsps3000 (0.64), Xbarc (0.56) and lowest shown by marker S19M93 (0.43)

4.2.2 Dendrogram analysis

Molecular cluster analysis generated two broad groups of wheat genotypes.

First group consisted of 15 genotypes and second group containing 25 genotypes. The first cluster contained 15 genotypes are further divided into two sub-groups A and B at 66% similarity. Sub-group A comprised of eleven genotypes which could be further divided into groups A1 and A2. A1 group consisted of 5 genotypes and A2 consisted of 6 genotypes. The second main cluster consisted of 25 genotypes which are further divided into three major sub-groups C, D, and E at 66% similarity. Sub-group C comprised of 22 genotypes could be further divided into two branches C1 and C2 consisted of 4 and 18 genotypes respectively. Cluster D is having only one member. Sub-group E comprised of two genotypes. Cluster analysis based on genetic similarity values (0.45-0.93). Cluster analysis provided a significant genetic variation and a clear resolution of relationships among all 40 genotypes of wheat.

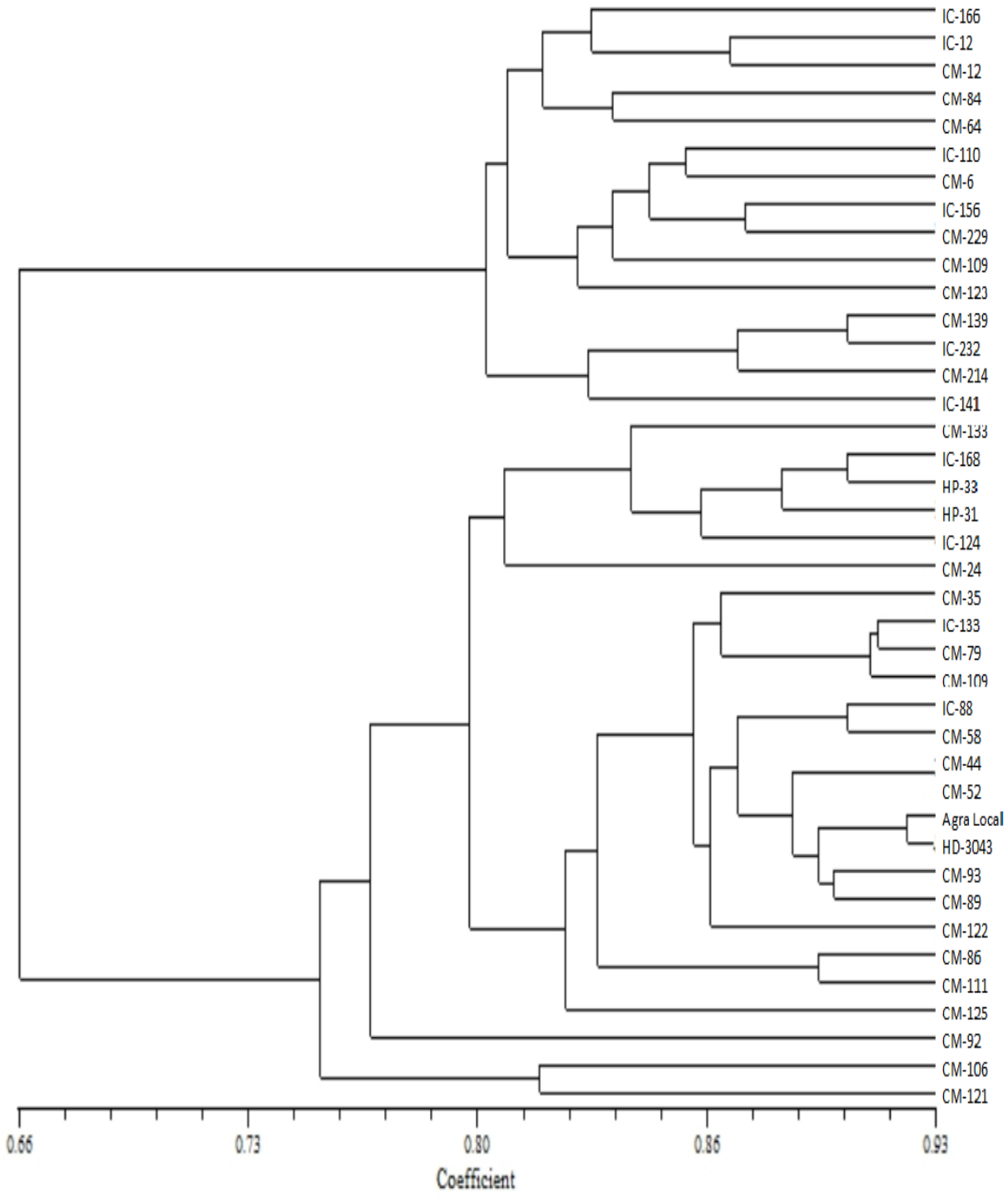


Fig 4.2: Dendrogram generated using UPGMA analysis showing relationship among 40 wheat genotypes using SSR markers.

Chapter-5

Discussion

Wheat is one of the oldest and most important cereal crop. For any successful crop improvement program, genetic diversity and germplasm characterisation has prime importance. To estimate the degree of variation in experimental material, various genetic tools are used with biometrical methods like genotypic (GCV) and phenotypic (PCV)/coefficients of variation. While lesser differences between these factors suggest that the environment has little influence on how traits are expressed, higher values of these parameters indicate the presence of a large level of heterogeneity. The heritable portion of variability is shown by the heritability indicator, which also contributes to genetic variation. a high degree of genetic variability paired with genetic advance as a percent of mean indicated that these traits are governed by additive genes and that selection will be beneficial for improvement of such traits. Development of new virulent races of pathogens is the main challenge in disease resistance programme, therefore, plant breeders have to put continuous efforts to develop new varieties with high resistance background. DNA markers are being used for genetic improvement of crops through incorporation of disease resistance genes. The benefit of using molecular markers is that these are not affected by environment thus increase the efficiency and accuracy for selection of desirable plants in a population. Therefore, existing plant breeding techniques along with available molecular markers can help a breeder in developing superior wheat varieties with resistance to biotic and abiotic stresses and to minimize yield losses due to such stresses. In present studies SSR markers have been used to screen the advanced breeding lines of wheat. The characteristic feature of SSR marker is that these are co-dominant in nature and have high reproducibility, high polymorphism, distributed over whole genome and are cost efficient. In present study, Genetic diversity and molecular analysis of 100 wheat genotypes were assessed for qualitative and quantitative traits. In this study, the main aim was to find the superior and highly diverse genotypes those can be utilized in further breeding programs to improve and to widen the genetic base of wheat. The findings of current study are discussed below, with references to relevant literature and explanations wherever, feasible:

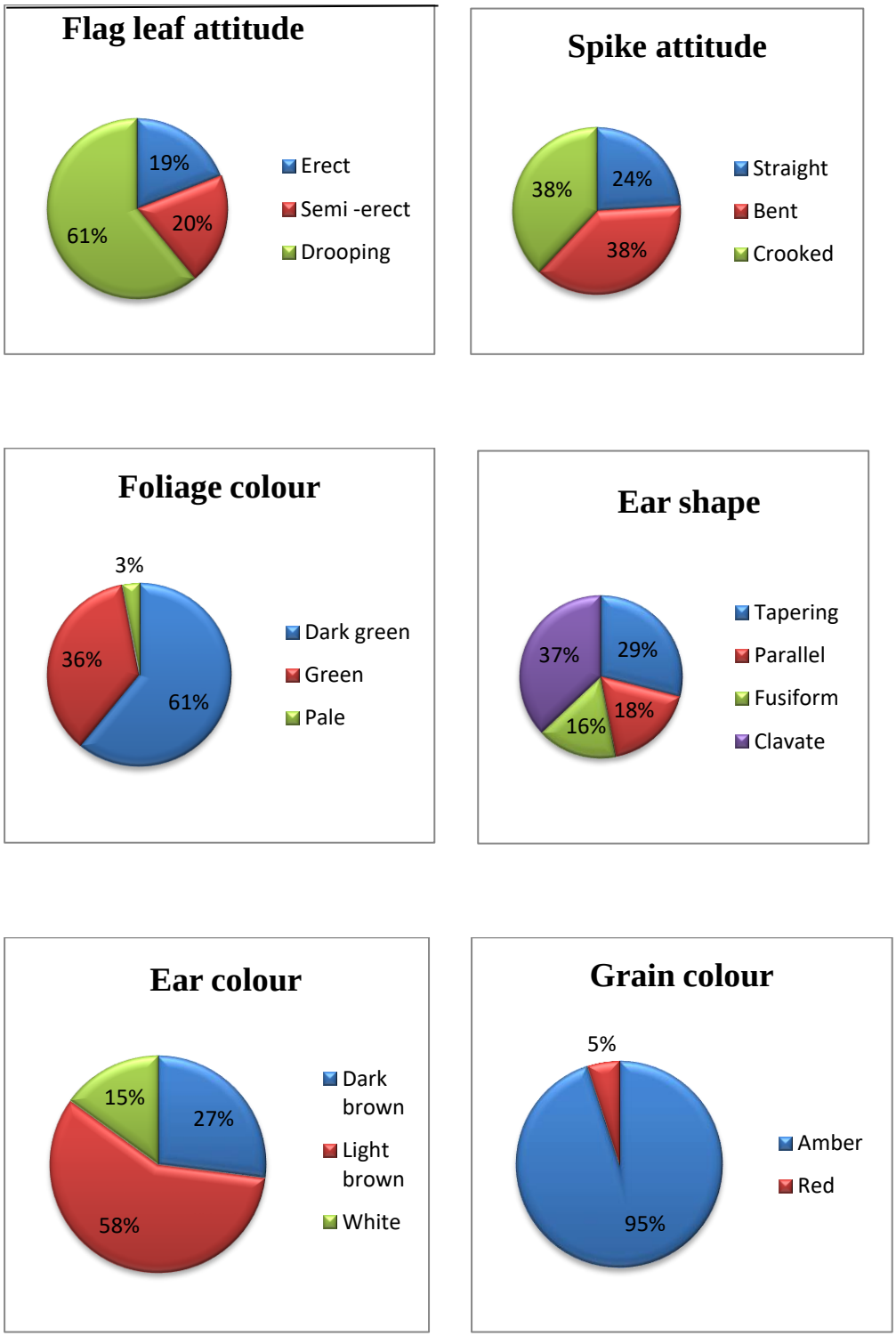
5.1 Analysis of variance for agro-morphological traits

Analysis of variance for ten agro-morphological traits revealed that mean sum of squares for treatments ignoring blocks and treatments eliminating blocks showed significant difference for the traits like days to 50% flowering, plant height, number of tillers per plant, flag leaf area, number of spikelets per spike, number of grains per spike, grain yield per plot, test weight and harvest index except chlorophyll content. The mean sum of squares for blocks (eliminating treatments) showed non-significant results for the traits days to 50% flowering, plant height, number of tillers per plant, flag leaf area, number of grains per spike, grain yield per plot and harvest index except number of spikelets per spike and test weight, indicating homogeneity of evaluation blocks while as for blocks (ignoring treatments) revealed significant result for the traits plant height, number of tillers per plant, flag leaf area, number of spikelets per spike, number of grains per spike, grain yield per plot, test weight and harvest index except days to 50% flowering and chlorophyll content. The mean sum of squares was significant for all the traits for check vs entries indicating that the test entries were significantly different from checks. For checks, the mean sum of squares showed significant result for most of the traits like, days to 50% flowering, chlorophyll content, flag leaf area, number of grains per spike, grain yield per plot, test weight and harvest index except plant height, number of tiller per plant and number of spikelets per spike. For test entries, the mean sum of squares was significant for most of the traits except chlorophyll content, number of spikelets per spike and harvest index. Selvarani and Gomathinayagam., 2000 ; Ahmed *et al*., 2013; Jaipal and Shekhawat., 2016 also found similar results while working on wheat.

5.2 Characterization of wheat genotypes for qualitative traits

The evaluation of 100 wheat genotypes for six qualitative traits was recorded to assess the diversity. For foliage colour , (61%) genotypes revealed dark green colour, (36%) showed green colour and only (3%) genotypes showed pale colour. Erect (19%), semi-erect (20%) and drooping (61%) type groups were recorded in flag leaf attitude. The spike attitude was classified into three groups, like Straight (24%), Bent (38%), and Crooked (38%). Ear colour was categorized into three groups viz., dark brown (27%), light brown (58%) and white (15%). Majority of wheat genotypes (95%) possess amber grain colour while only (5%) showed red grain colour. In case of ear shape it was divided into four viz., tapering (29%), parallel (18%), fusiform (16%) and clavate (37%). Similar findings were also reported by Kaduwal *et al*., 2019 for the above mentioned qualitative characters. Banjarey *et al*., 2022 also studied qualitative traits including the above mentioned traits.

Fig 5.1: Graphical representation of qualitative traits in wheat genotypes



5.3 Genetic parameters

5.3.1 Genotypic coefficient of variation and phenotypic coefficient of variation

For all the characters, a wide range of genotypic coefficients of variation (GCV) and phenotypic coefficient of variation (PCV) has been recorded which ranged from 1.83% to 25.88% and 3.27% to 30.75%. respectively. Highest value of genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) was observed for grain yield per plot. Moderate GCV and PCV was observed for test weight, number of tillers per plant, flag leaf area and number of grains per spike whereas, low GCV and PCV was observed for plant height , number of spikelets per spike, ,days to 50%flowering, harvest index and chlorophyll content. Similar results were found by Awan *et al.* (2014) ; Arya *et al*., (2017), Thakur et al., (2018), Bushan *et al*., (2013)

5.3.2 Heritability (bs)

Heritability estimates for all the characters ranged from 3.13 to 88.37 %. Maximum heritability was recorded for number of tillers per plant followed by chlorophyll content, test weight, plant height ,grain yield per plot, number of spikelets per spike and flag leaf area. Moderate heritability was observed for days to 50% flowering and number of grains per spike. Low heritability was observed for harvest index . Similar findings of high heritability for the traits number of tillers per plant, plant height, grain yield in wheat were also reported by Ajmal *et al*., (2009). Similar findings were reported by Ahmed *et al*., (2016) and Kashif and Khalid., (2004)

5.3.3 Genetic advance as per cent of mean

Perusal of results of present study showed that genetic advance as percent of mean varied from 3.58% to 35.29%. Maximum genetic advance as percentage of mean was observed for number of tillers per plant followed by test weight , flag leaf area and number of spikelets per spike. Moderate GAM was observed in harvest index followed by number of grains per spike ,chlorophyll content, grain yield per plot and plant height whereas low GAM was observed in days to 50% flowering. Similar findings were also reported by Dargicho *et al*., (2015). Grain yield per plot depicted high heritability coupled with high genetic advance suggesting additive gene action and thus selection is effective. Similar findings were reported by Gupta *et al*.,(2000); Jamil *et al*., (2017). Similar findings were also reported by Desheva *et al*. (2014) ; Dargicho *et al*., (2015); Fikre *et al.* (2015) and Kumar and Singh (2022)

5.4 Genetic divergence

Cluster analysis grouped 100 genotypes of wheat into four Clusters. Cluster I consisted highest no of entries (31) followed by cluster II (27), Cluster III (25) and Cluster IV (17). Similar results were also reported by Manschadi *et al*., (2008) and Khodadadi *et al*., (2011) . Highest intra-cluster distance was found in cluster III (4.41) followed by cluster IV (4.12) and cluster II (3.56) indicating the high diversity among genotypes while lowest intra-cluster distance was reported in cluster I (3.41), indicating low diversity among genotypes. Maximum inter-cluster distance was found between cluster I and cluster IV (4.91) followed by cluster II and cluster III (4.85), cluster I and cluster III (4.60), cluster III and cluster IV (4.26) and cluster I and cluster II (4.14), indicating if hybrids are produced from the genotypes of such clusters would have a high chances of having a diverse population in the segregating generations. Minimum inter-cluster distance was observed between cluster II and cluster IV (3.65) indicating the genotypes present in these clusters are genetically close, thus hybridization between genotypes of these clusters may not yield a fruitful product. Genotypes found in these clusters can be selected and used for hybridization to obtain transgressive segregants for a desired trait, which can further exploit to generate superior high yielding varieties. Significant level of genetic diversity for all traits has been observed for cluster means of most of agro-morphological traits. Highest cluster mean values for most of the trait such as number of spikelets per spike (20.03), number of grains per spike (59.70), grain yield per plot (444.08), test weight (41.57) and harvest index (34.28) exihibited by cluster II indicating its superiority over the other clusters. The same cluster showed lowest cluster mean with respect to days to 50% flowering (102.9), which is desirable trait for earliness. Similar results were reported by Kallimullah *et al*., (2012) Verma *et al*., (2014); Rehman *et al*., (2015) ; Rajshree and Singh (2018) ; Singh *et al*., (2020) and Brawed *et al*., (2022)

5.5 Phenotypic screening of yellow rust in wheat germplasm

Experimental material of present investigation comprised of one hundred genotypes of wheat including four checks were artificially inoculated in the field with the the mixture of races of *Puccinia striformis* to develop rust and to screen and acertain their response to infection and select resistant lines. The stripe rust severity on the wheat plants was recorded as percent of the rust infection according to the Modified Cobb's scale (Peterson *et al*.,1948). Persual of data revealed that disease response to stripe rust under field conditions ranged from 0-80S indicating disease response from immunity to

susceptible reaction. 38 wheat genotypes showed resistant disease reaction,14 wheat genotypes showed moderately resistant reaction, 29 showed moderately susceptible and 19 genotypes were found as susceptible in nature. Similar results were also reported by Yamin *et al*., 2021 who evaluated 30 wheat genotype for phenotypic screening of yellow rust resistance and they observed result that 6 genotypes were resistant, 6 moderately resistant, 13 moderately resistant to moderately susceptible, 2 moderately susceptible and 3 susceptible genotypes. Similar results were also found by Peterson *et al*., 1948; Singh *et al*., 2005 ; Rani *et al*.,(2019) and Azene *et al*., 2021

5.6 Molecular divergence

In the present investigation, 40 wheat genotypes were subjected to SSR analysis using 12 linked SSR markers. Out of 12 SSR markers 5 markers (Barc349, S19M93, Xbarc8, Xsps3000 and Barc181) showed polymorphism. In our study for Barc349, 100bp, 105bp, 120bp, 125bp, 135bp, 140bp and 150bp, size of amplicons was observed. However, amplicons of size 100bp, 105bp 120bp and 140bp has been reported by researchers, revealed that out of 40 wheat genotypes 30 were amplified within given product size and exihibited polymorphism and presence of yellow rust resistance. In case of marker S19M93, product size is 100bp and 140bp, while 40 wheat genotype amplicons were found at 100bp, 135bp, 140bp and 145bp, thus it has been observed that 36 wheat genotypes amplified within given product size and showed presence of yellow rust resistance. Xbarc8 is having product size of 240bp, 260bp and 280bp, while wheat genotypes were amplified at 240bp, 260bp, 265bp, 280bp and 285bp which revealed that out of 40 wheat genotypes 35 showed presence of yellow rust resistance. 240bp, 260bp and 280bp is the product size of marker Xsps3000 while 236bp, 240bp, 260bp and 290bp are the base pairs where wheat genotypes were amplified, thus results revealed that out of 40 wheat genotypes 33 amplified within given product size. For marker Barc181, product size is 175bp, 180bp and 200bp where DNA of 29 wheat genotypes was amplified within given range, thus revealed presence of yellow rust resistance. Genotypes amplifying bands other than reported amplicons with respective primers might have some different alleles/genes of Yr resistance and can be analysed in future breeding programs.

Molecular cluster analysis generated two groups of wheat genotypes. First group consisted of 15 genotypes and second group containing 25 genotypes. The first cluster containing 15 cultivars are further divided into two sub-groups A and B at 66% similarity coefficient. The second main cluster consisted of 25 cultivars which are further divided

into three major sub-groups C, D, and E at 66% similarity coefficient. Sub-group A comprising of eleven cultivars which could be further divided into groups A1 and A2. A1 group consisted of 5 cultivars IC-166, IC-12, CM-12, CM-84 and CM-64 where maximum similarity coefficient (0.87) occurred between genotype IC-166 and CM-12 and minimum similarity coefficient (0.81) occurred between genotype IC-12 and IC-110. A2 group consisted of 6 cultivars IC-110, CM-6, IC-156, CM-229, CM-109 and CM-123, where maximum similarity coefficient (0.88) occurred between IC-156 and CM-229 and minimum similarity coefficient (0.79) between CM-123 and IC-156. Sub-group B comprised of 4 cultivars CM-139, IC-232, CM-214 and IC-141, where the maximum similarity coefficient (0.91) occurred between CM-139 and IC-232 and minimum similarity coefficient (0.82) occurred between IC-141, CM-139 and CM-214. Sub-group C comprising of 22 genotypes that could be further divided into two branches C1 and C2. The C1 branch consists of 6 genotypes CM-133, IC-168, HP-33, HP-31, IC-124 and CM-24 revealed more genetic similarity among themselves in which maximum similarity coefficient (0.91) occurred between CM-133 and IC-168 and minimum similarity coefficient (0.78) occurred between CM-24 and HP-33.The C2 branch was the largest group in this study, including 18 genotypes in which maximum similarity coefficient (0.93) occurred between CM-44 and CM-52 and mimimum similarity coefficient (0.76) CM-86 and CM-111. CM-92 was the only one genotype belonging to sub-group D at a similarity coefficient of 0.76. Sub-group E comprised of only 2 genotypes CM-106 and CM-121 with the similarity value of 0.81 genetically close to each other. Cluster analysis based on genetic similarity values (0.45-0.93). Similar results were also reported by Prasad *et al*., 2000, wherein which genetic similarity coefficient value ranged from 0.05-0.88 and 20 and wheat genotypes were grouped into two clusters. Similar results were also reported by Huang *et al.* (2002); Ullah *et al*., (2011) and Drikvand *et al*. (2012)

Polymorphic information content (PIC) was used to analysize each marker and its discriminatory potential and is presented in table 4.10. PIC values ranged from 0.43 to 0.68. Highest PIC values was shown by marker Barc349 (0.68) followed by Barc181(0.66), Xsps3000 (0.64), Xbarc (0.56) and lowest shown by marker S19M93 (0.43). Similar results of PIC value ranged from 0.278-0.816 were also reported by Salem et al., (2008) and Ratiba *et al*., 2012 who reported two groups on cluster analysis of 40 genotypes.

Chapter 6

SUMMARY AND CONCLUSIONS

The present investigation entitled "Genetic diversity studies in advanced breeding lines of wheat (*Triticum aestivum* L.em Thell) " was conducted during 2022-23 at Research farm of division of Plant Breeding and Genetics, Chatha, SKUAST-J. One hundred genotypes of wheat were evaluated in Augmented Block Design with a plot size of $1.6m^2$. Recommended agronomic practices were adopted at all the stages of plant growth. The data was recorded as per standard DUS guidelines for all the qualitative and quantitative traits *viz*., days to 50%flowering flag leaf attitude, foliage colour, spike attitude, ear shape, ear colour, grain colour, plant height, chlorophyll content, number of tillers per plant, flag leaf area, snumber of spikelets per spike, number of grains per spike, grain yield per plot, test weight and harvest index.

- The analysis of variance revealed that the mean sum of squares for treatments (eliminating blocks) and treatments (ignoring blocks) showed significant result for all traits except chlorophyll content.
- The mean sum of squares for blocks (eliminating treatments) showed non-significant results except number of spikelets per spike and test weight, indicating homogeneity of evaluation blocks while as for blocks (ignoring treatments) revealed significant results for all traits except days to 50% flowering and chlorophyll content.
- The mean sum squares was significant for all the traits for check vs entries indicating that the test entries were significantly different from checks.
- The values of phenotypic coefficient of variation were relatively greater than genotypic coefficient of variation for all the traits, indicating that environment has a significant role in the expression of traits.
- Grain yield per plot depicted high heritability coupled with high genetic advance suggesting additive gene action and thus selection is effective.
- Cluster analysis grouped genotypes into four Clusters. Cluster I consisted highest no of entries (31) followed by Cluster II (27), Cluster III (25) and Cluster IV (17). Highest intra-cluster distance was observed in Cluster III (4.41) followed by Cluster IV (4.12),Cluster II (3.56) and Cluster I (3.41). The highest inter-cluster distance was

observed between cluster II and cluster IV indicating higher genetic divergence among the genotypes present in the different clusters.

- Highest values for cluster mean was observed in most of the traits such as number of spikelets per spike (20.03), number of grains per spike (59.70), grain yield per plot (444.08), test weight (41.57) and harvest index (34.28) exhibited in cluster II. The same cluster showed lowest cluster mean with respect to days to 50% flowering (102.9),which is preferred trait.
- Cluster analysis based on molecular data divided the dendrogram into two major clusters (groups). First group consisted of 15 genotypes whereas second group had 25 genotypes. First (A) and second (B) groups were further divided into 2 and 3 sub-groups, respectively. Similarity values obtained by UPGMA ranged from 0.45 to 0.93 depicting wider variability among the genotypes under study.
- Polymorphism Information Content (PIC) values ranged from 0.43 to 0.68 indicating variation among genotypes for the markers under study. Highest PIC value (0.68) was observed in marker Barc349 followed by Barc181 (0.66), Xsps3000 (0.64), Xbarc8 (0.56) and S19M93 (0.43), suggesting their usability in future research programs.

Based on the results of present study, following conclusions have been made:

- Selection from such diverse population will be effective for grain yield trait as it is governed by additive gene action.
- Cluster analysis grouped 100 genotypes into four clusters hence indicated sufficient diversity in material used. Hence, crosses could be made between parents of clusters having highest inter-cluster distance for getting potential hybrids for rust resistance and grain yield.
- Out of twelve SSR markers used, five markers showed polymorphism and hence, the yellow rust resistance in wheat genotypes.
- Fourteen genotypes namely, IC-166, IC-12, IC-156, IC-132, IC-88, CM-109, CM-139, CM-214, HP-33, HP-31, CM-24, CM-58, CM-93 and CM-121 have shown the presence of five markers viz., Barc349, S19M93, Xbarc8, Xsps3000 and Barc181 linked to *Yr*5, *Yr*5, *Yr*10, *Yr*15, and *Yr*24 respectively at molecular level clearly indicated that these genotypes possesses more than one gene conferring yellow rust resistance. Hence, it has been concluded that these fourteen genotypes could be

further used for breeding programmes for the development of rust resistant and high yielding varieties.

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CERTIFICATE – IV

Certified that all necessary corrections as suggested by the external examiner and advisory committee have been duly incorporated in the thesis entitled **"Genetic Diversity Studies in Advanced Breeding Lines of Wheat (*Triticum aestivum* L.em Thell)"**, submitted by **Ms. Mahpara Bashir,** Registration No. **J-21-M-824.**

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