# Population Structure and Genetic Diversity Studies for Terminal Heat Stress Tolerance in Indian Mustard [*Brassica juncea* (L.) Czern & Coss.]


**BY**

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## CERTIFICATE – I

This is to certify that this thesis entitled, **"Population structure and genetic diversity studies for terminal heat stress tolerance in Indian mustard "[*Brassica juncea* (L.) Czern & Coss.]"** submitted for the degree of **Doctor of Philosophy** in the subject of **Genetics and Plant Breeding** of the Chaudhary Charan Singh Haryana Agricultural University, Hisar, is a bonafide research work carried out by **Mr. Raju Ram Choudhary** under my supervision and that no part of this thesis has been submitted for any other degree.

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

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## CERTIFICATE – II

This is to certify that this thesis entitled **"Population structure and genetic diversity studies for terminal heat stress tolerance in Indian mustard [*Brassica juncea* (L.) Czern & Coss.]"** submitted by **Mr. Raju Ram Choudhary** to the Chaudhary Charan Singh Haryana Agricultural University, Hisar in partial fulfillment of the requirementsfor the degree of **Doctor of Philosophy** in the subject of **Genetics and Plant Breeding** has been approved by the Student's Advisory Committee after an oral examination on the same.

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# ABBREVIATIONS

| | |
|---|---|
| % | Per cent |
| µl | Microlitre |
| µM | Micromolar |
| A | Photosynthetic rate (µmol $CO_2/m^2/s$) |
| AMOVA | Analysis of Molecular Variance |
| ANOVA | Analysis of Variance |
| bp | Base pair |
| Caro | Carotenoid content (mg/g). |
| CD | Critical Difference |
| Chl | Total chlorophyll content (mg/g); |
| cM | Centi Morgan |
| cm | Centimeter |
| CTAB | Cetyl Trimethyl Ammonium Bromide |
| DNA | Deoxyribonucleic acid |
| dNTP | Deoxyribonucleotide triphosphate |
| D.F. | Degree of Freedom |
| E | Transpiration rate (mmol $H_2O/m^2/s$) |
| *e.g.* | For example |
| EDTA | Ethylene diamine tetra acetic acid |
| *et al*. | *et alia* (and others) |
| Fig. | Figure |
| g | Gram |
| gs | Stomatal conductance |
| GenAlEx | Genetic Analysis in Excel |
| GCV | Genotypic coefficient of variation |
| Gs | Stomatal conductance (mmol/$m^2/s$) |
| GWAS | Genome Wide Association Study |
| *i.e.* | That is |
| Kg | Kilogram |
| kg/ha | Kilogram per hectare |

| | |
|---|---|
| L | Ladder |
| LD | Linkage Disequilibrium |
| LG | Linkage Group |
| m | Meter |
| M | Molar |
| MAB | Marker Assisted Breading |
| MAS | Marker Assisted Selection |
| MTA | Marker Trait Association |
| Mb | Mega Byte |
| mg/l | Milligram per liter |
| mha | Million hectares |
| min. | Minute |
| ml | Milliliter |
| MLM | Mixed Linear Model |
| MLMM | Multi Locus Mixed Model |
| mM | Mill molar |
| mm | Millimeter |
| mt | Million tons |
| N | Normal |
| ng | Nano gram |
| OC | Oil content (%) |
| OECD | Organization for Economic Co-operation and Development |
| PCV | Phenotypic coefficient of variation |
| PCA | Principal Component Analysis |
| PCR | Polymerase Chain Reaction |
| pH | Negative logarithm of hydrogen ion concentration |
| RNase | Ribonuclease |
| rpm | Revolution per minute |
| Sr. No. | Serial number |
| S. E. | Standard Error |
| SSR | Simple Sequence Repeat |

| | |
|---|---|
| *Taq* | *Thermus aquaticus* |
| TASSEL | Trait Analysis by Association, Evolution and Linkage |
| TBE | Tris-Boric acid-EDTA |
| TE | Tris-EDTA |
| $T_m$ | Melting Temperature |
| Tris | 2-amino-2 (hydroxymethyl)-1, 3-propandiol |
| TSW | 1000-seed weight |
| U | Units |
| UPGMA | Unweighted Pair Group Method with Arithmetic Mean |
| UV | Ultra Violet |
| *Viz*. | videlicet (namely) |
| v/v | Volume/volume |
| w/v | Weight/ volume |

# CHAPTER-I

## *INTRODUCTION*

India, the second-largest nation in the world by population, has a wide variety of socio-economic conditions, geography, flora and fauna as well as a vast spectrum of climatic conditions (Hinz *et al.*, 2020). India is now one of the top producers of agricultural products in the world thanks to its extensive usage of agricultural land. Agriculture is India's most important industry and the foundation of its economy (Dolli and Divya, 2020). A third of India's 1.3 billion people's income comes from agriculture. Oilseed crops rank second in importance to grains in the Indian agricultural sector (Chakrabarty, 2022). India is the fourth-largest producer of oilseeds after the United States, China and Brazil accounting for roughly 19% of the global area and 2.7% of the global production (Thapa *et al.*, 2019). Only 10.5 MT of the 22.23 million metric tonnes (MT) of vegetable oil that India now requires is locally produced. Due to this, India is both the greatest producer and consumer of vegetable oils in the world, sourcing up to 60% of its domestic consumption from imports at a cost of up to INR 1.22 trillion annually (Anonymous, 2021). Additionally, as a result of India's continuously growing population and rising per capita use of vegetable oil, domestic edible oil demand will rise in the upcoming years. Despite a 6% annual growth in domestic demand for edible oils and fats, local production has only grown by 2% (Singh *et al.*, 2022b). The substantial lack of edible oils in the nation has been attributed to a number of problems including the population's rapid growth, abrupt climate change, rising household income, the low yield of oilseed crops and a complex illness called pest syndrome. The primary cause of India's enormous mismatch between supply and demand for edible oils is the low yield of oilseed crops (Singh *et al.*, 2022c).

Brassica oilseeds alone are the third most widely produced oilseed crop in India out of the seven edible oilseed crops grown here, contributing about 32% of the nation's total oil pool. In 2020–21, 34.71 million hectares of brassica oilseeds were grown worldwide, producing 73.21 million metric tonnes at a productivity of 2110 kg/ha (Singh *et al.*, 2022a). With an area of 6.70 million hectares and a production of 10.21 million metric tonnes, India is the world's third-largest producer of brassica oilseeds (19.76% of global production) (Singh *et al.*, 2022a, Singh *et al.*, 2022c). The most widely grown crop is Indian mustard [*Brassica juncea* (L.) Czern & Coss.], an allotetraploid (2n = 4x = 36, AABB), which accounts for 90% of the total area under cultivation for brassica oilseeds and 92% of its production in India. After crossing its diploid relatives *B. rapa* (2n = 20, AA) and *B. nigra* (2n = 16, BB), an amphidiploid crop known as *B. juncea* (2n = 36, AABB) emerged

thousands of years ago (Singh *et al.*, 2021a). In addition to being used in cooking, Indian mustard has a wide range of uses in the food and chemical sectors, as well as in bio fertilizers. India is now the world's top exporter of mustard seed meal, which is a good source of nutrition for poultry animals (Sharma *et al.*, 2022c).

In comparison to the global average (2110 kg/ha), India's average productivity of brassica oilseeds is much lower (1520 kg/ha). This is due to crop's extreme vulnerability to a variety of biotic and abiotic stresses, including disease, pests, heat, drought, cold stress, etc., is largely responsible for its extremely low productivity, which is expected to get worse in the near future owing to changing climatic conditions (Singh *et al.*, 2021a). Extreme temperatures are among the stresses that have the greatest negative effects on Indian mustard crop productivity. Although Indian mustard can withstand temperatures between 6 to 27 °C annually, it responds to light efficiently at temperatures between 15-20 °C. The plant reaches its maximal $CO_2$ exchange range at this temperature, and after that, it starts to decline (Kaushal *et al*., 2016; Singh and Bhajan, 2016). Early mustard planting results in heat stress at the seedling stage, which hinders seedling establishment, while delayed planting results in high temperatures at the seed filling stage, which limits assimilatory supplies to the developing seeds or reduces sink strength. However, high temperatures during the reproductive stage have a significant negative impact on blooming, siliqua formation, and seed development which causes Indian mustard to produce fewer seeds and mature more quickly (Sharma *et al.*, 2022b). There are significant yield losses in Indian mustard as a result of pod rupture and shattering brought on by high-temperature stress at the terminal stage. The majority of India's mustard-growing regions, especially those in the north, mature and harvest their crops in the month of March, and at that time, the temperature remains high (Thakur *et al.*, 2020). High temperatures produce toxic byproducts, change hormonal activity, and create a fatal environment for plant growth and development. These factors also cause aberrant phenotypes (Rahaman *et al.*, 2017). Plants can adapt to stressful environments by slowing down their growth and development, producing less, and altering their morphological, physiological, biochemical, and molecular characteristics. Even for a brief period, a temperature increase of 3–4 °C during the reproductive stages could result in a 15–35% yield reduction (Ul Hassan *et al.*, 2021). According to earlier research, the highest daily temperature during the flowering period (21–24 °C) increased by 3 °C caused a 430 kg/ha drop in seed output (Sandhu *et al*., 2019). High-yielding and better-performing varieties of Indian mustard that can endure environmental changes, grow in a variety of agro-climatic situations, and handle diverse

biotic and abiotic stress challenges must be developed in order to meet our demand for edible oil. Given the rising demand for edible oil and the low productivity of Indian mustard, which is impacted by multiple biotic and abiotic challenges, adopting plant breeding methods to improve the mustard's genetic makeup has become imperative.

It is a polygenic characteristic for plants to tolerate heat stress. In crops plants, the precise function of the genes in coping with heat stress has not yet been discovered. The scientific world is largely unaware of the short-term cure for heat stress tolerance because of the intricacy of physiological features and how they interact with the environment (Saini *et al.*, 2019; Wani *et al.*, 2020; Pant *et al.*, 2022). According to all of the earlier investigations, cultivars varied in terms of their morpho-physiological properties (Singh *et al*., 2018; Pandey *et al.*, 2020; Chugh *et al*., 2022). Information on *Brassica juncea* L.'s reaction to extreme heat is lacking (Meena *et al*., 2013; Singh *et al*., 2017; Tripathi *et al*., 2020; Sharma *et al*., 2022b). The effective use of germplasm resources can benefit from research on the genomic structure of traits that contribute to yield and traits associated to terminal heat stress resistance. Linkage disequilibrium, which is a non-random association of alleles at two loci, is the foundation of association mapping (AM), which uses ancestral recombination and natural genetic variability within a population to quantify the quantitative features (Sahu *et al*., 2018; Khedikar *et al*., 2020). Biparental crosses is a different approach to identify genetic variables and have a greater mapping resolution among a sizable population of unrelated people. This facilitates the discovery of shared genetic variations that regulate a common trait (Gupta *et al*., 2019; Kulwal and Singh, 2021). It is a relatively recent and effective genetic technique for identifying QTL and dissecting complicated traits in plants (Luo *et al*., 2021). It makes use of a sample of recombinant accessions from germplasm collections that have undergone numerous recombination cycles. To find connections between markers and traits, this technique has been used in numerous crop and animal species (Kumari *et al*., 2022). Association mapping would be a suitable strategy to identify the genomic areas linked to phenotypes impacted by heat stress because heat stress is a complicated trait (Ahmad *et al*., 2021; Tadesse *et al*., 2019). These findings led to the adoption of this research strategy to locate the genomic areas linked to the terminal heat stress features in a group of *B. juncea* accessions grown in the field. In this study, we had used largely "unexploited" *Brassica juncea* (L.) germplasm resources from the Oilseeds Section, CCS HAU, Hisar; ICAR-DRMR, Bharatpur; ICAR-IARI, New Delhi; and PAU, Ludhiana with the primary goal of searching for new genetic variation in traits related to terminal heat stress tolerance and their correlations with

genomic regions using an LD-based association mapping technique. The present research will aid in the understanding of the genetic basis of the terminal heat stress trait in this crop. Therefore, the present study was undertaken with the following objectives:

1. To investigate the morpho-physiological diversity among the genotypes of Indian mustard.
2. To estimate the population structure and linkage disequilibrium (LD) using SSR markers.
3. To study correlation and path analysis among various yield contributing and physiological traits.

# CHAPTER- II

## REVIEW OF LITERATURE

Indian mustard [*Brassica juncea* (L.) Czern & Coss.] is an important crop of the rapeseed-mustard group with a paramount contribution in area and production of total oilseeds. Terminal heat stress has now emerged as a major challenge in Indian mustard because of climate change. Breeding for terminal heat stress (THS) in Indian mustard is recognized as a major breeding objective for sustained productivity in the present global warming situation (Sandu *et al*., 2019). It is important now to identify variation for these traits in germplasm and utilize it in breeding programmes. The success of terminal heat stress tolerance depends upon the amount of genetic variability inherent in the donors. However, molecular diversity within Indian mustard varieties and germplasm collections has been very limited (Singh *et al*., 2021b).

Molecular markers aid in the uncovering of a number of novel alleles that otherwise remain hidden in the genome background (Tanksley and McCouch, 1997). The development of a set of molecular markers is urgently needed for terminal heat stress molecular characterization of Indian mustard varieties, germplasm accessions, and pre-breeding lines to give speed and precision. The relevant review of work done is presented here under the following headings:

2.1 Morpho-physiological diversity

2.2 Molecular diversity, population structure and linkage disequilibrium (LD)

2.3 Correlation and path analysis

### 2.1 Morpho-physiological diversity

Chauhan *et al*. (2009) investigated the effects of terminal heat stress on 22 advanced breeding lines/varieties of Indian mustard during 2008–09. The genotypes were grown in RBCD with three replications in two environments, viz., $E_1$ ($9^{th}$ November sowing) and $E_2$ ($25^{th}$ November sowing). The highest reduction in seed yield and its component traits ranged from 22.2% for seeds/siliqua to 69.2% for seed yield/plant. Four terminal high temperature-tolerant genotypes, as indicated by their low HSI for seed yield, were BPR 538-10 (0.33), NRCDR 2 (0.44), RH 0216 (0.57), and NPJ 112 (0.58). Genotypes BPR 2, BPR 141-B-205-43, and BPR 540-6 having tolerance to high temperatures for multiple characters were identified for utilization in future breeding programmes.

Singh *et al*. (2011) studied the genetic parameters for the various traits in 145 lines of Indian mustard. High heritability coupled with a high GCV was observed for seed yield, length of the main shoot, and days to maturity. The high magnitude of heritability and moderate genetic advance for days to 50% flowering indicated that improvement in this trait could be

done through the selection process.

Rameeh (2011) evaluated 36 *Brassica napus* genotypes, including four cultivars and 32 advanced lines in RBD with three replications. Analysis of variance indicated significant genetic variation for different seed yield contributing characters. Most variations among the genotypes were in siliquae on the main raceme and seeds per siliqua, with 25.3 and 18.0 per cent CV, respectively. Heritability (bs) estimates were high for siliquae per plant, seeds per siliqua, and siliquae on the main raceme (0.81, 0.77, and 0.70), respectively.

Kumar *et al.* (2013) screened fifteen *Brassica juncea* (L.) genotypes on the basis of physiological parameters, *viz*., relative water content (RWC), total chlorophyll content, membrane stability index (MSI), total carotenoid content, and yield. These genotypes were subjected to temperature stress by growing the crops at three dates of sowing, i.e., October 15, November 1, and November 15. MSI, RWC, chlorophyll, and carotenoid content in leaves as well as seed yield/plant declined at a slower rate in the Proagro, NDR 8801, and CS-52 genotypes compared to Pusa Agrani, EJ-15, and Pusa Tarak genotypes.

Meena *et al*. (2013) investigated the effects of high temperature stress among 18 advanced breeding lines and four varieties of Indian mustard during the terminal stage on crop growth and genetic parameters under normal ($E_1$) and late sown ($E_2$) conditions. The genotype, BPR 538-10 exhibited terminal heat tolerance for seed yield per plant, biological yield per plant, total dry matter (60 days after sowing), heat use efficiency, crop growth rate, and relative growth rate (40–60 DAS); RH 0216 possessed terminal heat stress tolerance only for biological yield per plant. Under $E_1$, heat use efficiency had a high PCV and a moderate GCV. According to this study, developing cultivars that are resistant to terminal heat stress require rapid leaf area expansion that results in high total dry matter production.

Sharma and Sardana (2013) evaluated 25 promising *Brassica juncea* genotypes for heat stress at the seedling stage in terms of seedling mortality and at the terminal stage on growth traits and yield in two environments: timely (3$^{rd}$ week of October) and late sown (3$^{rd}$ week of November). Genotypes RB-10, PR-2004-2, NPJ-93, NRCDR-2, and CS-810-5-2-SP showed >30% seedling mortality and were rated as thermo-tolerant. Two genotypes, namely RGN-152 and RL-2047, showed less than 30% yield reduction in the present investigation. RL-2047 showed terminal heat tolerance for seed yield per plant. The average heat susceptibility index (HSI) was 0.28, and heat tolerance efficiency (HTE) was 84.8 % in the heat-tolerant genotypes. The identified genotypes are suitable donors for crossing programmes to develop heat-tolerant mustard genotypes.

Singh *et al*. (2014) investigated the effects of heat stress during the terminal stage on

the morpho-physiological characters and seed yield of 43 germplasm lines of rapeseed-mustard during 2010–11. These genotypes were sown in an augmented design at two dates of sowing, i.e., $D_1$ (Oct. 26, 2010) and $D_2$ (November 26, 2010), to allow the crop exposure to high temperature at the terminal stage, i.e., the grain filling stage. The genotypes, BPR-549-9, BPR-540-6 and BPR-349-9 showed tolerance to high temperature at terminal stage based on a lower reduction in seed yield, a lower stress intensity and a medium transpiration rate.

Kavita and Pandey (2017) conducted a field experiment with the objective of screening advanced breeding lines (genotypes) of Indian mustard for different physiological parameters, *viz*., chlorophyll content, membrane stability index (MSI %), relative water content (RWC %), electrolyte leakage and yield related to terminal heat tolerance. The study indicated that genotypes, 'Pusa Bold', 'NRCHB-101' and 'PRO-5222' had tolerance to high temperature at terminal stage. The correlation coefficient between test weight and chlorophyll content (0.843), MSI (0.832) and RWC (0.323) was positive but was negative for electrolyte leakage (- 0.564).

Singh *et al.* (2017) attempted crosses in line x tester mating scheme (13 heat-tolerant lines x four heat susceptible testers) for genetic analysis of yield traits. Next year, 52 $F_1$s along with their 17 parents were sown at three different sowing dates, i.e., early (25 September, $E_1$), timely (25 October, $E_2$), and late (25 November, $E_3$), to expose all genotypes to seedling stress as well as terminal heat stress. The lines, namely Urvashi in $E_1$, PR 08-5 in $E_2$, and PRL 08-6 in $E_3$, as well as the testers, namely RH 0304 in $E_1$, $E_2$, and JMWR 08-3 in $E_3$, exhibited the highest GCA for seed yield. The most outstanding heterotic specific crosses for different environments were Urvashi x RH 0304 in $E_1$, PR 08-5 x JMWR 08-3 in $E_2$ and PRL 08-6 x RH 0304 in $E_3$ for seed yield along with four to six component traits.

Singh *et al.* (2018) evaluated 31 released varieties of *Brassica juncea* for 15 morphological traits. These varieties were evaluated for three years in the RCBD. All the genotypes were grouped into five different clusters on the basis of multivariate analysis following Euclidean distance and the UPGMA method. The varieties grouped into different clusters (RLM 619, Geeta, Rohini, CS 52, and Laxmi of cluster 1 and RH 30, RH 781, RH 819, Varuna, Sanjuncta Asech, and Vasundhara of cluster 5) may be used as parents for hybridization and are likely to show a wide spectrum of variability in $F_2$ and $F_3$ generations.

Srivastava *et al*. (2019) conducted an experiment on 38 genotypes of Indian mustard along with Giriraj as check to estimate the genetic variability. Days to 50% flowering, number of siliqua/plant, siliqua length, seeds/siliqua, 1000-seed weight, seed yield/plant, seed yield/plot, biological yield and seed yield (q/ha) showed high heritability along with high genetic advance indicating additive gene action for these traits. As a result, direct selection for these traits may be fruitful. For seeds/siliqua, a larger genotypic coefficient of variation and a

higher phenotypic coefficient of variation were observed.

Pandey *et al*. (2020) evaluated 40 accessions of Indian mustard germplasm for yield as well as quality traits. The high estimates of phenotypic and genotypic coefficients of variation were found for harvest index and secondary branches per plant. The higher estimates of heritability, coupled with higher genetic advance, were observed for secondary branches per plant and harvest index.

Sandhu *et al.* (2020) evaluated a fixed diversity stock of 486 *Brassica juncea* lines under late planted conditions to expose the crop to terminal heat stress for two subsequent years. They calculated the selection indices using different combinations of traits and expected genetic advancement at 5 % selection intensity. Set-1 comprised of five traits *viz*., plant height, number of secondary branches, number of siliquae on main shoot, seed yield and test weight which recorded the highest genetic advance (8.612) in comparison to other trait combinations. This recommended that in early generation ($F_2/F_3$), a plant breeder may base his selection on four yield related traits, *viz*., plant height, number of secondary branches, number of siliquae on main shoot, test weight and this will save the time and resources to predict seed yield.

Tripathi *et al.* (2020) studied the effect of terminal heat stress on variability in physiological traits of twelve Indian mustard genotypes. Physiological parameters were recorded at the anthesis and siliquae initiation stages. Three genotypes, *viz*., DRMRIJ 16-3, RGN 403, and RH 1556, recorded higher chlorophyll stability indexes (0.53%, 0.52%, and 0.46%), net photosynthesis rates (33.5, 33.0, and 32.6 µ mol/$m^2$/s), relative water contents (60.8%, 59.7%, and 59.1%), and canopy temperature depressions (3.5, 3.7, and 3.5) at the post-flowering stage, while higher chlorophyll content (41.3, 40.6, and 39.2 SPAD) at the siliquae initiation stage. The genotypes DRMRIJ 16-3, RGN 403, and RH 1556 had tolerance to high temperature stress with delayed sowing, as they maintained higher values of all the studied physiological parameters.

Patel *et al*. (2021) assessed the seed yield and quality attributes of the 45 genotypes of *Brassica juncea* (L.). The results of the analysis of variance showed that there was variability for every attribute among the genotypes under investigation, with the mean sum of squares owing to genotypes being significant for each of the eighteen characters analyzed. The high values of genotypic and phenotypic coefficients of variation for the number of branches per plant, seed yield per plant, myristic acid, palmitic acid and stearic acid indicated the potential variability available for these traits. The number of branches per plant, number of siliquae per plant, seeds per siliqua, siliqua length, 1000-seed weight, seed yield per plant, myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid and glucosinolate showed high heritability estimates along with high genetic advance indicating the dominant role of additive

gene action for their expression.

Chugh *et al.* (2022) evaluated a set of 45 Indian mustard genotypes, comprising introgression lines, landraces, wild species, induced mutants, advance breeding lines, and the released varieties/cultivars under normal and stressed environments for yield stability during 2016–2018. The higher values of the heat tolerance index, geometric mean productivity, yield stability index, relative stability index, and yield production score index with a lower heat susceptibility index indicated tolerance to high temperatures in some genotypes. Based on the yield performance and derived traits, six genotypes, namely, HLM-41-13-2 and ELM-38 with zero erucic acid quality, MCN-05-8, an induced mutant, germplasm lines PCR-3 and CSR-1163, and MCN-08-2, a released variety, were rated as promising for heat stress tolerance at both locations. Biplot analysis indicated that genotypes in groups I and IV are tolerant of high yield potential and stability in a stressed environment, irrespective of location.

Sharma *et al*. (2022a) investigated genetic variability among a panel of 145 Indian mustard germplasm accessions using 11 agro-morphological traits. The result of the variance analysis exhibited significant differences among all the studied genotypes indicating the presence of a high degree of variability. The phenotypic coefficient of variation for seed yield was the highest (CV = 28.26%) among all the studied traits followed by biological yield (CV =25.72%). Days to maturity ranged from 130 to 141 days (CV = 1.88%) which was the minimum variation exhibited by Indian mustard genotypes. Cluster analysis using morphological traits grouped all 145 accessions into two major clusters.

Sharma *et al*. (2022b) evaluated 145 genotypes of Indian mustard along with six checks in an augmented design for terminal heat stress tolerance screening using 12 agro-morphological traits. Terminal heat stress caused a significant reduction in performance of all traits except fruiting zone length and oil content. Seed yield/plant showed the highest reduction (33.92%) followed by test weight (21.28%). Test weight and secondary branches were associated with seed yield under late sown conditions. Based on stress tolerance indices, yield under stress, geometric mean productivity, and yield index, five lines, *viz*., DRMR 2094, DRMR 59, DRMR 2071, DRMR 2129 and DRMR 2136 were identified as high yielding and terminal heat stress tolerant whereas, based on the heat stress susceptibility index, DRMR 1347, DRMR 1190, and DRMR 1154 were the top-performing lines tolerant to terminal heat stress.

**2.2 Molecular diversity, population structure and linkage disequilibrium (LD)**

Delourme *et al*. (2013) analyzed the four segregating doubled haploid populations of oilseed rape to develop an integrated genetic map for oilseed rape by high throughput SNP genotyping. The precise integrated map comprise 5764 SNPs and 1603 PCR markers. With a

total genetic length of 2250 cM, the integrated map contained a density of 3.27 markers (2.56 SNPs) per cM. Overall, spring oilseed rape types exhibited higher levels of polymorphism and quicker LD decay than "00" winter oilseed rape types.

Singh *et al*. (2013a) evaluated the genetic diversity among 44 Indian mustard genotypes by using A and B genome specific SSR markers. Phenotypic data was recorded on 12 yield and yield contributing traits. Out of the 143 primers tested, 134 reported polymorphisms and a total of 355 alleles were amplified. Genotypes were grouped into four clusters based on genetic distances. Both the clustering patterns based on Jaccard's similarity and Manhattan dissimilarity coefficients, independently, discriminated the genotypes effectively as per their pedigree and origin. The correlation between phenotypic and genetic distance matrices was observed to be very low (r = 0.11). Hence, for diversity studies, reliability of molecular markers is worth proving and SSR markers are stronger tools than quantitative traits in discriminating *B. juncea* genotypes.

Cai *et al*. (2014) evaluated a panel of 192 inbred lines of *B. napus* from all over the world and genotyped those using 451 single-locus SSR markers and 740 AFLP markers. Using the model controlling both population structure and relative kinship (Q + K), a total of 43 associations ($P < 0.001$) were detected using the means of the six yield-related traits across 3 years, with two to fourteen markers associated with individual traits. Among these, 18 markers were repeatedly detected in at least 2 years, and 12 markers were located within or close to QTLs identified in previous studies. Six markers were found to be commonly associated with correlated traits.

Akhatar and Banga (2015) used a Diversity Fixed Foundation Set comprising 48 inbred lines of *Brassica juncea* (L.) for association mapping. Canonical analysis demonstrated the importance of primary branches and seed size as the traits of significance for the drought susceptibility index. Microsatellite markers (158), representing all 18 chromosomes were used to assess the population structure, linkage disequilibrium (LD), the association panel and marker–trait associations (MTAs). Thirteen significant associations were detected between the molecular markers and agronomic traits. A single marker, SB1822-1 was repeatedly detected for seed size and grain yield and was localized at 17.5 cM (centi-Morgan) on chromosome B3. Among the favourable alleles, SB1822-1 had the average positive phenotypic effect for seed size and grain yield. Marker cnu316-3 had maximum positive phenotypic effects on grain yield.

Luo *et al*. (2015) examined the genetic architecture of the harvest index in an association panel of 155 *Brassica napus* accessions using 35,791 high-throughput SNPs. Plant height, branch count, biomass production per plant, harvest index and seed yield per plant were the five characteristics that were phenotyped in the four conditions. On the C genome, nine

SNPs were shown to be strongly associated with harvest index; five of these SNPs were also found to be significantly connected with seed yield. The 3.42 per cent of the phenotypic variation in the harvest index was explained by these nine SNPs.

Gyawali *et al*. (2016) conducted GWAM studies for sclerotinia resistance in *B. napus*. The genotyping of 152 accessions with 84 SSR markers provided 690 polymorphic loci for GWAM. The general linear model in TASSEL best fitted the data when adjusted for population structure (STRUCTURE), GLM + Q. After correction for a positive false discovery rate, 34 loci were significantly associated with both disease traits, of which 21 alleles contributed to resistance and the remaining enhanced susceptibility. The phenotypic variation explained by the loci ranged from 6 to 25 %. Five loci mapped to published quantitative trait loci conferring Sclerotinia resistance in Chinese lines.

Bawa (2019) evaluated 64 *Brassica juncea* (L.) genotypes for morphological traits and Alternaria blight resistance during 2016–17 and 2017–18. All the genotypes were genotyped with 82 SSR markers. Marker A03_25410649 explained 28.8 per cent of the phenotypic variation for Alternaria blight, while markers Ni4_A06 and Ni3-E06 were found significantly associated with a major QTL/gene explaining 20.3 per cent and 26.4 per cent of the phenotypic variation, respectively for seed test weight. For siliqua per plant, 8 MTAs were observed, showing a range of phenotypic variation from 11 per cent to 16 per cent.

Sandhu *et al*. (2019) examined a fixed diversity stock of 491 genotypes for a wide range of variations in seed yield under natural terminal heat stress. A panel of 96 genotypes was constituted from this stock on the basis of their differential responses to heat susceptibility index and seed yield reduction under natural terminal heat stress. The constituted panel was evaluated for validation under controlled conditions for ten seed yield-related traits. Double digest restriction site associated DNA sequencing of 71 genotypes identified 18,258 SNPs after filtration. The least square means of all the traits under NS and THS conditions and the best linear unbiased predictors along with identified SNPs were used for a genome-wide association study. A total of 34 SNPs under NS, 24 SNPs under THS, and 30 SNPs using BLUP values were found to be associated with all seed yield-related traits. Chromosome B05 harbored the maximum number of SNPs (nine), followed by chromosomes A07 and A09 (eight SNPs each). This was the first report on the identification of 24 marker-trait associations detected for SY and its component traits under terminal heat stress conditions.

Shyam *et al.* (2020) conducted an experiment on 48 Indian mustard genotypes to identify low and high erucic acid contents on the basis of SSR markers. Out of 50 SSR markers, only 23 were found to be polymorphic. A total of 109 alleles were identified, with an average of 4.47 alleles per locus for polymorphic SSR markers. Genetic diversity varied from a

minimum of 0.55 for SSR marker Na10-D07 to a maximum of 0.77 for BRMS-098 with a mean value of 0.68. All 48 genotypes fell into three major clusters in the dendrogram. The number of sub-populations (K) was identified based on delta K values and maximum likelihood and divided into three groups using a membership probability threshold of 0.80.

Thakur *et al*. (2020) analyzed the population structure and genetic diversity of a panel of 78 *Brassica carinata* accessions using 212 SSR markers. A total of 139 (65.57%) SSRs resulted in polymorphic amplicons, with allele numbers ranging from 1–7 (average 3.03 alleles per marker). The average PIC values and gene diversity (He) values were 0.29 and 0.37 per SSR marker, respectively. Overall, lower PIC values and gene diversity values indicated the presence of lesser genetic diversity among Ethiopian mustard genotypes. The UPGMA-based dendrogram divided all the 78 accessions into two major groups, whereas, three subpopulations/subgroups were predicted by population structure analysis.

Akhtar *et al*. (2021) studied the effect of nitrogen levels on the timely transition to flowering, maturity and plant height in the Indian mustard population which comprised 92 diverse genotypes. A genotyping-by-sequencing approach helped identify 406,888 SNP markers, undertake GWAS and identified 282 significant MTAs. Annotation of the genomic region(s) within 50 kb of the peak SNPs facilitated prediction of 30 candidate genes belonging to light perception, floral meristem identity circadian, plant development, flowering regulation, and gibberellic acid pathways. These included over one copy of each of AGL24, AP1, FVE, FRI, GID1A, and GNC, while, A02 FLC and CO on chromosomes B08, GNC, CO, AGL24, FLC, CDF1, and FAF2 appeared to influence the variation for plant height.

Singh *et al.* (2021b) conducted an experiment to study the genetic diversity as well as population structure between 16 genotypes of Indian mustard resistant to Sclerotinia stem rot using SSR markers. A total of 114 alleles were generated via 48 polymorphic primers, with a mean value of 2.38 alleles per primer. The average values of expected gene heterozygosity (He) and PIC from all the polymorphic primers were 0.50 and 0.43, respectively. All the genotypes were categorized into three major clusters depending on Jaccard's dissimilarity coefficients. The Evanno method for population structure revealed the presence of three populations ($SP_1$, $SP_2$, and $SP_3$) at maximum K. $SP_1$ is mainly comprised of resistant/highly resistant genotypes.

Singh *et al*. (2021c) evaluated the 95 diverse genotypes of *B. juncea* for genetic diversity by using 70 random primers. Out of 70 SSR markers, 44 were found to be polymorphic, which amplified 157 alleles with a mean value of 3.57 alleles per locus and 0.48 of average polymorphic information content. The observed heterozygosity (Ho) and expected heterozygosity (He) values were 0.81 and 0.54, respectively. The primer BG 109 showed the highest effective multiplex ratio (EMR) value and marker index (MI) value, while BG 99

showed the highest discriminating power value (D). Jaccard's dissimilarity coefficient ranged from 0.137 to 0.77 and based on the dissimilarity coefficient, M 13 and RC 47 were the most diverse genotypes (0.77).

Singh *et al.* (2021d) evaluated the polymorphic potential of 350 SSR markers to derive a set of SSR markers for the characterization of 46 Indian mustard genotypes. Out of a total of 350 SSR markers, 310 produced polymorphic amplicons with a 3.22 average number of alleles per locus. PIC values ranged from 0.24 (Ol09A01) to 0.75 (nia-m141a), with an average PIC value of 0.40 per locus. The UPGMA-dendrogram grouped all the genotypes into two main clusters, while the structure analysis formed three sub-populations having admixture of alleles.

Rahman *et al.* (2022) estimated the extent of linkage disequilibrium (LD) in 383 globally distributed *B. napus* germplasm samples using 8,502 SNP markers. They divided the germplasm collection into five sub-populations ($P_1$ to $P_5$) according to geographic and growth habit-related patterns. All subpopulations showed moderate genetic diversity with an average PIC value (H = 0.22) and Shannon's information index (I = 0.34). The pairwise Fst (average pairwise divergence between subpopulations) comparison revealed a great degree of divergence (Fst > 0.24) between rutabaga, spring and winter types. Admixture model based structure analysis, neighbor-joining tree analysis and principal component analysis placed all sub-populations into three different clusters. The average LD was 0.03 and decayed to half maximum within 45 kb of the entire genome. The C genome's LD decay was slower (93 kb) than the A genome's (21 kb), which was confirmed by the availability of larger haplotype blocks in the "C" genome than the "A" genome. The collection would be used as a valuable resource for association mapping studies to uncover genes relevant to crop development and for the selection of parents for hybrid breeding as a result of LD pattern and population structure.

Sharma *et al*. (2022a) investigated population structure and genetic diversity among a panel of 145 Indian mustard germplasm accessions using 11 agro-morphological traits and 182 SSR markers. Out of 235 SSR random primers, 182 (77.45%) SSRs resulted in polymorphic amplicons with a 3.97 average number of alleles per marker. The average PIC value and gene diversity (He) were 0.39 and 0.46 per marker, respectively. Cluster analysis using SSR markers and agro-morphological traits grouped all 145 accessions into two major clusters. Population structure analysis grouped all the genotypes into three subpopulations with varying degrees of admixture genotypes. AMOVA inferred that 88% of the total variation resides within the groups.

Yadav *et al.* (2022) conducted an experiment of genetic diversity on 76 genotypes of *B. juncea*, including exotic lines, cultivars, advanced breeding lines, registered genetic stocks and germplasm lines. 50 SSR (120 SSRs and 20 EST-SSR) were found to be polymorphic, with

126 alleles amplified. Using phenotypic and molecular data, the dendrogram was constructed based on the Manhattan dissimilarity coefficient, Jaccard's similarity coefficient and the linkage algorithm UPGMA. All the genotypes were grouped into five and eight clusters based on their dissimilarity matrix and population structure, respectively.

**2.3 Correlation and Path analysis**

Rameeh (2011) evaluated 36 *Brassica napus* genotypes, including four cultivars and 32 advanced lines in RBD with three replications. Siliquae/plant had a significant positive correlation (0.80**) and a significant positive direct effect (0.85) on seed yield.

Singh *et al.* (2011) studied the character association for various yield contributing traits among the 145 lines (10 parents + 45 $F_1$s + 45 $F_2$s + 45 $F_3$s) of *Brassica juncea*. Seed yield had a significant positive association with plant height, days to 50 % flowering, days to maturity, main shoot length, number of siliquae on main shoot and test weight both at genotypic and phenotypic levels. Seed yield also exhibited significant and positive correlation with per cent oil content only at genotypic level indicating that these are the major yield contributing traits.

Singh *et al.* (2013b) studied the trait association analysis among 50 genotypes of Indian mustard taken from different parts of the country under timely sown ($E_1$) and late sown ($E_2$) conditions. Seed yield per plant showed a positive and significant correlation with the number of seeds per silique, siliqua length, main shoot length and test weight in $E_1$, whereas in $E_2$ it was positively correlated with number of siliquae on main shoot, main shoot length, plant height and test weight. Under both conditions, the days to 50% flowering had the greatest positive direct and indirect effects on seed yield.

Shekhawat *et al.* (2014) conducted an experiment on 60 genotypes of *Brassica juncea* in RBD with two replications to study character association for 13 yield and its component characters. There was a strong positive and significant correlation of seed yield/plant with the number of siliquae per plant, test weight and number of seeds per siliqua while, it was negatively and significantly correlated with days to 50% flowering and days to maturity. Out of these associations, days to 50% flowering was significantly and positively correlated with days to maturity, while the correlation between days to maturity and number of seeds/siliqua was negative and significant. According to path analysis, the number of siliquae/plant, test weight, and number of seeds/siliquae had positive and high direct effects on seed yield/plant, indicating selection of these characters can be used to improve the seed yield of Indian mustard.

Luo *et al.* (2015) evaluated 155 *Brassica napus* genotypes in four environments. The harvest index was found to be highly correlated with seed yield but only weakly with plant height.

Kumar *et al*. (2018) evaluated 41 exotic lines of Indian mustard (*Brassica juncea*) in irrigated environments. Correlation and path analysis among nine quantitative characters; plant height, number of primary branches, number of secondary branches, number of siliquae per plant, siliqua length (cm), number of seeds per siliqua, number of siliquae per plant, total seed yield (g) and test weight (g). The seed yield/plant was found to be positively related to siliqua length. The number of seeds per plant and test weight had greater phenotypic direct effects on total seed yield per plant indicating that indirect selection for these traits could improve seed yield.

Srivastava *et al*. (2019) estimated trait associations between different traits using 38 genotypes of Indian mustard and Giriraj as a check. Seed yield (q/ha) was positively correlated at the genotypic level with plant height, number of secondary branches per plant, number of siliquae per plant, length of siliqua, number of siliqua on the main raceme, test weight, seed yield per plant and biological yield; thus, these traits could be taken into consideration for direct selection. At the phenotypic level, path coefficient analysis showed that plant height, number of primary branches/plant, number of secondary branches/plant, number of seeds per siliqua, length of main raceme, and number of siliquae on main raceme had a positive direct effect on seed yield (q/ha). RVM-2 (17.3 q/ha), RGN-73 (16.4 q/ha), JD-6 (16.2 q/ha), RGN-298 (15.9 q/ha), and RGN-48 (15.8 q/ha) were discovered to have higher seed yield than Giriraj.

Rout *et al*. (2019) carried out an experiment with 71 genotypes of Indian mustard under sub-Himalayan conditions during *Rabi* 2017–18. The results of the trait association study revealed that secondary branches per plant and siliquae per plant had positive and significant associations with seed yield per plant. Path coefficient analysis indicated that penetration force exhibited the highest direct effect on seed yield. Furthermore, the number of siliquae/plant and secondary branches/plant had a strong direct positive effect and a positive relationship with seed yield/plant. On the basis of all eleven traits taken together, the genotype PRD-2013-9 was found to be the best.

Tiwari (2019) evaluated 25 genotypes of Indian mustard to estimate the trait association among seed yield per plant and eight other traits. The genotypic correlation coefficients being higher than the phenotypic correlation coefficients indicated that there was a strong inherent association between the various characters studied. The number of seeds per siliqua and the number of siliquae per plant were the major characters that had the greatest direct contribution to seed yield and plant.

Pandey *et al*. (2020) evaluated 40 genotypes of Indian mustard for the identification of yield contributing traits. At both the genotypic and phenotypic levels, seed yield per plant was positively and significantly correlated with the number of seeds per silique, 1000-seed weight

and harvest index. Path coefficient analysis identified 1000-seed weight and plant height as important components having a high order of direct effect, while seedling dry weight via vigour index-II and seedling length via vigour index-II are important components having a high order of indirect effect on seed yield per plant. The traits mentioned above that had significant direct and indirect yield components should be taken into account when creating an efficient selection strategy for developing high yielding genotypes of mustard.

Singh *et al*. (2020) conducted an experiment to study the genetic relationship between eleven quantitative traits of 95 Indian mustard genotypes. The results showed that plant height, number of primary branches per plant, number of secondary branches per plant and number of siliquae on the main shoot length were all positively and significantly related to seed yield per plant. Selection based on these traits would ultimately improve seed yield.

Chugh and Sharma (2021) evaluated 49 advanced breeding lines of *Brassica juncea* sown under timely and late conditions. In timely sown crop, there was a significant and strong correlation between SPAD value (flowering stage) and total chlorophyll (siliquing stage). A positive correlation was found between SPAD value and total chlorophyll content at the flowering stage and carotenoid content at siliqua forming stage.

Patel *et al*. (2021) evaluated 45 genotypes of *Brassica juncea* for seed yield and quality traits during *Rabi* 2019–20. Association analysis revealed a significant positive correlation of seed yield per plant with number of seeds per siliqua, number of siliquae per plant, length of the siliqua, myristic acid and erucic acid. Path analysis revealed positive direct effects of seeds per siliqua and number of siliquae per plant towards seed yield per plant, while, number of branches per plant and myristic acid had a positive indirect effect on seed yield/plant via number of siliquae per plant suggesting that the selection for such traits would be more effective for improving seed yield in Indian mustard.

Choudhary *et al*. (2022) investigated trait association studies for seed yield and its components in 68 genotypes of Indian mustard. According to the findings, there was a substantial positive correlation between plant height, number of primary and secondary branches/plant, length of the main shoot, number of siliquae on the main shoot, and number of seeds per siliquae. The strongest genotypic association between number of siliquae on the main branch and seed yield/plant (0.704**) suggested that direct selection of high seed yield would be beneficial. According to path coefficient analysis at genotypic level, number of secondary branches/plant (0.448) and number of siliquae on the main shoot (0.367) had the highest positive direct effects on seed yield per plant.

# CHAPTER–III

# MATERIALS AND METHODS

## 3.1 Experiment plant material and crop cultivation

The experimental plant material for the present study comprised a diverse panel of 154 Indian mustard genotypes (Table 3.1) selected from released cultivars, advanced breeding lines, registered genetic stocks and germplasm lines available at Oilseeds Section, Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar; ICAR - Directorate of Rapeseed and Mustard Research, Bharatpur; Punjab Agricultural University, Ludhiana; and ICAR-Indian Agricultural Research Institute, New Delhi. These 154 genotypes (including four checks, *viz*., RH 1566, RH 1499-30, RH 749, and RH 8812) were phenotyped under timely (sown on October 15; represented normal sown conditions; abbreviated as NS) and late sown (sown on November 15; represented late sown conditions to expose the crop to terminal heat stress; abbreviated as THS) conditions. Each genotype was sown in a plot of two rows of 4-meter length at a row-to-row distance of 45 cm and plant-to-plant spacing of 15 cm in augmented block design during *Rabi* seasons 2020–21 and 2021–22. All the recommended package of practices has been adopted to raise a healthy crop.

**Table 3.1: List of 154 *Brassica juncea* genotypes (including checks)**

| S.No. | Genotype | Pedigree | Source |
|---|---|---|---|
| 1 | RH 30 | P 26 x 3-1 | CCS HAU, Hisar, Haryana |
| 2 | RH 0119 | Pusa Bold x Rajat (PCR 7) | CCS HAU, Hisar, Haryana |
| 3 | RH 406 | RH 9608 x RH 8812 | CCS HAU, Hisar, Haryana |
| 4 | RH 725 | RH 781 x RH 9617 | CCS HAU, Hisar, Haryana |
| 5 | PBR 91 | (RLM 511/PR 18 x CM 1) | PAU, Ludhiana, Punjab |
| 6 | RH 761 | DHR 9738 x RH 30 | CCS HAU, Hisar, Haryana |
| 7 | RH 8113 | T 59 x RC 781 | CCS HAU, Hisar, Haryana |
| 8 | PBR 97 | (DIR 202 x RLM 619/Varuna) | PAU, Ludhiana, Punjab |
| 9 | RH 9304 | RH 839 x RH 30 | CCS HAU, Hisar, Haryana |
| 10 | RH 9801 | Selection from RC 1670 | CCS HAU, Hisar, Haryana |
| 11 | RB 50 | Laxmi x RH 9617 | CCS HAU, Hisar, Haryana |
| 12 | RL 1359 | RLM 514 x Varuna | PAU, Ludhiana, Punjab |
| 13 | PM 21 | Pusa Bold x ZEM 2 | IARI, New Delhi |
| 14 | PM 22 | Pusa Basanti x ZEM 2 | IARI, New Delhi |
| 15 | PM 24 | (Pusa Bold x LEB 15) x LES 29 | IARI, New Delhi |
| 16 | PM 25 | SEJ 8 x Pusa Jagannath | IARI, New Delhi |
| 17 | PM 26 | VEJ Open x Pusa Agrani | IARI, New Delhi |
| 18 | PM 28 | SEJ 8 x Pusa Jagannath | IARI, New Delhi |
| 19 | PDZM 31 | PM 21 (LES 1-27) x NUDHYJ 3 | IARI, New Delhi |
| 20 | Pusa Vijay | Synthetic *B. juncea* x VSL 5 | IARI, New Delhi |
| 21 | Pusa Jai Kisan | Somaclone variant of Varuna | IARI, New Delhi |
| 22 | Pusa Karishma | Pusa Basanti x ZEM 1 | IARI, New Delhi |
| 23 | Pusa Bold | Varuna x BIC 1780 | IARI, New Delhi |
| 24 | Pusa Jagannath | Varuna x synthetic *B. juncea* | IARI, New Delhi |
| 25 | BPR 349-9 | PCR 20 x RH 30 | DRMR, Bharatpur, (Raj.) |
| 26 | BPR 540-6 | MDOC 8 x PCR 7 | DRMR, Bharatpur, (Raj.) |

| | | | |
|---|---|---|---|
| 27 | BPR 543-2 | TM 2 x PCR 9202 | DRMR, Bharatpur, (Raj.) |
| 28 | BPR 549-9 | Chapka x PCR 9301 | DRMR, Bharatpur, (Raj.) |
| 29 | DRMR 150-35 | RH 819 x Pusa bold | DRMR, Bharatpur, (Raj.) |
| 30 | DRMR 1165-40 | EC 552583 x BPR 897-4-3 | DRMR, Bharatpur, (Raj.) |
| 31 | NRCDR 2 | MDOC 43 x NBPGR 36 | DRMR, Bharatpur, (Raj.) |
| 32 | NRCDR 601 | NBPGR 272 x RK 9903 | DRMR, Bharatpur, (Raj.) |
| 33 | RGN 48 | RSM 204 x B 75 | ARS, Sriganganagar (Raj.) |
| 34 | RGN 145 | Varuna x Tobin | ARS, Sriganganagar (Raj.) |
| 35 | RGN 229 | HEB-3 x Laxmi | ARS, Sriganganagar (Raj.) |
| 36 | RGN 236 | SBG 00-01 x Laxmi | ARS, Sriganganagar (Raj.) |
| 37 | RGN 298 | RGN 96 x Pusa Bold | ARS, Sriganganagar (Raj.) |
| 38 | CS 56 | RH 851 x Pusa Bold | CSSRI, Karnal |
| 39 | Radhika | Ashirwad x DRMR 2486 | DRMR, Bharatpur, (Raj.) |
| 40 | Shivani | Selection from local germplasm | BAU, Ranchi |
| 41 | Aravali | Krishna x RS 50 | DRMR, Bharatpur, (Raj.) |
| 42 | Varuna | Selection from Varanasi Local | DRMR, Bharatpur, (Raj.) |
| 43 | Pant Rai 18 | Selection from Kranti | GBPUAT, Pantnagar |
| 44 | Pant Rai 19 | Krishna 2-1 x HS 027-1 | GBPUAT, Pantnagar |
| 45 | Pant Rai 20 | Selection from Kranti | GBPUAT, Pantnagar |
| 46 | RH 781 | (RL 18 x P 26/3-1) x RL 18 | CCS HAU, Hisar, Haryana |
| 47 | Giriraj | HB 9908 x HB 9916 | DRMR, Bharatpur, (Raj.) |
| 48 | RH 819 | Prakash x Bulk pollen | CCS HAU, Hisar, Haryana |
| 49 | RVM 2 | Selection from Chambal region | RVSKVV, Gwalior |
| 50 | CS 52 | Selection from DIRA 343 | CSSRI, Karnal |
| 51 | IC 122287 | Indigenous collection | DRMR, Bharatpur, (Raj.) |
| 52 | IC 333591 | Indigenous collection | DRMR, Bharatpur, (Raj.) |
| 53 | IC 470135 | Indigenous collection | DRMR, Bharatpur, (Raj.) |
| 54 | IC 491390 | Indigenous collection | DRMR, Bharatpur, (Raj.) |
| 55 | RH 847 | RH-9906 x RH-9806 | CCS HAU, Hisar, Haryana |
| 56 | RH 1400-2 | Selection from MCN 13-91 | CCS HAU, Hisar, Haryana |
| 57 | RC 2 | Germplasm line | CCS HAU, Hisar, Haryana |
| 58 | RC 5 | Germplasm line | CCS HAU, Hisar, Haryana |
| 59 | RC 12 | Germplasm line | CCS HAU, Hisar, Haryana |
| 60 | RC 48 | Germplasm line | CCS HAU, Hisar, Haryana |
| 61 | RC 81 | Germplasm line | CCS HAU, Hisar, Haryana |
| 62 | RC 91 | Germplasm line | CCS HAU, Hisar, Haryana |
| 63 | RC 104 | Germplasm line | CCS HAU, Hisar, Haryana |
| 64 | RC 106 | Germplasm line | CCS HAU, Hisar, Haryana |
| 65 | RC 108 | Germplasm line | CCS HAU, Hisar, Haryana |
| 66 | RC 118 | Germplasm line | CCS HAU, Hisar, Haryana |
| 67 | RC 134 | Germplasm line | CCS HAU, Hisar, Haryana |
| 68 | RC 162 | Germplasm line | CCS HAU, Hisar, Haryana |
| 69 | RC 280 | Germplasm line | CCS HAU, Hisar, Haryana |
| 70 | RC 330 | Germplasm line | CCS HAU, Hisar, Haryana |
| 71 | RC 448 | Germplasm line | CCS HAU, Hisar, Haryana |
| 72 | RC 449 | Germplasm line | CCS HAU, Hisar, Haryana |
| 73 | RC 587 | Germplasm line | CCS HAU, Hisar, Haryana |
| 74 | RC 713 | Germplasm line | CCS HAU, Hisar, Haryana |
| 75 | RC 734 | Germplasm line | CCS HAU, Hisar, Haryana |
| 76 | RC 806 | Germplasm line | CCS HAU, Hisar, Haryana |
| 77 | RC 840 | Germplasm line | CCS HAU, Hisar, Haryana |
| 78 | RC 904 | Germplasm line | CCS HAU, Hisar, Haryana |
| 79 | RLC 1 | QM 4 x Pusa Bold | PAU, Ludhiana, Punjab |
| 80 | RLC 2 | QM 4 x Pusa Bold | PAU, Ludhiana, Punjab |
| 81 | RLC 3 | JM 06003 x JM 06020 | PAU, Ludhiana, Punjab |
| 82 | RLM 619 | Mutant of RL 18 | PAU, Ludhiana, Punjab |
| 83 | PBR 357 | (PBR 91 x RLM 514) x Bio 902 | PAU, Ludhiana, Punjab |
| 84 | RH 1916 | RH 0832 x Pusa Karishma | CCS HAU, Hisar, Haryana |
| 85 | RH 1917 | RH 0832 x Pusa Karishma | CCS HAU, Hisar, Haryana |
| 86 | RH 1918 | RH 0832 x Pusa Karishma | CCS HAU, Hisar, Haryana |

| | | | |
|---|---|---|---|
| 87 | RH 1919 | RH 0832 x Pusa Karishma | CCS HAU, Hisar, Haryana |
| 88 | RH 1922 | RH 0832 x Pusa Karishma | CCS HAU, Hisar, Haryana |
| 89 | RH 1923 | RH 0832 x Pusa Karishma | CCS HAU, Hisar, Haryana |
| 90 | RH 1924 | RH 1140 x RH-0919 | CCS HAU, Hisar, Haryana |
| 91 | RH 1927 | RH 0749 x NPJ 22 | CCS HAU, Hisar, Haryana |
| 92 | RH 1928 | RH 0749 x NPJ 22 | CCS HAU, Hisar, Haryana |
| 93 | RH 1929 | RH 0749 x DRMRIJ 11-275 | CCS HAU, Hisar, Haryana |
| 94 | RH 1930 | RH 0749 x DRMRIJ 11-275 | CCS HAU, Hisar, Haryana |
| 95 | RH 1931 | RH 0749 x RH 1365 | CCS HAU, Hisar, Haryana |
| 96 | RH 1932 | RH 1152 x RH 0919 | CCS HAU, Hisar, Haryana |
| 97 | RH 1934 | JM 6011 x RH 1365 | CCS HAU, Hisar, Haryana |
| 98 | RH 1935 | RH 1152 x RH 0919 | CCS HAU, Hisar, Haryana |
| 99 | RH 1936 | RH 1130 x T 6342 | CCS HAU, Hisar, Haryana |
| 100 | RH 1937 | RH 1130 x T 6342 | CCS HAU, Hisar, Haryana |
| 101 | RH 1938 | RH 1130 x T 6342 | CCS HAU, Hisar, Haryana |
| 102 | RH 1939 | RH 1130 x T 6342 | CCS HAU, Hisar, Haryana |
| 103 | RH 1940 | RH 1130 x T 6342 | CCS HAU, Hisar, Haryana |
| 104 | RH 2001 | TM 114 x RH 0401 (YS) | CCS HAU, Hisar, Haryana |
| 105 | RH 2002 | CS 52 x Kranti | CCS HAU, Hisar, Haryana |
| 106 | RH 2003 | RH 1135 x RH 8814 | CCS HAU, Hisar, Haryana |
| 107 | RH 2004 | RH 1135 x RH 8814 | CCS HAU, Hisar, Haryana |
| 108 | RH 2005 | RH 1135 x RH 8814 | CCS HAU, Hisar, Haryana |
| 109 | RH 2006 | TM 215 x RH 1235 | CCS HAU, Hisar, Haryana |
| 110 | RH 2007 | TM 215 x RH 0401 (YS) | CCS HAU, Hisar, Haryana |
| 111 | RH 2008 | TM 106 x RH 0401 (YS) | CCS HAU, Hisar, Haryana |
| 112 | RH 2012 | RH 1140 x RH 1372 | CCS HAU, Hisar, Haryana |
| 113 | RH 2013 | RH 1140 x RH 1372 | CCS HAU, Hisar, Haryana |
| 114 | RH 2014 | RH 1140 x RH 1372 | CCS HAU, Hisar, Haryana |
| 115 | RH 2015 | RH 1140 x RH 1372 | CCS HAU, Hisar, Haryana |
| 116 | RH 2016 | RH 1143 x RH 1372 | CCS HAU, Hisar, Haryana |
| 117 | RH 2017 | RH 1143 x RH 1372 | CCS HAU, Hisar, Haryana |
| 118 | RH 2018 | RH 1143 x RH 1372 | CCS HAU, Hisar, Haryana |
| 119 | RH 2019 | RH 1143 x RH 1372 | CCS HAU, Hisar, Haryana |
| 120 | RH 2020 | RH 1143 x RH 1372 | CCS HAU, Hisar, Haryana |
| 121 | RH 2021 | JM 6011 x RC 781 | CCS HAU, Hisar, Haryana |
| 122 | RH 2022 | JM 6011 x RC 781 | CCS HAU, Hisar, Haryana |
| 123 | RH 2023 | JM 6011 x RC 781 | CCS HAU, Hisar, Haryana |
| 124 | RH 2024 | JM 6011 x RC 781 | CCS HAU, Hisar, Haryana |
| 125 | RH 2025 | JM 6011 x RC 781 | CCS HAU, Hisar, Haryana |
| 126 | RH 2026 | JM 6011 x RC 781 | CCS HAU, Hisar, Haryana |
| 127 | RH 2027 | JM 6011 x RC 781 | CCS HAU, Hisar, Haryana |
| 128 | RH 2028 | JM 6011 x RC 781 | CCS HAU, Hisar, Haryana |
| 129 | RH 2029 | JM 6011 x RC 781 | CCS HAU, Hisar, Haryana |
| 130 | RH 2030 | JM 6011 x RC 781 | CCS HAU, Hisar, Haryana |
| 131 | RH 2031 | JM 6011 x RC 781 | CCS HAU, Hisar, Haryana |
| 132 | RH 2032 | RH 1142 x RH 7846 | CCS HAU, Hisar, Haryana |
| 133 | RH 2033 | RH 1201 x RH 7846 | CCS HAU, Hisar, Haryana |
| 134 | RH 2034 | RH 1201 x RH 7846 | CCS HAU, Hisar, Haryana |
| 135 | RH 2035 | RH 1201 x RH 7846 | CCS HAU, Hisar, Haryana |
| 136 | RH 2036 | RH 1201 x RH 7846 | CCS HAU, Hisar, Haryana |
| 137 | RH 2038 | RH 1203 x RH 7846 | CCS HAU, Hisar, Haryana |
| 138 | RH 2039 | RH 1203 x RH 7846 | CCS HAU, Hisar, Haryana |
| 139 | RH 2040 | RH 1203 x RH 7846 | CCS HAU, Hisar, Haryana |
| 140 | RH 2041 | RH 1203 x RH 7846 | CCS HAU, Hisar, Haryana |
| 141 | RH 2042 | RH 1203 x RH 7846 | CCS HAU, Hisar, Haryana |
| 142 | RH 2043 | RH 1203 x RH 7846 | CCS HAU, Hisar, Haryana |
| 143 | RH 2044 | RH 1203 x RH 7846 | CCS HAU, Hisar, Haryana |
| 144 | RH 2045 | RH 1203 x RH 7846 | CCS HAU, Hisar, Haryana |
| 145 | RH 2046 | RH 1203 x RH 7846 | CCS HAU, Hisar, Haryana |
| 146 | RH 2047 | RH 1203 x RH 7846 | CCS HAU, Hisar, Haryana |

| | | | |
|---|---|---|---|
| 147 | RH 2048 | RH 1203 x RH 7846 | CCS HAU, Hisar, Haryana |
| 148 | RH 2049 | RH 1203 x RH 7846 | CCS HAU, Hisar, Haryana |
| 149 | RH 2050 | RH 1203 x RH 7846 | CCS HAU, Hisar, Haryana |
| 150 | RH 2051 | RH 1203 x RH 7846 | CCS HAU, Hisar, Haryana |
| 151 | RH 1566 | RH 0734 X RH 0202 | CCS HAU, Hisar, Haryana |
| 153 | RH 1499-30 | RH 0788 x RH 0270 | CCS HAU, Hisar, Haryana |
| 152 | RH 749 | RH 781 x RH 9617 | CCS HAU, Hisar, Haryana |
| 154 | RH 8812 | PR 15 x RH 30A | CCS HAU, Hisar, Haryana |

### 3.2 Experimental site and weather conditions:

The present investigation entitled "**Population structure and genetic diversity studies for terminal heat stress tolerance in Indian mustard "[*Brassica juncea* (L.) Czern & Coss.]"** was conducted at Oilseeds Section, Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar, India. Geographically Hisar is situated at 29.09° N latitude and 75.43° E longitude and at elevation of 215.2 m above mean sea level. It lies on the outer margins of the south-west (SW) monsoon region. It has tropical monsoonal climate and is characterized as arid type of climate. The main characteristics of climate in Hisar district are its dryness, extremes temperature and scanty rainfall. The average annual rainfall is around 452 mm. The soil exhibits mixed pattern of Aeolian and Alluvial deposits. The fortnightly averaged meteorological data at CCS Haryana Agricultural University, Hisar during *Rabi* season of 2020-21 and 2021-22 is given in Figure 3.1.

### 3.3 Observations recorded:

#### 3.3.1. Morphological parameters:

All of the following attributes were observed on five competitive plants that were randomly chosen from the plot, with the exception of days to 50% flowering and days to maturity, which were observed over the entire plot.

**3.3.1.1. Days to 50% flowering:** It was calculated by adding up the number of days between the date of sowing and the day that half of the plants began to flower.

**3.3.1.2. Days to maturity:** It was recorded by counting days from the date of sowing to the date when 50 per cent of the siliquae on the main branch turned purplish and reached physiological maturity.

**3.3.1.3. Plant height (cm):** It was measured from ground level to the tip of the main branch at the time of maturity.

**3.3.1.4. Number of primary branches/plant:** It was recorded by counting the primary branches bearing siliquae on each plant at the time of maturity.

**3.3.1.5. Number of secondary branches/plant:** It was recorded by counting the secondary branches bearing siliquae on each plant at the time of maturity.

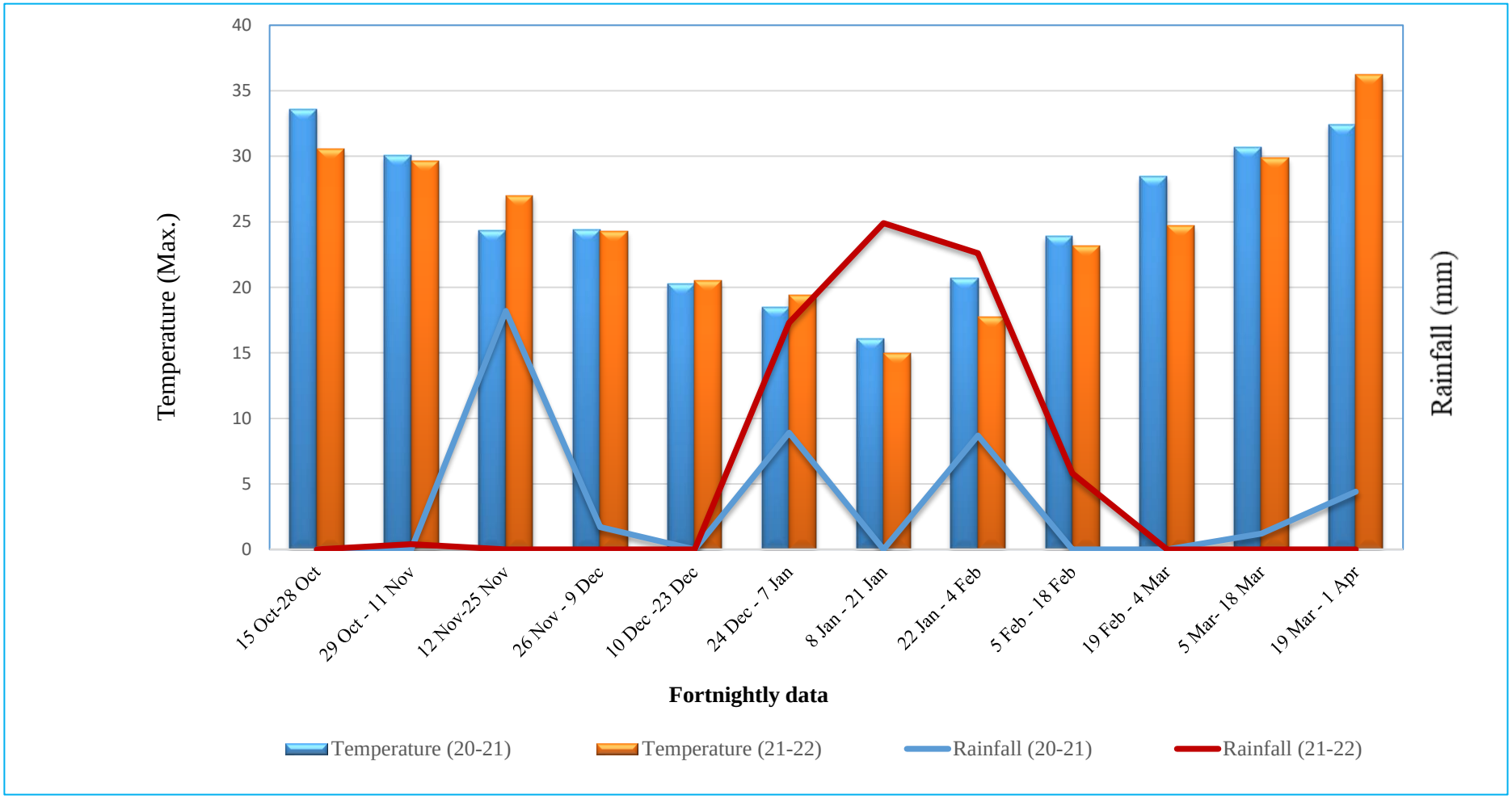


**Figure 3.1: Weekly averaged meteorological data during crop season of 2020-21 and 2021-22 at CCS Haryana Agricultural University, Hisar**

**3.3.1.6. Main shoot length (cm):** It was measured from the node of the first primary branch to the tip of the plant at the time of maturity.

**3.3.1.7. Number of siliquae on main shoot**: It was recorded by counting the seed bearing siliquae present on the main shoot.

**3.3.1.8. Siliqua length (cm)**: The length of a siliqua was measured from the base to the tip of five randomly selected siliquae from each plant and averaged.

**3.3.1.9. Number of seeds/siliqua**: It was measured by counting the seeds present in five siliquae and later taking the average of them.

**3.3.1.10. Seed yield/plant (g)**: It was calculated by weighing and averaging the clean seed of harvested siliqua from each of the five sample plants.

**3.3.1.11. 1000-seed weight (g):** The 1000-seed weight was determined by weighing thousands of seeds and was expressed in grams (g).

**3.3.1.12. Oil content (%):** Seed samples were taken from five selected plants in each of the paired rows, and oil content was determined through the Soxhlet Method of Oil Extraction. Oven-dried seed samples were ground into a fine powder using a pastle and mortar. Then, stapled the 2-gram sample of seed powder in Whatman Filter paper and weighed the packed sample again, noting its original weight ($W_1$). Following that, an equal number of samples, i.e., 5–6 samples, were placed in the sock extractor by filling the container up to 2/3 with petroleum ether. Instrument was kept working for 8–9 hours with continuous supervision, especially for water supplies. After 8–9 hours, sample packets were removed and kept to dry in the air at room temperature. Again, the used dried samples ($W_2$) were weighted, and oil content (%) was calculated using the following formula:

$$\text{Oil content (\%)} = \frac{W1-W2}{W1} \times 100$$

Where,

$W_1$ = Oven dried fresh weight of crushed seed sample in gram.

$W_2$ = Air dried Soxhlet used seed sample weight in gram.

**3.3.2. Physiological parameters:**

**3.3.2.1. Total Chlorophyll content (Hiscox and Israelstam, 1979):**

100 mg of fresh leaf (taken 20 days before maturity) from the sample was weighed and placed in 15 ml of DMSO in a test tube. It was incubated overnight at room temperature. The following morning, 3.0 ml of chlorophyll extract was transferred to a cuvette, and absorbance was measured with a spectrophotometer at 645 nm and 663 nm against DMSO as a blank. Calculation of chlorophyll content (mg/g) was done by using Wellburn's equation (Wellburn, 1994):

$$\text{Chlorophyll a (mg/g)} = 12.19 \times A_{663} - 3.45 \times A_{645} \times \text{volume} / 1000 \times \text{weight}$$

Chlorophyll b (mg/g) = 21.99 x $A_{645}$ - 5.32 x $A_{663}$ x volume /1000 x weight

Total Chlorophyll (mg/g) = Chlorophyll a + Chlorophyll b

**3.3.2.2. Carotenoids content (Hiscox and Israelstam, 1979):**

100 mg of fresh leaf (taken 20 days before maturity) of the sample was weighed and placed in 15 ml of DMSO in a test tube. It was incubated overnight at room temperature. The following morning, 3.0 ml of chlorophyll extract was transferred to a cuvette, and absorbance was measured with a spectrophotometer at 480 nm, 645 nm, and 663 nm, with DMSO as a blank. Wellburn's equation (Wellburn, 1994) is used to calculate the total carotenoids content:

Total carotenoids (mg/g) = (1000*$A_{480}$ -2.14*Ca-70.16*Cb)/220

Where, Ca – Chlorophyll a content (mg/g)

Cb – Chlorophyll b content (mg/g)

**3.3.2.3. Photosynthetic rate (µmol $CO_2$ $m^{-2}$ $s^{-1}$):**

The rate of photosynthesis was measured with the help of an infrared gas analyzer (CID 301, USA). The net exchange of $CO_2$ between a leaf and the atmosphere was measured directly by enclosing the leaf in the assimilation chamber, and the rate at which the $CO_2$ concentration changed over a definite time interval was monitored. The system automatically calculates the rate of photosynthesis on the basis of preloaded flow and leaf area as per the chamber used. Measurements were taken between 11:00 a.m. to 1:00 p.m. in triplicate for all the treatments.

**3.3.2.4. Stomatal conductance (mmol $m^{-2}$ $s^{-1}$):**

Stomatal conductance was measured directly (same as photosynthetic rate) using an infrared gas analyzer (CID 301, USA).

**3.3.2.5. Transpiration rate (µmol $H_2O$ $m^{-2}$ $s^{-1}$):**

Leaf transpiration rate was measured directly (same as photosynthetic rate) by using an infrared gas analyzer (CID 301, USA).

**3.3.2.6. Heat Susceptibility Index (HSI):**

The heat susceptibility index (HSI) was the mean values of these traits under optimum and heat stress conditions, and it was calculated using the formula proposed by Fischer and Maurer (1978):

$$HSI = (1-YD/YP)/D$$

Where, YD = Mean seed yield in heat stress

YP = Mean seed yield in normal conditions.

D = 1 - (mean YD of all genotypes / mean YP of all genotypes).

## 3.4 SSR marker-based Genotyping

### 3.4.1.Plant Material

For the present study, young leaves were collected from 4-5-week-old seedlings of 154 genotypes (including four checks) for DNA extraction used for genotyping during the *Rabi* season, 2020-21.

### 3.4.2.Chemicals and reagents

All chemicals required for DNA extraction, PCR amplification, and all other chemicals used in the present study were of molecular grade and procured as per university rules from different companies like Sigma Chemicals, Thermo Fisher Scientific, Genetix, Bangalore Genei, E. Merck, and SRL India Ltd.

### 3.4.3.Glassware

The glassware used in the present study was of borosilicate quality. Oven-dried (180 °C) conical flasks, bottles, pipettes, beakers, volumetric flasks, and measuring cylinders of different sizes (50 ml; 500 ml; 1000 ml) were used for reagents and solutions.

### 3.4.4.Methods

#### 3.4.4.1.Genomic DNA isolation

Genomic DNA was isolated from young leaves of 154 genotypes of Indian mustard by the cetyl trimethyl ammonium bromide (CTAB) extraction method of Saghai-Maroof *et al.* (1984).

#### 3.4.4.2.DNA Extraction Reagents

To isolate genomic DNA from Indian mustard leaves, the following reagents were used:

**a) CTAB extraction buffer:** Freshly prepared by combining all reagents (except β-mercaptoethanol) in sterilized distilled water. After preparation, CTAB buffer was incubated at 65°C until it became a transparent, homogeneous mixture. β- Mercaptoethanol was added at room temperature just before use. The following reagents were used in the preparation of 100 ml of extraction buffer:

| | | |
|---|---|---|
| 1M Tris (pH 8.0) | : | 20 ml |
| dH20 | : | 43 ml |
| 5M NaCl | : | 28 ml |
| 0.5 M EDTA (pH 8.0) | : | 4 ml |
| CTAB powder | : | 2 g |
| PVP | : | 1 g |
| ß-mercaptoethanol | : | 2 g (Added just before use) |

**b) Tris-EDTA (TE) buffer:** Prepared by dissolving the following components and then autoclaved.

| | |
|---|---|
| Tris (pH 8.0) | : 10 mM |
| EDTA (pH 8.0) | : 1 mM |

**c) RNase (10 mg/ml):** Ready to use Thermo Scientific RNase A (#EN0531) solution.

**Procedure:**

- The 20 ml of the CTAB extraction buffer (preparation described above) was added to 50-ml sterile polypropylene oak ridge tubes.
- Five grams of Indian mustard leaf tissue were homogenized to prepare a fine powder in a sterilized, pre-chilled mortar and pestle using liquid nitrogen.
- The samples were then well mixed with the extraction buffer (CTAB) by gently inverting the 50 ml oak ridge tubes several times after pouring the powdered samples into them. The samples were then incubated in a water bath at 65 °C for two hours.
- By gently spinning the tubes at intervals of 15-20 minutes, the contents were often mixed. After the samples had been incubated, they were cooled to room temperature before being mixed with an equal volume (20 ml) of CI solution (chloroform: isoamyl alcohol) in a 24:1 ratio.
- After adding CI, the sample tubes were again gently mixed by inverting the tubes several times to thoroughly mix up the solution.
- The samples were then centrifuged at 10,000 rpm for about 20 minutes. The top aqueous phase (supernatant) was transferred to a new, previously-sterilized oak ridge tube after centrifugation, and then an equivalent volume of the chloroform: isoamyl alcohol (24:1) solution was added once again.
- After being thoroughly mixed, the solution was centrifuged once more for 20 minutes at 10,000 rpm. After the second centrifugation, the supernatant was transferred to another pre-sterile oak-ridge tube, and the volume was doubled by adding an equal volume of pre-chilled (-20 °C) iso-propanol.
- The solution was mixed and left at -20°C overnight to precipitate. After that, the next morning, the solution was centrifuged at 10,000 rpm for 10 minutes.
- The DNA was pelleted and washed with 70 per cent ethanol for 10 minutes. After that, the pellet was air-dried.
- The dried pellet was dissolved in 300–400 µL of TE buffer and allowed for complete suspension of DNA.

**1. RNase Treatment**

To remove RNA contamination, DNA samples were treated with 1 µL of RNase A solution (10 mg/ml) per 100 µL of DNA samples. The samples were then incubated in a water bath at 37 °C for 3 h. The samples were then kept at -20°C until their subsequent use.

**2. Quantitative and qualitative determination of DNA**

**3.4.4.4.1.Agarose gel electrophoresis**

Agarose gel electrophoresis was used to determine the quantity and quality of the isolated genomic DNA. This method was preferred over the spectrophotometric method, which fails to provide reliable estimation because of the presence of sheared DNA and other chemicals such as chloroform that may contribute to UV absorbance at 260 nm.

Reagents:

1) Agarose (Low melting Temperature)
2) 10X TBE buffer (Autoclaved)

| | |
|---|---|
| Tris | 108.0 g |
| Boric acid | 55.0 g |
| 0.5 M EDTA | 40 ml |
| Final volume | 1000 ml |

3) 6X Loading dye

| | |
|---|---|
| Sucrose | 40 g |
| Bromophenol blue | 250 mg |
| Xylene cyanol | 250 mg |
| Final volume | 100 ml |

Loading dye solution was stored at $4^0$C in the refrigerator.

**Procedure:**

In the agarose gel electrophoresis method, DNA was quantified by visual comparison with Lambda DNA standards of known concentration in 0.8 per cent agarose gels stained with ethidium bromide (EtBr). An appropriate amount of agarose was heated in 1X TBE and then cooled to a manageable temperature under running tap water. Ethidium bromide was added to the gel at a rate of 6 µg/100 ml and gently mixed before being poured into a gel casting plate fitted with the required well number and size comb. Once the gel has settled, sealing tapes are removed from opposite ends, the gel plate is placed in the electrophoresis chamber, submerged using 1X TBE buffer, and the combs are removed. Using a micropipette, DNA samples were mixed with sterile distilled water and a 6X loading dye solution (1:4:1) and loaded into wells alongside Lambda DNA. Electrophoresis was carried out at constant voltage (3 volts

per cm of gel). The samples were then visualized under UV light and photographed using G: Box Syngene Gel Documentation System.

**3.4.4.5. Polymerase chain reaction (PCR) amplification**

The PCR amplification reactions were carried out in the Bio-Rad T100TM Thermal Cycler and the Biometra TAdvanced Thermal Cycler. The optimization of the PCR reaction was done by using varying concentrations of template DNA (50, 100, and 200 ng) and primers (0.1 µM, 0.25 µM, 0.5 µM and 0.8 µM) in a reaction volume of 10 µl. The optimized reaction mixture (10 µl) contained the following mixtures:

| | | |
|---|---|---|
| 100 ng DNA template | : | 1 µl |
| 0.25 µM Primers | : | 0.25 µl |
| 25 mM $MgCl_2$ | : | 1 µl |
| 10mM dNTP | : | 0.2 µl |
| 6X PCR buffer | : | 1 µl |
| Taq polymerase (2U/ µl) | : | 0.125 µl and |
| Nuclease free water | : | 6.175 µl. |

A total of 237 SSR markers were used for PCR amplification of the template DNA (APPENDIX-I). These SSR markers were obtained from the Chinese cabbage BAC clone-derived SSR markers.

The PCR reaction (10 µl) was set up in PCR plates under following reaction conditions:

i. 94°C for 2:00 minutes (initial denaturation)

ii. 94 °C for 1:00 minute (denaturation)

iii. 58 °C for 1:00 minute (primer annealing)

iv. 72 °C for 1:30 minutes (primer extension)

v. 72°C for 7 minutes (final primer extension)

vi. Hold at 4ºC for infinity

The amplification reaction was set to repeat steps (ii) to (iv) for 35 times (cycles), and after completion of all cycles, the product was kept at 4°C till further use.

**3.4.4.6. Allele scoring**

DNA banding patterns of all 154 genotypes of Indian mustard were observed under UV light with staining of electrophoretic gels in ethidium bromide (1 µl/ 50 ml). The presence

of an amplified band in each position was scored as 1 and the absence as 0. The size (in nucleotides base pairs) of the amplified bands was determined based on their migration relative to the standard 100-bp DNA ladder.

### 3.5 Data analysis:

#### 3.5.1 Statistical analysis

The data for various characters were analyzed on the mean basis. The data were subjected to following statistical analysis:

#### 3.5.2 Analysis of variance (ANOVA):

Data with respect to check varieties were subjected to an analysis of variance as per the augmented design (Federer, 1956) to obtain adjusted trait values for four checks as well as for 150 test genotypes. To obtain the estimate of error, the following ANOVA was used (Tables 3.2 and 3.3).

**Table 3.2: A two-way table of check genotypes for augmented design**

| **Check varieties** | **Blocks** | | | | |
|---|---|---|---|---|---|
| | **$B_1$** | **$B_2$……** | **$B_{10}$** | **Total** | **Mean ($X_i$)** |
| $C_1$ | $X1_1$ | $X1_2$…… | $X1_{10}$ | $C_1$ | $X_1$ |
| $C_2$ | $X2_1$ | $X2_2$…… | $X2_{10}$ | $C_2$ | $X_2$ |
| $C_3$ | $X3_1$ | $X3_2$……. | $X3_{10}$ | $C_3$ | $X_3$ |
| $C_4$ | $X4_1$ | $X4_2$…… | $X4_{10}$ | $C_4$ | $X_4$ |
| Total | $B._1$ | $B._2$…… | $B._{10}$ | G | **M** |

Where, $C_1$, $C_2$, $C_3$ and $C_4$ stand for RH 1566, RH 749, RH 1499-30 and RH 8812, respectively.

Xij = character value of $i^{th}$ check in $j^{th}$ block

B.j = $\sum$Xij = sum over checks in $j^{th}$ block.

Ci. = $\sum$ Xij = sum of $i^{th}$ check over all the blocks.

G = $\sum$Ci = $\sum$B.j = grand total of all checks.

Xi = Ci / b = mean of the $i^{th}$ check

M = $\sum$X i = G/b = sum of all check means

b = number of blocks

c = number of checks

**Table 3.3: Joint ANOVA of augmented design over multiple years and date of sowing**

| **source of variation** | **d.f.** | **Mean squares** | **F-test** |
|---|---|---|---|
| **Year (Y)** | y-1 | $MS_Y$ | $MS_Y$/ $MS_E$ |
| **Sowing date (S)** | s-1 | $MS_S$ | $MS_S$/ $MS_E$ |
| **Y × S** | (y-1)(s-1) | $MS_{YS}$ | $MS_{YS}$/ $MS_E$ |
| **Block** | ys (b-1) | $MS_B$ | $MS_B$/ $MS_E$ |
| **Genotype (G)** | g-1 | $MS_G$ | $MS_G$/ $MS_E$ |
| **G × Y** | (g-1)(y-1) | $MS_{GY}$ | $MS_{GY}$/ $MS_E$ |
| **G × S** | (g-1)(s-1) | $MS_{GS}$ | $MS_{GS}$/ $MS_E$ |
| **G × Y × S** | (g-1)(y-1)(s-1) | $MS_{GYS}$ | $MS_{GYS}$/ $MS_E$ |
| **Error** | ys(b-1)(c-1) | $MS_E$ | - |

For the joint ANOVA in multiple locations and years, the linear model was

$$yijk = \mu + \tau i + \nu j + (\tau\nu)ij + \omega k + (\tau\omega)ik + (\nu\omega)jk + (\tau\nu\omega)ijk + \varepsilon ijk$$

where, yijk is the observation of the $i^{th}$ genotype (i = 1, 2, …, g) at the $j^{th}$ sowing date (j = 1, 2, …, s) in the $k^{th}$ year (k = 1, 2, …, y); μ is the overall mean; τi is the genotype effect; νj is the sowing date effect; ωk is the year effect; (τν)ij, (τω)ik, (νω)jk, and (τνω)ijk are the interaction effects between genotypes and sowing date, between genotypes and years, between sowing date and years and between all three factors, respectively; and εijk is the joint experimental error estimated based on the pooled ANOVA of four control cultivars.

### 3.5.3 Parameters of ANOVA

**a) Mean:** Mean was worked out by dividing total sum of all the values by number of corresponding observations.

$$\bar{X} = \frac{\sum xi}{n}$$

Where,

∑Xi= Sum of all the observations.

n= Total number of observations.

**b) Standard Error of difference $SE_{(d)}$**

Standard error of difference for two means was calculated with the help of error meansquare from ANOVA table

$$SE\,(d) = \sqrt{\frac{2MSe}{r}}$$

**c) Critical difference**

Critical difference was calculated to compare the means of various genotypes. It was calculated with the help of standard error of difference and t value at error degree of freedom at 1% and 5% level of significance

$$CD = SE_{(d)} \times t_{(1\% \,\&\, 5\%)} \text{ error degree of freedom}$$

**d) Range**

Range for each character was worked out by depicting the lowest and highest values.

### 3.5.4 Genotypic and phenotypic variance (GCV and PCV)

Phenotypic coefficient of variance and genotypic coefficient of variability were calculated by the method explained by Singh and Chaudhary (1985).

The genotypic variance represents the component of variance due to geneticdifferences among the genotypes and calculated as follows:

Genotypic variance of character (xi) = $\sigma^2$gii = "mgii- meii" /"R

Where, Mgii = mean square due to genotypes

Meii = mean square due to errors

$\sigma^2$ = genotypic variance,

R = replication

Phenotypic variance can be estimated by totaling the genetic and environmental variances and calculated as follows:

Phenotypic variance of character xi = $\sigma^2$pii = $\sigma^2$gii + $\sigma^2$eii

Where, $\sigma^2$pii = phenotypic variance

$\sigma^2$gii = genotypic variance

$\sigma^2$eii = error variance

Phenotypic coefficient of variation (PCV): √ ("$\sigma^2$p×100")/"X"

Genotypic coefficient of variation (GCV): √ ("$\sigma^2$g × 100")/"X"

### 3.5.5 Heritability and genetic advance

The ratio of genetic variance to total variance is known as heritability. Heritability in the broad sense was valid for homozygous lines. Heritability and genetic advance as per cent of mean were estimated by the method given by Burton and Devane (1953) as follows:

Heritability in broad sense H(bs): Vg / Vp or Vg / Vg + Ve

Vg = genotypic component of variance

Vp = phenotypic component of variance and

Ve = environmental component of variance

Genetic advance as per cent of mean (GS) ="[(K) (σ p) (H) × 100]" /"Mean"

Where, K= selection differential

σp = phenotypic standard deviation

H= heritability in broad sense

The genetic advance as per cent of mean was classified as suggested by Johnson *et al.* (1955) which were as follows:

Low: <10%

Moderate: 10-20%

High: >20 %

### 3.5.6 Correlation coefficient analysis

The statistic that measures the relationship between two or more variables is known

as correlation. Correlation coefficients measure the mutual relationship between various plant character pairs and determine the component character on which selection can be based for improvement in yield. The phenotypic and genotypic variances and covariances were calculated according to Al-Jibouri *et al.* (1958).

$$\text{Phenotypic correlation} = rij\ (P) = "\sigma^2 pij" / \sqrt{("\sigma^2 pii \times \sigma^2 pjj")}$$

Where, $\sigma^2 pij$ = Phenotypic co-variance of character xi and xj

$xj\sigma^2 pii$ = Phenotypic variance of character and

$xi\sigma^2 pjj$= Phenotypic variance of character xj

$$\text{Genotypic correlation} = rij\ (G) = "\sigma^2 gij" / \sqrt{("\sigma^2 gii \times \sigma^2 gjj")}$$

Where, $\sigma^2 gij$ = Genotypic co-variance of character xi and xj

$xj\sigma^2 gii$ = Genotypic variance of character xi and

$\sigma^2 gjj$ = Genotypic variance of character xj

Significance of phenotypic correlations was tested at 5 per cent and 1 per cent levels of significance against the expected value from Fisher's table at (n-2) degree of freedom.

### 3.5.7 Path coefficient analysis:

The cause-and-effect interrelationship between two variables cannot be estimated from a simple correlation coefficient analysis. Therefore, the data were subjected to a standard regression analysis known as "path analysis" to unravel whether the association of different characters with yield is due to their direct effects or a consequence of their indirect effects via some other characters. The path coefficient analysis was carried out according to the method suggested by Wright (1921) and used by Dewey and Lu (1959).

These values were obtained by solving the following set of 'p' simultaneous equations using OPSTAT software.

$$P_{01} + P_{02}\, r_{12} + \text{- - - - -} + P_{0P}\, r_{1P} = r_{01}$$

$$P_{01} + P_{12}\, r_{12} + \text{- - - - -} + P_{0P}\, r_{2P} = r_{02}$$

$$\downarrow$$

$$P_{01} + r_{1P} + P_{02}\, r_{2P} + \text{- - - - - -} + P_{0P} = r_{0P}$$

Where,

$P_{01}$, $P_{02}$, - - - - - - $P_{0P}$ are the direct effects of variables 1,2- - - - - - p on the dependent variable 0 and $r_{12}$, $r_{13}$, - - - - - - $r_{1P}$ - - - - - - $r_{P(P-1)}$ are the possible correlation coefficients between various independent variables and $r_{01}$, $r_{02}$, $r_{03}$ - - - - $r_{0P}$ are the correlations between dependent and independent variables.

The indirect effects of the $i^{th}$ variable *via* $j^{th}$ variable are attained as ($P_{oj}$ x $r_{ij}$). The

contribution of remaining unknown factor is measured as the residual factor, which is calculated as given below:

$$P^2_{0X} = 1 - [P^2_{01} + 2P_{01}P_{02}r_{12} + 2P_{01}P_{03}r_{13} + - - - - - - + P^2_{02} + 2P_{02}P_{03}r_{23} + - - - - - - + P^2_{0P}]$$

$$\text{Residual factor} = \sqrt{(P^2_{0X})}$$

### 3.5.8 SSR markers scoring and genetic diversity assessment

Molecular weights of SSR primer amplified products were estimated in base pairs (bp), and data was analyzed using POWERMARKER v. 3.25 (Liu and Muse, 2005) and polymorphism information content (PIC), major allele frequency, number of alleles per marker, availability, and gene diversity for each marker were calculated. The genetic distance calculated by Nie *et al*. (1983) among Indian mustard genotypes was used to construct the Neighbor joining tree. For better representation, the dendrogram file obtained was further visualized and edited using MEGA7 (Kumar *et al*., 2016).

### 3.5.9 Analysis for population structure

The analysis for population structure of 154 Indian mustard genotypes was performed using STRUCTURE software version 2.3.4 (Pritchard *et al*., 2000). An admixture model was selected to estimate the number of sub-groups present in the populations. Initially, 10 runs for each value of K ranging from 1 to 10 were conducted with a burn-in of 10,000 iterations and an equal number of replications using STRUCTURE. Finally, optimum numbers of sub-groups (K) were estimated using a web-based tool for population structure analysis, i.e., STRUCTURE HARVESTER (Earl, 2012), which is based on the approach of Evanno *et al*., (2005).

### 3.5.10 Analysis of molecular variance

Analysis of molecular variance for predefined sub-populations by structure analysis and parameters used for diversity assessment, like the total number of alleles per locus (Na), the number of effective alleles per locus (Ne), Shannon‘s information index (I), observed gene diversity (h), and unbiased gene diversity (uh), were calculated using GenAlex version 6.5 (Peakall and Smouse, 2007).

### 3.5.11 Steps in association mapping

- First step: selection of germplasms, cultivars, or advanced breeding lines with a wide range of genetic diversity
- Second step: phenotyping of the selected population
- The third step is to combine genotypic data from germplasm with molecular marker information (SSR markers).
- Final step: data analysis

**Data analysis key points**

- Markers that possess less than 5% minor allele frequency should be removed from the marker groups to avoid the lower resolution of the association within the alleles (Myles *et al*., 2009).
- Linkage disequilibrium determination, assessment of the population structure and kinship, development or selection of the regression model
- The better model is selected on the basis of the smallest mean square difference (MSD) between the observed and expected P-value.
- A general linear model (GLM) and a mixed linear model (MLM) are used to control the population structure, where GLM is used to control only fixed effects and MLM to control both fixed (SNP and population structure effects) and random (kinship) effects (Yu *et al*., 2006).
- As MLM deals with unbalanced data across multiple trials and shows reliable inference through the correlation of the model between genetic and environmental effects, it is used in GWAS to avoid bias within the population structure and relatedness.
- The molecular markers present within the close proximity of traits of interest are known as "significant markers" and used as "marker tags" in this approach (Abdurakhmonov *et al*., 2008).
- Significant markers (based on P-value) could be subsequently used in a stepwise regression model to identify major QTL. Finally, markers from each side of the major QTL will be blasted to identify candidate genes.

*CHAPTER-IV*

***RESULTS***

The experimental results of the present investigation entitled **"Population structure and genetic diversity studies for terminal heat stress tolerance in Indian mustard [*Brassica juncea* (L.) Czern & Coss.]"** are presented and discussed under the following headings

4.1 Analysis of variance

4.2 Mean performance and range

4.3 Studies on genetic variability components

4.4 Correlation and path coefficient analysis

4.5 Selection of terminal heat tolerant genotypes based on heat susceptibility index and per cent seed yield reduction

4.6 Genetic diversity

4.7 Molecular characterization

4.8 Population structure and AMOVA

4.9 Linkage disequilibrium

4.10 Association mapping

## 4.1. Analysis of variance:

In the present study, four experiments in two consecutive years with two dates of sowing (timely and late) were conducted to evaluate a set of 154 Indian mustard genotypes (Table 3.1). A fixed model for all factors; year, location and genotype was used to conduct a joint ANOVA. The joint ANOVA results for seed yield/plant revealed considerable differences among sowing dates (S) and genotypes (G) (Table 4.1), showing substantial environmental differences during different dates of sowing while all other remaining morphological and physiological traits also showed the significant differences among genotypes (G) and sowing dates (S). Significant genetic variation among the 154 genotypes was observed, although significant interactions also existed between genotypes (G), years (Y), sowing date (S) and interaction (Y × S, Y × G and S × G) and among all three factors interaction (G × Y × S).

## 4.2. Mean performance and range:

### 4.2.1. Mean performance and range of yield and its component in different environments:

**Table 4.1: Joint ANOVA of augmented design over multiple years and date of sowing for morphological and physiological traits**

| SV | df | Mean square | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | DF | DM | PH | NPB | NSB | MSL | NSMS | SL | NSS | OC | TSW | SYP | A | Gs | E | Chl | Caro |
| **Y** | 1 | 684.80** | 144.10** | 1287.80** | 0.09 | 24.20** | 1.00 | 306.70** | 8.37** | 5.99** | 7.71** | 13.72** | 0.75 | 65.70** | 0.036** | 2.19 | 0.00 | 0.01 |
| **S** | 1 | 59302** | 82508** | 40662** | 239.32** | 2108** | 18506** | 10392** | 25.54** | 654** | 34.18** | 125** | 4033** | 10466** | 7.718** | 2933** | 9.665** | 6.15** |
| **G** | 149 | 27.70** | 27.70** | 216.60** | 2.08** | 16.54** | 139.60** | 58.50** | 1.51** | 1.72** | 0.33** | 3.85** | 67.10** | 21.80** | 0.022** | 4.25* | 0.057** | 0.23** |
| **Y × S** | 1 | 31.30** | 4460.80** | 503.20** | 0.01 | 5.25** | 1.40 | 19.60 | 0.01 | 2.97** | 0.13* | 0.63 | 1.57 | 4.50 | 0.001 | 7.17** | 0.002 | 0.04* |
| **Y × G** | 149 | 5.50** | 14.20** | 44.20* | 0.13* | 2.10** | 39.50** | 24.40** | 0.13** | 0.29** | 0.03 | 0.22 | 4.27 | 3.30** | 0.002** | 0.44 | 0.003** | 0.01** |
| **S × G** | 149 | 7.60** | 5.50** | 52.70** | 0.17** | 2.10** | 32.00** | 15.30** | 0.07 | 0.52** | 0.15** | 0.28** | 3.00 | 6.00** | 0.005** | 2.25** | 0.008** | 0.04** |
| **Y × S × G** | 149 | 5.20** | 5.50** | 28.60 | 0.05 | 1.12** | 21.30* | 8.80 | 0.05 | 0.22** | 0.03 | 0.17 | 1.20 | 1.20 | 0.001* | 0.41 | 0.002 | 0.01 |
| **Error#** | 108 | 3.30 | 3.44 | 30.50 | 0.09 | 0.47 | 15.25 | 8.14 | 0.07 | 0.14 | 0.03 | 0.17 | 3.52 | 1.86 | 0.001 | 0.62 | 0.002 | 0.01 |

**Significant at $P \leq 0.01$ and *Significant at $P \leq 0.05$. Y-Year; S-Sowing date; G-Genotype; #Error variance was estimated from combined ANOVA for pooled data of checks (replicated) over the environments. DF-Days to 50% flowering; DM-Days to maturity; PH-Plant height (cm); NPB-Number of primary branches/plant; NSB-Number of secondary branches/plant; MSL-Main shoot length (cm), NSMS-Number of siliquae on main shoot; SL-Siliqua length (cm); NSS-Number of seeds/siliqua; OC - Oil content (%); TSW-1000-seed weight (g); SYP-Seed yield/plant (g); A–Photosynthetic rate (µmol $CO_2/m^2/s$); Gs – Stomatal conductance (mmol/$m^2$/s); E – Transpiration rate (mmol $H_2O/m^2/s$); Chl – Total chlorophyll content (mg/g); Caro – Carotenoid content (mg/g).

The mean performance and range of different morphological and physiological traits in 154 genotypes of Indian mustard from pooled data of the timely sown and late sown environments are given in APPENDIX-II and APPENDIX-III. Mean performance of morphological and physiological traits have been compared year wise as well as environment wise and the results of the comparisons are shown as under (Table 4.2). Comparison of morphological and physiological traits between different environments showed that all the traits varied from timely to late sown environment except siliqua length (cm) and oil content (%) which have not changed significantly.

**4.2.2.1 Days to 50% flowering:** The mean performance of days to 50% flowering was found highest in late sown environment of year 2020-21 followed by late sown environment of year 2021-22, timely sown environment of 2020-21 and timely sown environment of 2021-22 (Figure 4.1). The environment wise pooled data from year 2020-21 and 2021-22 were analysed to study the comparison between timely and late sown environment and it showed that timely sown environment recorded least days to 50% flowering (50 days) while, late sown environment took more days (around >20 days) due to low temperature faced by crop during early growth stage (Table 4.2).

**4.2.2.2 Days to maturity:** The mean days to maturity was found highest in timely sown environment of year 2021-22 followed by timely sown and late sown environment of year 2020-21 while, lowest mean value of days to maturity was observed in late sown environment of year 2021-22 (Figure 4.2). The results further revealed that late sown environment crop took less number of days to maturity (127 days) as compared to timely sown (141 days) environment (Table 4.2).

**4.2.2.3 Plant height (cm):** The highest average plant height was observed in timely sown environment of year 2021-22 followed by timely sown environment of year 2020-21 and late sown environment of year 2021-22. The minimum average plant height was observed in late sown environment of year 2020-21 (Figure 4.3). On the basis of pooled data, highest average of plant height was recorded in timely sown (206 cm) environment as compared to late sown (191 cm) environment (Table 4.2).

**4.2.2.4 Number of primary branches/plant:** The number of primary branches/plant were observed highest in timely sown environment of both years while, lowest branches were recorded in late sown environment of both the years (Figure 4.4). The results further revealed that number of primary branches/plant was more in timely sown (5) environment as compared to late sown (4) environment (Table 4.2).

**4.2.2.5 Number of secondary branches/plant:** The mean performance of number of secondary branches/plant was recorded highest in both years under timely sown environments

while, it was minimum in both years under late sown environments (Figure 4.5). The pooled data showed that mean number of secondary branches/plant was higher under timely sown (13) environment as compared to late sown (9) environment (Table 4.2).

**4.2.2.6 Main shoot length (cm):** The average main shoot length was observed highest (82 cm) in timely sown environment of both the year 2020-21 and 2021-22 while, lowest average main shoot length (71 cm) was observed in late sown environment of both the year 2020-21 and 2021-22 (Figure 4.6). The pooled data showed that timely sown environment had significantly higher main shoot length (83 cm) as compared to late sown (72 cm) environment (Table 4.2).

**4.2.2.7 Number of siliquae on main shoot:** The average number of siliquae on main shoot were observed highest in timely sown environment of year 2021-22 followed by timely sown environment of year 2020-21 and late sown environment of year 2021-22 while, minimum was observed in late sown environment of year 2020-21 (Figure 4.7). The pooled data revealed that timely sown environment had more number of siliquae on main shoot (53) as compared to late sown (45) environment (Table 4.2).

**4.2.2.8 Siliqua length (cm):** The siliqua length was found highest in timely sown environment of year 2020-21 followed by timely sown environment of year 2021-22, late sown environment of year 2020-21 and 2021-22 (Figure 4.8). The environment wise (timely and sown) pooled data did not differ significantly for siliqua length (Table 4.2).

**4.2.2.9 Number of seeds/siliqua:** The average number of seeds/siliqua were found highest in timely sown environment of both the years 2020-21 and 2021-22 while, lowest were found in late sown environment of both the years 2020-21 and 2021-22. The average pooled data of timely and late sown environment of both the years 2020-21 and 2021-22 showed that timely sown environment had significantly more number of seeds/siliqua (14) as compared to late sown (12) environment (Table 4.2).

**4.2.2.10 Oil content (%):** The mean performance of oil content was recorded highest in timely sown environment of year 2021-22 followed by timely sown environment of 2020-21, late sown environment of 2021-22 and 2020-21 (Figure 4.10). The environment wise (timely and sown) pooled data did not differ significantly for per cent oil content (Table 4.2).

**4.2.2.11 1000-seed weight (g):** The average 1000-seed weight was found highest in timely sown environment of year 2021-22 followed by the timely sown environment of the year 2020-21, late sown environment of year 2021-22 and late sown environment of 2020-21 (Figure 4.11). The average pooled data of timely and late sown environment of both the years 2020-21 and 2021-22 showed that timely sown environment had significantly higher 1000-seed weight (5.46 g) as compared to late sown (4.55 g) environment (Table 4.2).

**4.2.2.12 Seed yield/plant (g):** The average seed yield/plant was found highest under timely sown environment of both the year 2020-21 and 2021-22 followed by late sown environment of year 2021-22 and late sown environment of year 2020-21 (Figure 4.12). The environment wise pooled data showed that timely sown environment had significantly higher seed yield/plant (22.7 g) as compared to late sown (17.5 g) environment (Table 4.2).

**4.2.2.13 Photosynthetic rate (µmol $CO_2/m^2/s$):** The average photosynthetic rate was found highest under timely sown environment of the year 2021-22 followed by timely sown environment of year 2020-21, late sown environment of year 2021-22 and late sown environment of year 2020-21 (Figure 4.13). The environment wise (timely and late sown) pooled data showed timely sown environment had significantly higher photosynthetic rate (17.05 µmol $CO_2/m^2/s$) as compared to late sown (8.70 µmol $CO_2/m^2/s$) environment (Table 4.2).

**4.2.2.14 Stomatal conductance (mmol/$m^2$/s):** The highest average stomatal conductance (0.46 mmol/$m^2$/s) was observed under timely sown environment of year 2021-22 followed by timely sown environment of year 2020-21, late sown environment of year 2021-22 and late sown environment of year 2020-21 (Figure 4.14). The environment wise pooled data of both the years showed that timely sown environment had significantly higher stomatal conductance (0.45 mmol/$m^2$/s) as compared to late sown (0.23 mmol/$m^2$/s) environment (Table 4.2).

**4.2.2.15 Transpiration rate (mmol $H_2O/m^2/s$):** The average transpiration rate was found highest under late sown environment of year 2020-21 followed by late sown environment of year 2021-22, timely sown environment of year 2021-22 and timely sown environment of year 2020-21 (Figure 4.15). The pooled data revealed that late sown environment genotypes had higher transpiration rate (7.70 mmol $H_2O/m^2/s$) as compared to timely sown environment (3.28 mmol $H_2O/m^2/s$) genotypes (Table 4.2).

**4.2.2.16 Total chlorophyll content (mg/g):** The highest average total chlorophyll content was observed under timely sown environment of both years 2020-21 and 2021-22 (1.17 mg/g) followed by late sown environment of year 2020-21 and late sown environment of year 2021-22 (Figure 4.16). The pooled data showed that timely sown environment had higher chlorophyll content (1.17 mg/g) as compared to late sown (0.92 mg/g) environment (Table 4.2).

**4.2.2.17 Carotenoid content (mg/g):** The highest average carotenoid content was found under late sown environment of year 2020-21 followed by late sown environment of year 2021-22, timely sown environment of year 2021-22 and timely sown environment of year 2020-21 (Figure 4.17). The environment wise (timely and late sown) pooled data revealed late sown environment genotypes accumulated higher carotenoid content (2.34 mg/g) as compared to late sown environment (2.14 mg/g) genotypes (Table 4.2).

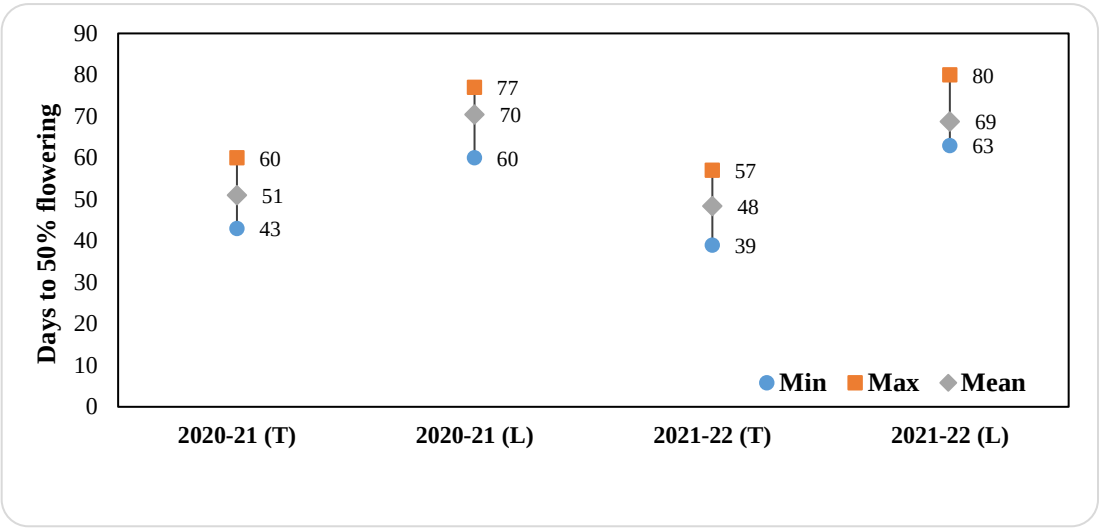


**Figure 4.1 Mean performance of days to 50% flowering across the environments**

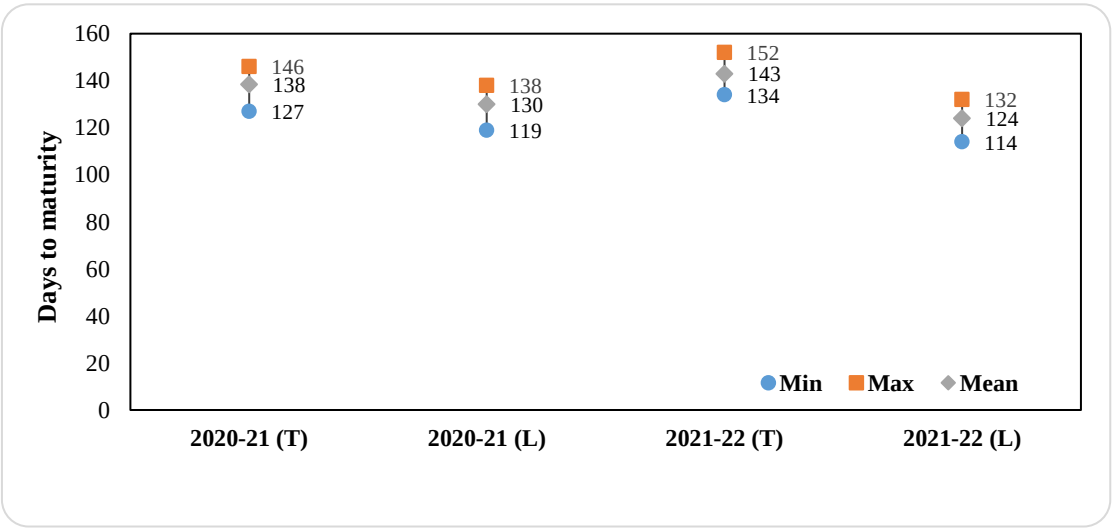


**Figure 4.2 Mean performance of days to maturity across the environments**

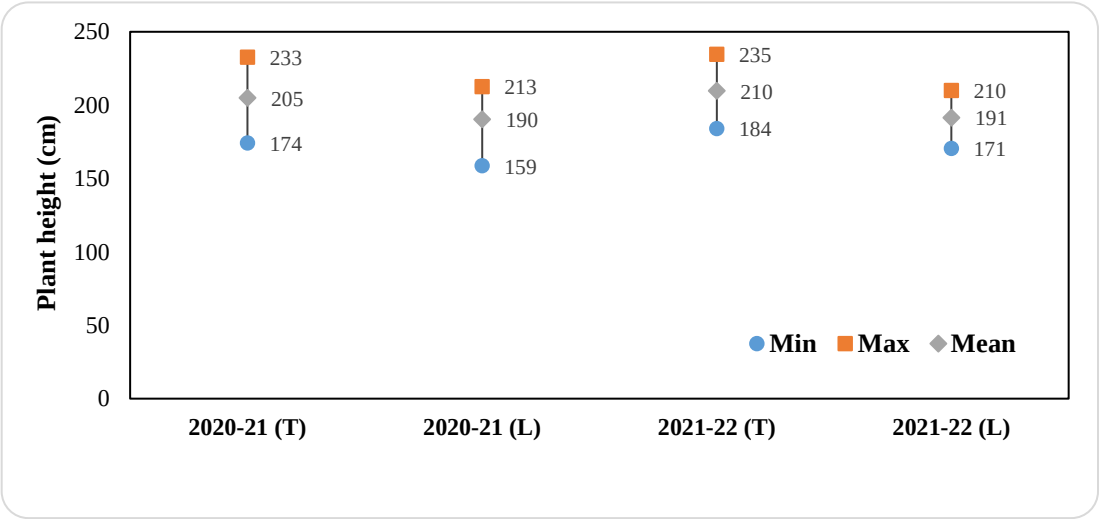


**Figure 4.3 Mean performance of plant height (cm) across the environments**

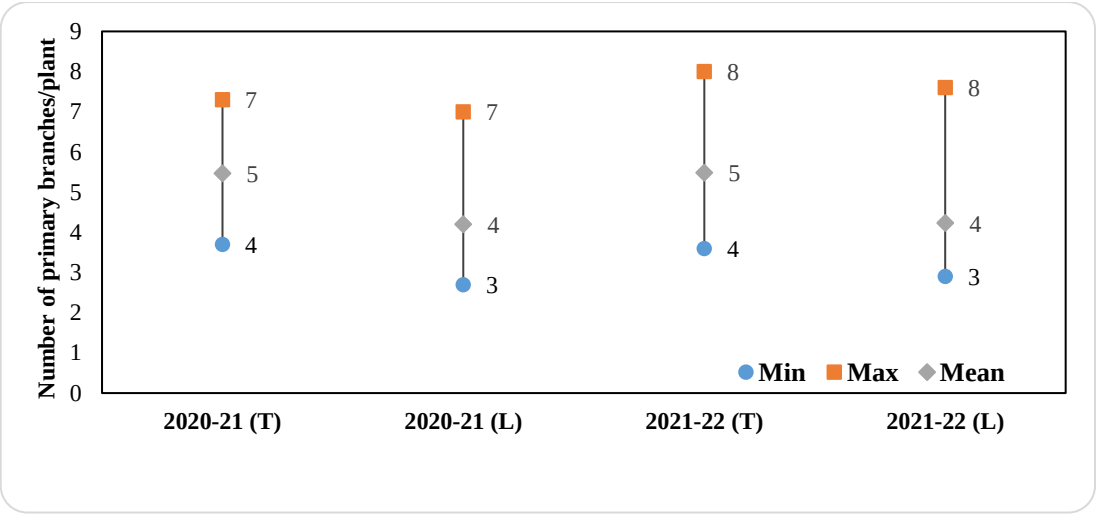


**Figure 4.4 Mean performance of number of primary branches/plant across the environments**

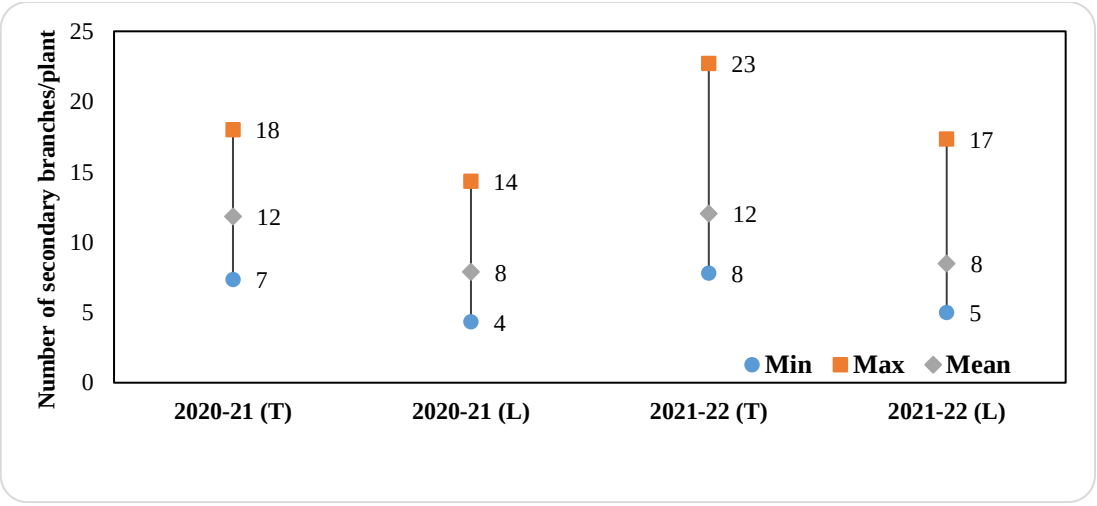


**Figure 4.5 Mean performance of number of secondary branches/plant across the environments**

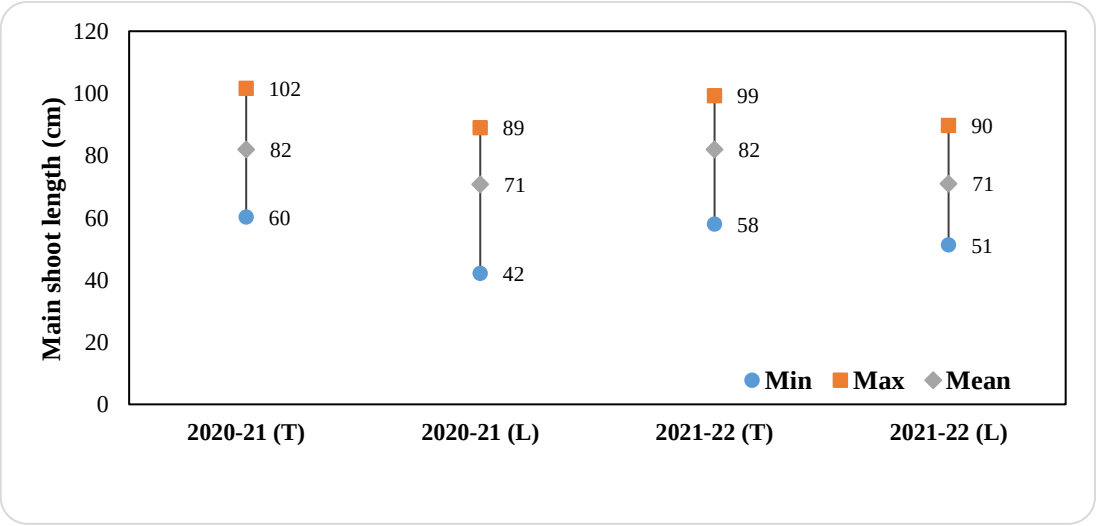


**Figure 4.6 Mean performance of main shoot length (cm) across the environments**

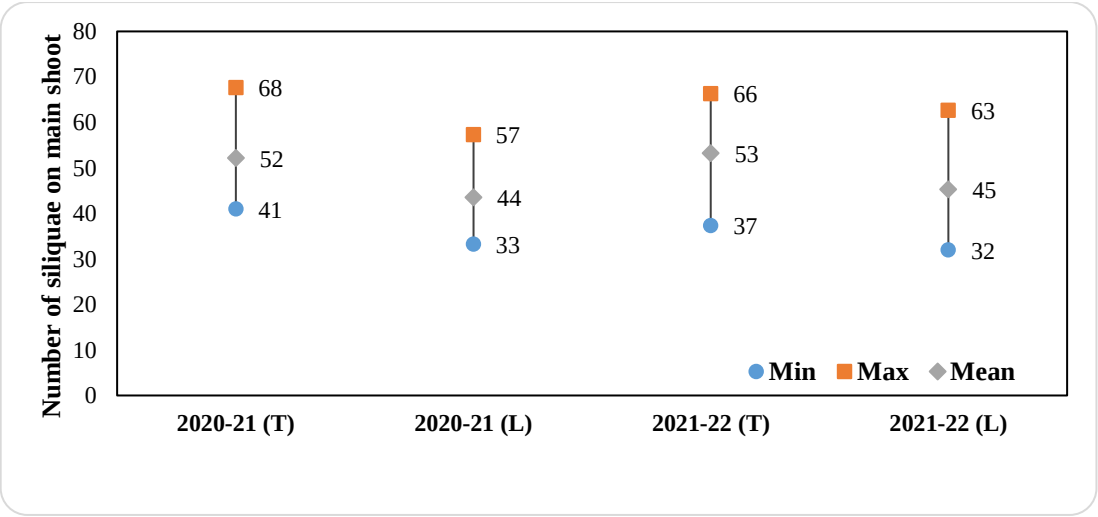


**Figure 4.7 Mean performance of number of siliquae on main shoot across the environments**

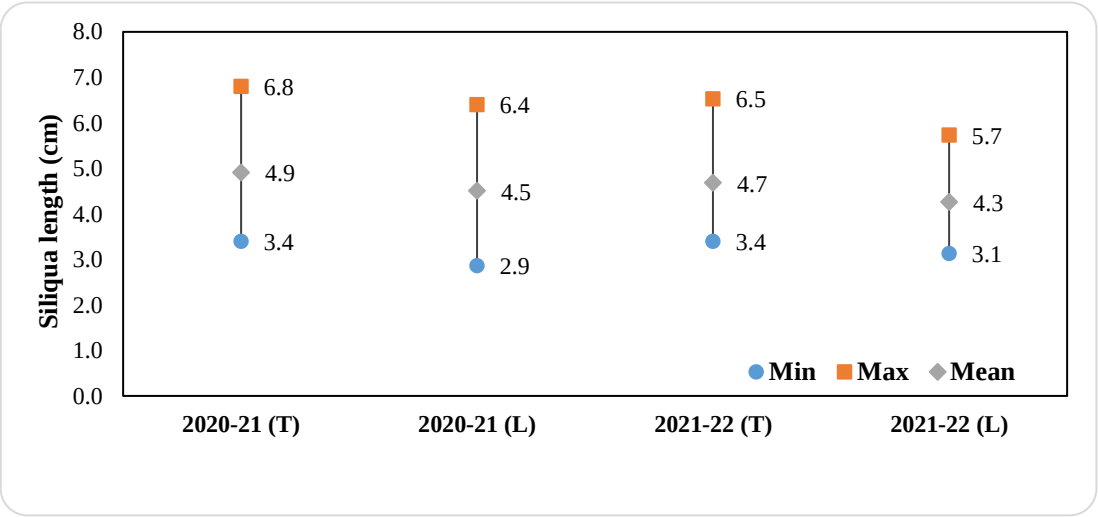


**Figure 4.8 Mean performance of siliqua length (cm) across the environments**

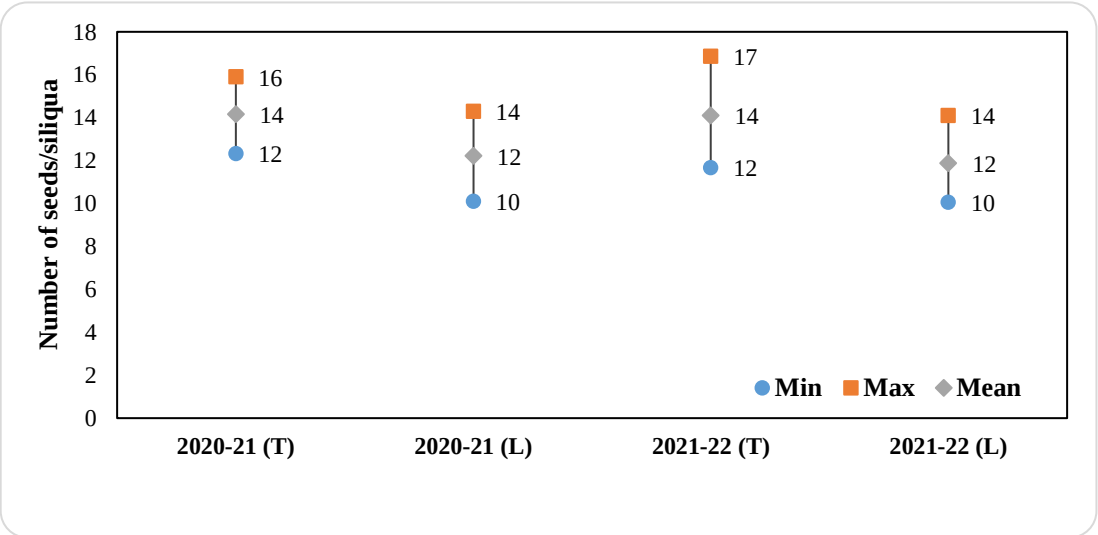


**Figure 4.9 Mean performance of number of seeds/siliqua across the environments**

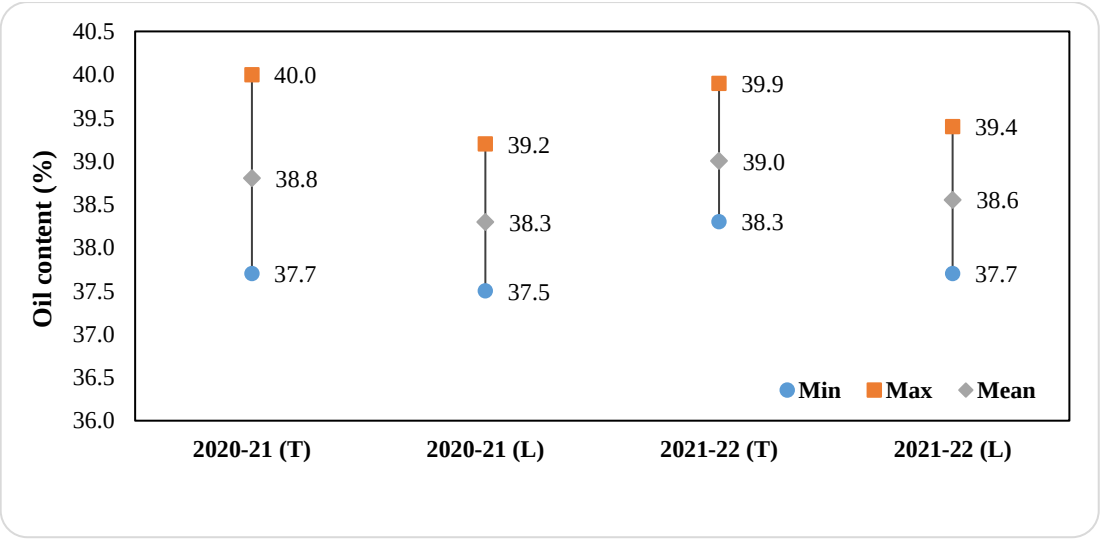


**Figure 4.10 Mean performance of oil content (%) across the environments**

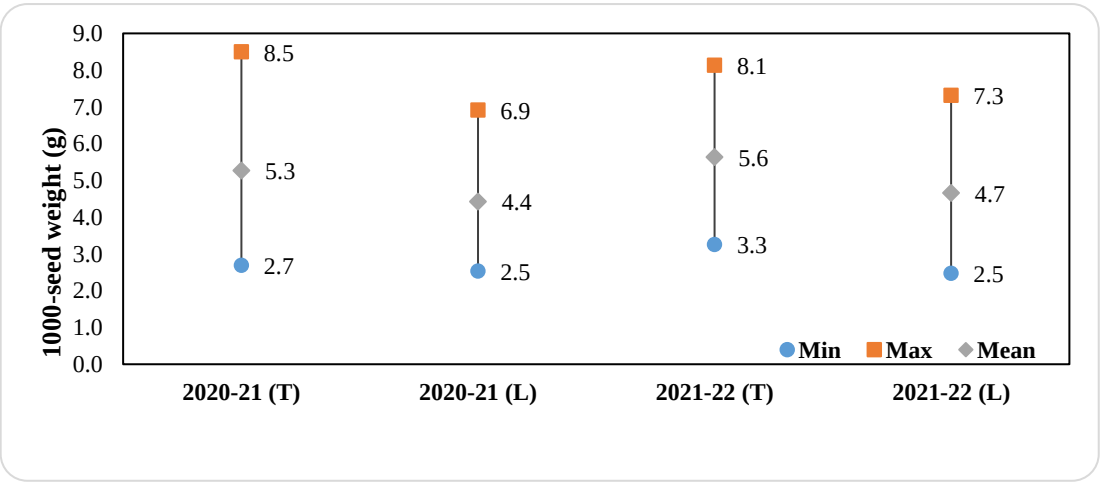


**Figure 4.11 Mean performance of test weight (g) across the environments**

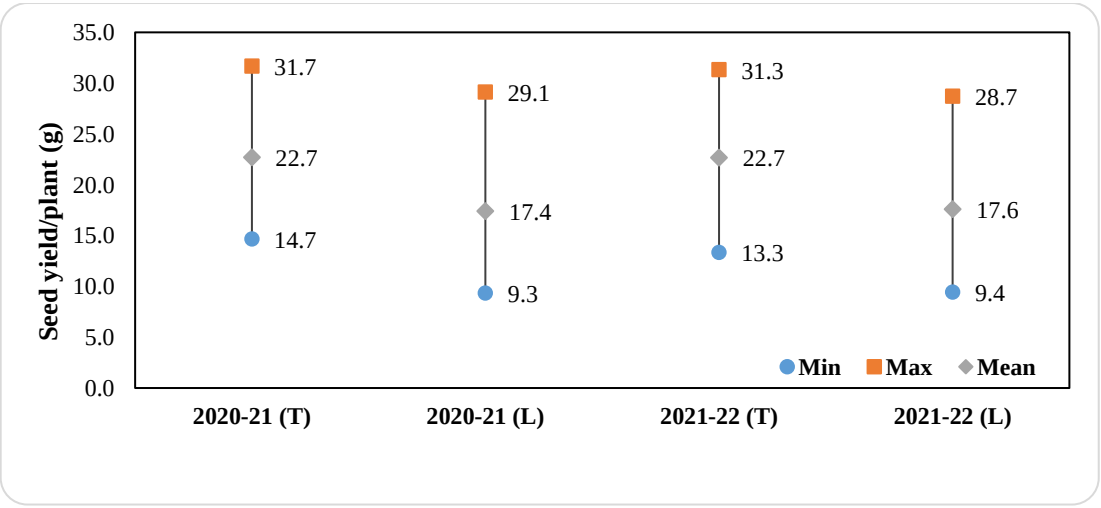


**Figure 4.12 Mean performance of seed yield/plant (g) across the environments**

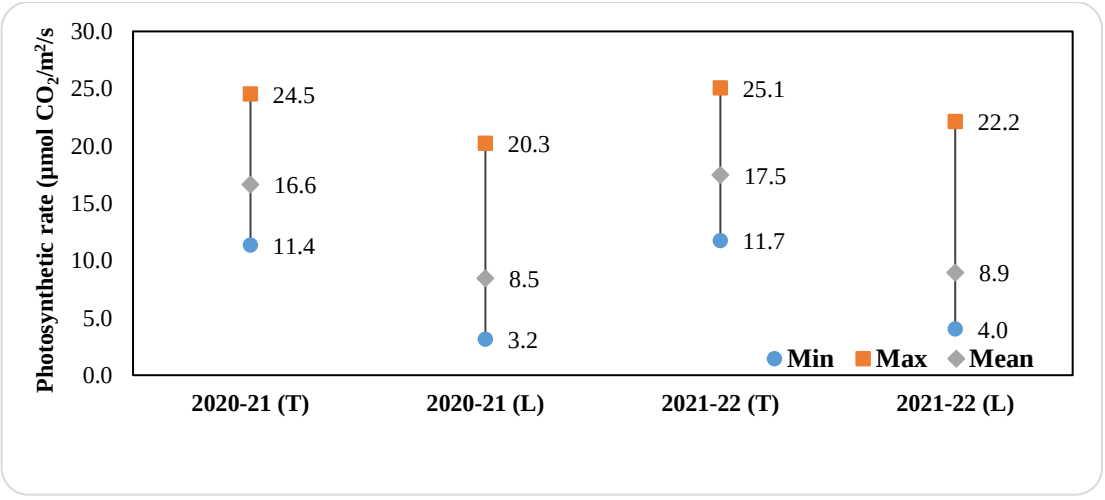


**Figure 4.13 Mean performance of photosynthetic rate across the environments**

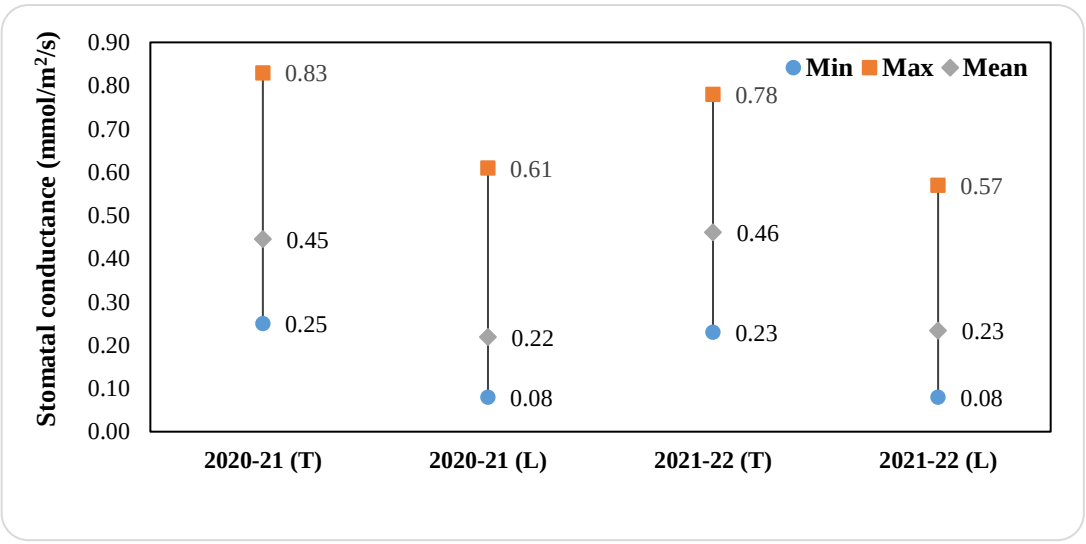


**Figure 4.14 Mean performance of stomatal conductance across the environments**

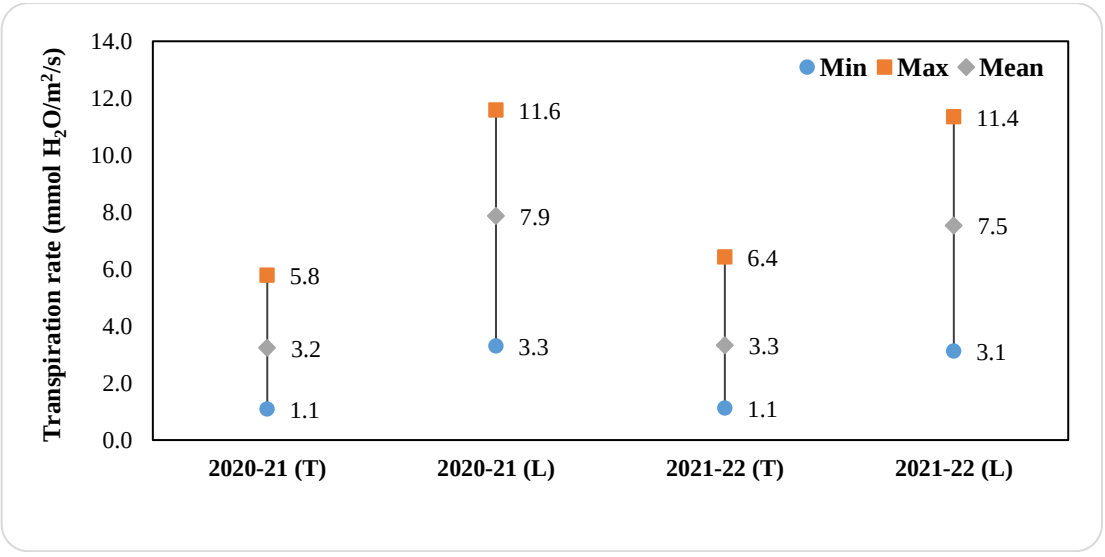


**Figure 4.15 Mean performance of transpiration rate across the environments**

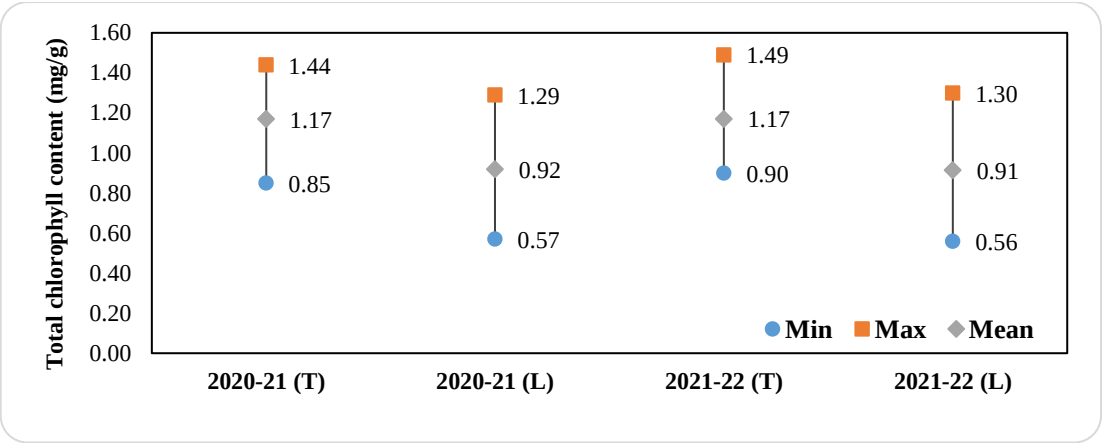


**Figure 4.16 Mean performance of stomatal conductance across the environments**

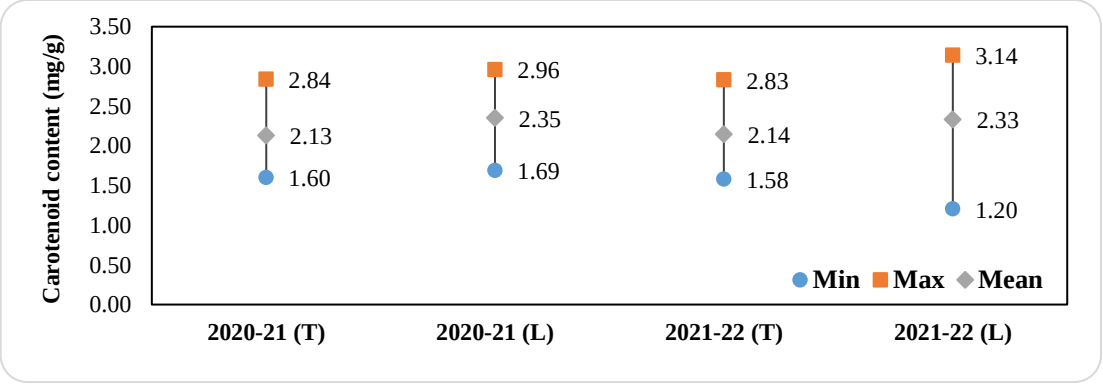


**Figure 4.17 Mean performance of carotenoid content across the environments**

**Table 4.2 Mean performance and range of various traits across the environments**

| Trait | Timely sown | | | Late sown | | | Per cent yield reduction |
|---|---|---|---|---|---|---|---|
| | Mean | Min. | Max. | Mean | Min. | Max. | |
| **DF** | 49.72 | 42.00 | 57.00 | 69.60 | 61.50 | 78.50 | +39.98 |
| **DM** | 140.70 | 132.50 | 147.50 | 127.25 | 118.50 | 133.50 | -9.56 |
| **PH** | 206.40 | 172.15 | 242.17 | 191.34 | 158.00 | 231.50 | -7.30 |
| **NPB** | 5.27 | 4.00 | 8.83 | 4.43 | 3.17 | 6.00 | -15.94 |
| **NSB** | 12.76 | 7.83 | 22.67 | 9.44 | 5.02 | 15.00 | -26.02 |
| **MSL** | 83.22 | 59.15 | 100.50 | 72.28 | 46.77 | 84.83 | -13.15 |
| **NSMS** | 53.02 | 40.33 | 66.83 | 45.39 | 33.00 | 57.85 | -14.39 |
| **SL** | 4.78 | 3.40 | 11.03 | 4.38 | 3.07 | 5.93 | -8.37 |
| **NSS** | 13.84 | 11.73 | 16.23 | 11.92 | 9.50 | 14.40 | -13.87 |
| **OC** | 38.90 | 38.10 | 39.95 | 38.43 | 37.70 | 39.30 | -1.21 |
| **TSW** | 5.46 | 3.08 | 7.81 | 4.55 | 2.63 | 7.12 | -16.67 |
| **SYP** | 22.69 | 14.00 | 30.89 | 17.50 | 9.55 | 28.40 | -22.87 |
| **A** | 17.05 | 11.55 | 24.80 | 8.70 | 3.60 | 21.20 | -48.97 |
| **Gs** | 0.45 | 0.26 | 0.80 | 0.23 | 0.10 | 0.59 | -48.89 |
| **E** | 3.28 | 1.15 | 5.81 | 7.70 | 3.22 | 11.18 | +134.76 |
| **Chl** | 1.17 | 0.88 | 1.46 | 0.92 | 0.57 | 1.28 | -21.37 |
| **Caro** | 2.14 | 1.61 | 2.84 | 2.34 | 1.51 | 3.05 | +9.35 |

DF-Days to 50% flowering; DM-Days to maturity; PH-Plant height (cm); NPB-Number of primary branches/plant; NSB-Number of secondary branches/plant; MSL-Main shoot length (cm), NSMS-Number of siliquae on main shoot; SL-Siliqua length (cm); NSS-Number of seeds/siliqua; OC-Oil content (%); TSW-1000-seed weight (g); SYP-Seed yield/plant (g); A–Photosynthetic rate (µmol $CO_2/m^2/s$); Gs – Stomatal conductance (mmol/$m^2$/s); E – Transpiration rate (mmol $H_2O/m^2/s$); Chl – Total chlorophyll content (mg/g); Caro – Carotenoid content (mg/g).

### 4.3 Studies on genetic variability components:

In plant breeding, genetic variability is the basis for selection and important for improvement of any desired trait. Out of total variation present for a desired trait, only heritable portion is desirable for gain in selection. The selection process is aided by higher heritability, resulting in responsive to selection. The variability was estimated for various morphological and physiological traits among 150 Indian mustard genotypes. Phenotypic co-efficient of variance (PCV), genotypic coefficient of variance (GCV), heritability (broad sense), genetic advance and genetic advance as a percentage of mean are presented in Table 4.3.

In the present study, the extent of variability was calculated in terms of coefficient of variation. The maximum (34.02 %, 31.41%) phenotypic and genotypic variability was recorded for photosynthetic rate and minimum (0.74%, 0.68%) for oil content, respectively. The PCV and GCV were classified as per Sivasubramaniam and Madhva Menon (1973); less than 10 % = low, 10-20% = moderate and more than 20 % = high. The high PCV was observed for photosynthetic rate (34.02%), stomatal conductance (31.54%), and transpiration rate (23.02%), seed yield/plant (20.32%) and number of secondary branches/plant (20.20%). The moderate PCV was recorded for number of primary branches/plant (14.89%), siliqua length (13.39%), 1000-seed weight (19.57%), total chlorophyll content (14.58%) and carotenoid content (12.91%) while, days to flowering (4.41%), days to maturity (2.04%), plant height (3.70%), main shoot length (7.72%), number of siliquae on main shoot (7.86%), number of seeds/siliqua (5.00%) and oil content (0.74%) were observed with low PCV.

High GCV was observed for photosynthetic rate (31.41%) and stomatal conductance (28.01%) while, moderate GCV was observed for number of primary branches/plant (14.49), number of secondary branches/plant (19.78), siliqua length (13.04), 1000-seed weight (18.99%), seed yield/plant (18.76%), transpiration rate (16.39%), total chlorophyll content (13.36%) and carotenoid content (11.55%). Low GCV was observed for days to 50% flowering (4.09%), days to maturity (1.83%), plant height (3.43%), main shoot length (7.31%), number of siliquae on main shoot (6.46%), number of seeds/siliqua (4.85%) and oil content (0.68%).

Estimates of genetic advance and heritability together give an idea of the gene action involved in the expression of various polygenic traits and serve as a reliable criterion for selection programmes. The heritability was classified as suggested by Johnson *et al.* (1955); low (0-30 %), moderate (31-60 %) and high (> 60 %). The high heritability was observed for days to 50% flowering (86.01%), days to maturity (80.65%), plant height (85.88%), number of primary branches/plant (94.64%), number of secondary branches/plant (95.84%), main shoot length (89.67%), number of siliquae on main shoot (67.55%), siliqua length (94.93%), number of seeds/siliqua (94.01%), oil content (84.34%), 1000-seed weight (94.23%), seed yield/plant (85.21%), photosynthetic rate (85.23%), stomatal conductance (78.87%), total chlorophyll

content (83.93%) and carotenoid content (80.12%) while, transpiration rate (50.68%) was observed with moderate heritability. In the present genotypes, all of the morphological and most of physiological traits under study exhibited high heritability except transpiration rate indicates that larger proportion of phenotypic variance has been attributed to genotypic variance and rigorous selection could be made for these attributes on the basis of phenotypic expression.

**Table 4.3 Estimates of genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability (broad sense) and genetic advance (GA) for different morphological and physiological traits in Indian mustard genotypes**

| Trait | Coefficient of variation (%) | | Heritability (Broad sense) | Genetic Advance | Genetic advance (% of mean) |
|---|---|---|---|---|---|
| | PCV | GCV | | | |
| **DF** | 4.41 | 4.09 | 86.01 | 4.67 | 7.83 |
| **DM** | 2.04 | 1.83 | 80.65 | 4.38 | 3.39 |
| **PH** | 3.70 | 3.43 | 85.88 | 13.04 | 6.55 |
| **NPB** | 14.89 | 14.49 | 94.64 | 1.41 | 29.07 |
| **NSB** | 20.20 | 19.78 | 95.84 | 4.02 | 39.94 |
| **MSL** | 7.72 | 7.31 | 89.67 | 10.93 | 14.29 |
| **NSMS** | 7.86 | 6.46 | 67.55 | 5.33 | 10.96 |
| **SL** | 13.39 | 13.04 | 94.93 | 1.21 | 26.22 |
| **NSS** | 5.00 | 4.85 | 94.01 | 1.27 | 9.70 |
| **OC** | 0.74 | 0.68 | 84.34 | 0.50 | 1.29 |
| **TSW** | 19.57 | 18.99 | 94.23 | 1.91 | 38.04 |
| **SYP** | 20.32 | 18.76 | 85.21 | 7.2 | 35.73 |
| **A** | 34.02 | 31.41 | 85.23 | 5.36 | 59.81 |
| **Gs** | 31.54 | 28.01 | 78.87 | 0.12 | 51.31 |
| **E** | 23.02 | 16.39 | 50.68 | 1.81 | 24.07 |
| **Chl** | 14.58 | 13.36 | 83.93 | 0.23 | 25.24 |
| **Caro** | 12.91 | 11.55 | 80.12 | 0.50 | 21.33 |

DF-Days to 50% flowering; DM-Days to maturity; PH-Plant height (cm); NPB-Number of primary branches/plant; NSB-Number of secondary branches/plant; MSL-Main shoot length (cm), NSMS-Number of siliquae on main shoot; SL-Siliqua length (cm); NSS-Number of seeds/siliqua; OC-Oil content (%); TSW-1000-seed weight (g); SYP-Seed yield/plant (g); A–Photosynthetic rate (µmol $CO^2/m^2/s$); Gs – Stomatal conductance (mmol/$m^2$/s); E – Transpiration rate (mmol $H_2O/m^2/s$); Chl – Total chlorophyll content (mg/g); Caro – Carotenoid content (mg/g).

Genetic gain under selection for morphological and physiological traits depends on the extent of genetic advance as per cent of 5% mean. Johnson *et al.* (1955) classified genetic advance as percentage of mean (GAM) values as low (0-10%), moderate (10-20%) and high (>20%). The high genetic advance as per cent of mean was observed for number of primary branches/plant (29.07%), number of secondary branches/plant (39.94%), siliqua length (26.22%), 1000-seed weight (38.04%), seed yield/plant (35.73%), photosynthetic rate (59.81%), stomatal conductance (51.31%), transpiration rate (24.07%), total chlorophyll content (25.24%) and carotenoid content (21.33%) while, medium genetic advance as per cent of mean was observed for main shoot length (14.29%) and number of siliquae on main shoot (10.96%). Low genetic advance as per cent of mean was recorded for days to 50% flowering (7.83%), days to maturity (3.39%), plant height (6.55%), number of seeds/siliqua (9.70%) and oil content (1.29%).

## 4.4 Correlation and path analysis:

For the effective selection procedure, it is very important to discover the type of relationship between various morphological and physiological traits with seed yield. Correlation analysis was performed to determine the nature of the relationship between various morpho-physiological variables and seed yield/plant (Table 4.4 and Figure 4.18). Although correlation shows the degree of link between characters, it is widely acknowledged that it falls short of the researcher's goals since it eliminates characters that indirectly affect seed yield.

Wright's (1921) path coefficient analysis illustrated the significant significance of such characteristics as splitting the correlation coefficient into direct and indirect effects under such circumstance. The trait seed yield/plant was considered as response variable whereas, remaining morpho-physiological traits were considered as causal variables. The path coefficient analysis results are presented in Table 4.5.

### 4.4.1 Correlation coefficient analysis:

The seed yield/plant was significantly and positively correlated with plant height (0.210), number of primary branches/plant (0.891), number of secondary branches/plant (0.559), main shoot length (0.370), number of siliquae on main shoot (0.316), siliqua length (0.613), number of seeds/siliqua (0.552), 1000-seed weight (0.729), photosynthetic rate (0.647), stomatal conductance (0.507), total chlorophyll content (0.747) and carotenoid content (0.536) while, negatively correlated with days to 50% flowering (-0.011) and transpiration rate (-0.125). 1000-seed weight was significantly and positively correlated with days to maturity (0.200), number of primary branches/plant (0.586), number of secondary branches/plant (0.243), main shoot length (0.351), siliqua length (0.779), number of seeds/siliqua (0.244), seed yield/plant (0.729), photosynthetic rate (0.356), stomatal conductance (0.373), total chlorophyll content (0.516) and total carotenoid content (0.359). The oil content was found negatively correlated with most of the traits.

Days to maturity had significant and positive correlation with plant height (0.340) and 1000-seed weight. The number of primary branches/plant was found significantly and positively correlated with plant height (0.185), number of secondary branches/plant (0.559), main shoot length (0.340), number of siliquae on main shoot (0.330), siliqua length (0.450), number of seeds/siliqua (0.487), 1000-seed weight (0.586), seed yield/plant (0.891), photosynthetic rate (0.728), stomatal conductance (0.445), total chlorophyll content (0.732) and carotenoid content (0.485). The secondary branches/plant was found significant positively correlated with primary branches/plant (0.559), number of siliquae on main shoot (0.302), siliqua length (0.183), number of seeds/ siliqua (0.439), 1000-seed weight (0.243), seed yield/plant (0.559), photosynthetic rate (0.341), stomatal conductance (0.208), total chlorophyll content (0.502) and carotenoid content (0.266).

**Table 4.4 Correlation coefficient between different morphological and physiological traits in Indian mustard**

| Trait | DF | DM | PH | NPB | NSB | MSL | NSMS | SL | NSS | OC | TSW | SYP | A | Gs | E | Chl | Caro |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **DF** | **1.000** | | | | | | | | | | | | | | | | |
| **DM** | 0.811** | **1.000** | | | | | | | | | | | | | | | |
| **PH** | 0.413** | 0.340** | **1.000** | | | | | | | | | | | | | | |
| **NPB** | -0.082 | 0.027 | 0.185* | **1.000** | | | | | | | | | | | | | |
| **NSB** | -0.042 | -0.028 | 0.148 | 0.559** | **1.000** | | | | | | | | | | | | |
| **MSL** | -0.243** | -0.174* | -0.025 | 0.340** | -0.005 | **1.000** | | | | | | | | | | | |
| **NSMS** | -0.122 | -0.134 | 0.255** | 0.330** | 0.302** | 0.107 | **1.000** | | | | | | | | | | |
| **SL** | -0.062 | 0.023 | 0.136 | 0.450** | 0.183* | 0.280** | 0.073 | **1.000** | | | | | | | | | |
| **NSS** | -0.125 | -0.130 | 0.183* | 0.487** | 0.439** | 0.155 | 0.369** | 0.324** | **1.000** | | | | | | | | |
| **OC** | 0.076 | 0.009 | -0.033 | -0.031 | 0.017 | -0.103 | 0.012 | -0.062 | -0.124 | **1.000** | | | | | | | |
| **TSW** | 0.022 | 0.200* | 0.153 | 0.586** | 0.243** | 0.351** | 0.083 | 0.779** | 0.244** | -0.033 | **1.000** | | | | | | |
| **SYP** | -0.011 | 0.093 | 0.210** | 0.891** | 0.559** | 0.370** | 0.316** | 0.613** | 0.552** | -0.035 | 0.729** | **1.000** | | | | | |
| **A** | -0.179* | -0.114 | 0.099 | 0.728** | 0.341** | 0.324** | 0.319** | 0.270** | 0.405** | -0.019 | 0.356** | 0.647** | **1.000** | | | | |
| **Gs** | -0.049 | 0.004 | 0.080 | 0.445** | 0.208** | 0.277** | 0.145 | 0.310** | 0.335** | -0.027 | 0.373** | 0.507** | 0.554** | **1.000** | | | |
| **E** | 0.034 | 0.088 | -0.130 | -0.204* | -0.311** | 0.245** | -0.146 | -0.023 | -0.233** | -0.076 | 0.061 | -0.125 | -0.223** | 0.185* | **1.000** | | |
| **Chl** | -0.056 | 0.040 | 0.100 | 0.732** | 0.502** | 0.265** | 0.309** | 0.397** | 0.502** | -0.083 | 0.516** | 0.747** | 0.625** | 0.469** | -0.208** | **1.000** | |
| **Caro** | 0.091 | 0.155 | 0.160* | 0.485** | 0.266** | 0.139 | 0.091 | 0.299** | 0.363** | 0.045 | 0.359** | 0.536** | 0.456** | 0.388** | -0.136 | 0.529** | **1.000** |

**Significant at $P \leq 0.01$ and *Significant at $P \leq 0.05$; DF-Days to 50% flowering; DM-Days to maturity; PH-Plant height (cm); NPB-Number of primary branches/plant; NSB-Number of secondary branches/plant; MSL-Main shoot length (cm), NSMS-Number of siliquae on main shoot; SL-Siliqua length (cm); NSS-Number of seeds/siliqua; OC-Oil content (%); TSW-1000-seed weight (g); SYP-Seed yield/plant (g); A–Photosynthetic rate (µmol $CO^2/m^2/s$); Gs – Stomatal conductance (mmol/$m^2/s$); E – Transpiration rate (mmol $H_2O/m^2/s$); Chl – Total chlorophyll content (mg/g); Caro – Carotenoid content (mg/g).

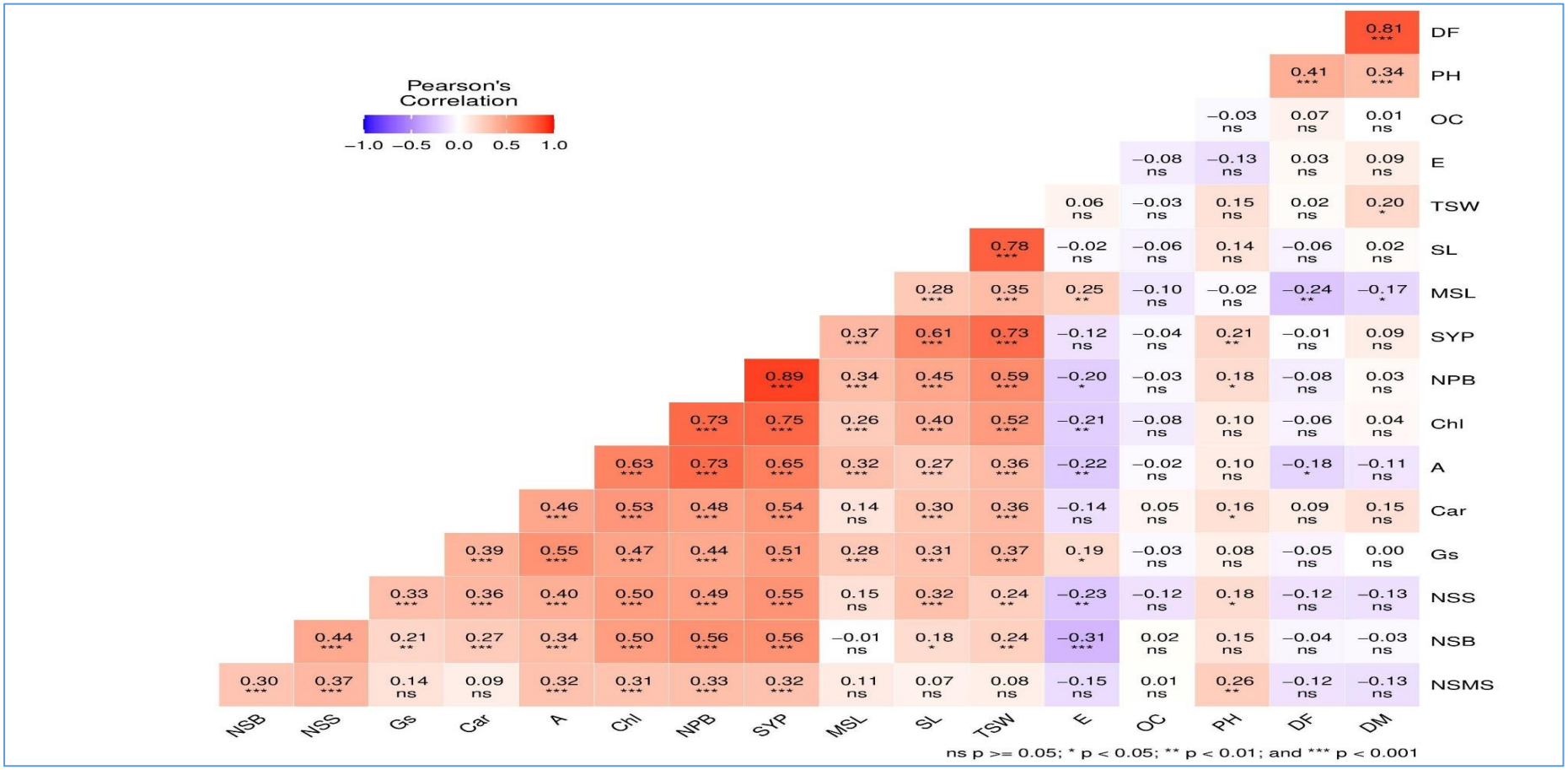


**Figure 4.18: Correlation plot diagram for different morpho-physiological traits in Indian mustard**

DF-Days to 50% flowering; DM-Days to maturity; PH-Plant height (cm); NPB-Number of primary branches/plant; NSB-Number of secondary branches/plant; MSL-Main shoot length (cm), NSMS-Number of siliquae on main shoot; SL-Siliqua length (cm); NSS-Number of seeds/siliqua; OC-Oil content (%); TSW-1000-seed weight (g); SYP-Seed yield/plant (g); A–Photosynthetic rate (µmol $CO_2/m^2/s$); Gs – Stomatal conductance (mmol/$m^2$/s); E – Transpiration rate (mmol $H_2O/m^2/s$); Chl – Total chlorophyll content (mg/g); Caro – Carotenoid content (mg/g).

**Table 4.5 Path coefficient based on correlation analysis for physiological and yield component traits indicating direct effect (diagonal and bold) and indirect effect (above and below diagonal) on seed yield in Indian mustard.**

| Trait | DF | DM | PH | NPB | NSB | MSL | NSMS | SL | NSS | OC | TSW | A | Gs | E | Chl | Caro |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **DF** | **0.078** | 0.063 | 0.032 | -0.006 | -0.003 | -0.019 | -0.010 | -0.005 | -0.010 | 0.006 | 0.002 | -0.014 | -0.004 | 0.003 | -0.004 | 0.007 |
| **DM** | -0.005 | **-0.006** | -0.002 | 0.000 | 0.000 | 0.001 | 0.001 | 0.000 | 0.001 | 0.000 | -0.001 | 0.001 | 0.000 | -0.001 | 0.000 | -0.001 |
| **PH** | -0.007 | -0.006 | **-0.018** | -0.003 | -0.003 | 0.000 | -0.005 | -0.002 | -0.003 | 0.001 | -0.003 | -0.002 | -0.001 | 0.002 | -0.002 | -0.003 |
| **NPB** | -0.041 | 0.014 | 0.092 | **0.497** | 0.278 | 0.169 | 0.164 | 0.224 | 0.242 | -0.016 | 0.291 | 0.362 | 0.221 | -0.101 | 0.364 | 0.241 |
| **NSB** | -0.005 | -0.003 | 0.017 | 0.063 | **0.113** | -0.001 | 0.034 | 0.021 | 0.050 | 0.002 | 0.028 | 0.039 | 0.024 | -0.035 | 0.057 | 0.030 |
| **MSL** | -0.013 | -0.009 | -0.001 | 0.018 | 0.000 | **0.052** | 0.006 | 0.014 | 0.008 | -0.005 | 0.018 | 0.017 | 0.014 | 0.013 | 0.014 | 0.007 |
| **NSMS** | -0.004 | -0.004 | 0.009 | 0.011 | 0.010 | 0.004 | **0.033** | 0.002 | 0.012 | 0.000 | 0.003 | 0.011 | 0.005 | -0.005 | 0.010 | 0.003 |
| **SL** | -0.006 | 0.002 | 0.014 | 0.046 | 0.019 | 0.028 | 0.007 | **0.102** | 0.033 | -0.006 | 0.079 | 0.027 | 0.032 | -0.002 | 0.040 | 0.030 |
| **NSS** | -0.014 | -0.014 | 0.020 | 0.053 | 0.047 | 0.017 | 0.040 | 0.035 | **0.108** | -0.013 | 0.026 | 0.044 | 0.036 | -0.025 | 0.054 | 0.039 |
| **OC** | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | -0.001 | 0.000 | -0.001 | -0.001 | **0.009** | 0.000 | 0.000 | 0.000 | -0.001 | -0.001 | 0.000 |
| **TSW** | 0.005 | 0.044 | 0.033 | 0.128 | 0.053 | 0.077 | 0.018 | 0.170 | 0.053 | -0.007 | **0.218** | 0.078 | 0.081 | 0.013 | 0.113 | 0.078 |
| **A** | -0.002 | -0.001 | 0.001 | 0.006 | 0.003 | 0.003 | 0.003 | 0.002 | 0.004 | 0.000 | 0.003 | **0.009** | 0.005 | -0.002 | 0.005 | 0.004 |
| **Gs** | -0.002 | 0.000 | 0.003 | 0.019 | 0.009 | 0.012 | 0.006 | 0.013 | 0.014 | -0.001 | 0.016 | 0.023 | **0.042** | 0.008 | 0.020 | 0.016 |
| **E** | 0.001 | 0.002 | -0.004 | -0.006 | -0.009 | 0.007 | -0.004 | -0.001 | -0.006 | -0.002 | 0.002 | -0.006 | 0.005 | **0.027** | -0.006 | -0.004 |
| **Chl** | -0.003 | 0.002 | 0.005 | 0.038 | 0.026 | 0.014 | 0.016 | 0.021 | 0.026 | -0.004 | 0.027 | 0.033 | 0.024 | -0.011 | **0.052** | 0.028 |
| **Caro** | 0.005 | 0.009 | 0.009 | 0.029 | 0.016 | 0.008 | 0.005 | 0.018 | 0.021 | 0.003 | 0.021 | 0.027 | 0.023 | -0.008 | 0.031 | **0.059** |

**Residual: 0.097**

DF-Days to 50% flowering; DM-Days to maturity; PH-Plant height (cm); NPB-Number of primary branches/plant; NSB-Number of secondary branches/plant; MSL-Main shoot length (cm), NSMS-Number of siliquae on main shoot; SL-Siliqua length (cm); NSS-Number of seeds/siliqua; OC-Oil content (%); TSW-1000-seed weight (g); A– Photosynthetic rate (µmol $CO^2/m^2/s$); Gs – Stomatal conductance (mmol/$m^2$/s); E – Transpiration rate (mmol $H_2O/m^2/s$); Chl – Total chlorophyll content (mg/g); Caro – Carotenoid content (mg/g).

The photosynthetic rate had significant and positive association with number of primary branches/plant (0.728), number of secondary branches/plant (0.341), main shoot length (0.324), number of siliquae on main shoot (0.319), siliqua length (0.270), number of seeds/siliqua (0.405), 1000-seed weight (0.356), seed yield/plant (0.647), stomatal conductance (0.554), total chlorophyll content (0.625) and carotenoid content (0.456). The total chlorophyll content was found significant positively correlated with number of primary branches/plant (0.732), number of secondary branches/plant (0.502), main shoot length (0.265), number of siliquae on main shoot (0.309), siliqua length (0.397), number of seeds/siliqua (0.502), 1000-seed weight (0.516), seed yield/plant (0.747), photosynthetic rate (0.625), stomatal conductance (0.469) and carotenoid content (0.529).

### 4.4.2 Path coefficient analysis:

#### 4.4.2.1 Direct effects:

The maximum direct effect was contributed by the number of primary branches/plant (0.497) followed by 1000-seed weight (0.218), number of secondary branches/plant (0.113), number of seeds/siliqua (0.108), siliqua length (0.102), days to 50% flowering (0.078), carotenoid content (0.059), total chlorophyll content (0.052), main shoot length (0.052), stomatal conductance (0.042), number of siliquae on main shoot (0.033), oil content (0.009) and photosynthetic rate (0.009). Days to maturity (-0.006) and plant height (-0.018) had a negative direct effect on seed yield/plant. The most crucial traits for selection to increase seed yield are those that have a direct positive effects on seed yield/plant.

#### 4.4.2.2 Indirect effects:

The number of primary branches/plant has a positive indirect effect on seed yield through the number of secondary branches/plant, main shoot length, number of siliqua on main shoot, siliqua length, number of seeds/siliqua, thousand seed weight, photosynthetic rate, stomatal conductance, total chlorophyll content and carotenoid content while, number of secondary branches/plant has a positive indirect influence on seed yield/plant via the number of primary branches/plant, number of siliquae on main shoot, siliqua length, number of seeds/siliqua, 1000-seed weight, photosynthetic rate, stomatal conductance, total chlorophyll content and carotenoid content. The photosynthetic rate and total chlorophyll content has a positive indirect effect on seed yield/plant via days to maturity, number of primary branches/plant, number of secondary branches/plant, main shoot length, number of siliquae on main shoot, siliqua length, number of seeds/siliqua, 1000-seed weight, stomatal conductance, total chlorophyll content and carotenoid content.

## 4.5 Selection of terminal heat tolerant genotypes based on heat susceptibility index and seed yield reduction per cent:

Heat susceptibility index (HSI) for seed yield/plant (g) and per cent seed yield reduction

(YD %) for the genotypes was calculated using the formula suggested by Fischer and Maurer (1978) and Sandhu *et al*. (2019), respectively. Based on the HSI and YD %, top ten genotypes *viz*; RH 2041, RH 2020, RH 2050, BPR 349-9, RH 2049, PM 26, RH 2034, RH 1400-2, RH 2015 and RH 1935 were identified as superlative in respect of terminal heat tolerance (Table 4.6).

**Table 4.6 Heat Susceptibility Index (HSI) and per cent yield reduction (YD %) of 10 top tolerant genotypes and five top susceptible genotypes to terminal heat stress under field conditions**

| Class | Sr. No. | Genotype | 2020-21 | | 2021-22 | |
|---|---|---|---|---|---|---|
| | | | HSI | YD% | HSI | YD% |
| Heat Tolerant | 1. | RH 2041 | 0.18 | 4.12 | 0.41 | 9.18 |
| | 2. | RH 2020 | 0.33 | 7.56 | 0.21 | 4.73 |
| | 3. | RH 2050 | 0.19 | 4.35 | 0.29 | 6.38 |
| | 4. | BPR 349-9 | 0.45 | 10.39 | 0.31 | 6.81 |
| | 5. | RH 2049 | 0.35 | 8.05 | 0.36 | 8.04 |
| | 6. | PM 26 | 0.35 | 7.93 | 0.40 | 8.85 |
| | 7. | RH 2034 | 0.53 | 12.07 | 0.41 | 9.13 |
| | 8. | RH 1400-2 | 0.53 | 12.26 | 0.43 | 9.52 |
| | 9. | RH 2015 | 0.44 | 10.09 | 0.58 | 12.85 |
| | 10. | RH 1935 | 0.18 | 4.18 | 0.76 | 16.81 |
| Heat Susceptible | 1. | Radhika | 1.77 | 40.66 | 1.97 | 43.88 |
| | 2. | Varuna | 1.62 | 37.10 | 2.05 | 45.60 |
| | 3. | RGN 145 | 1.71 | 39.13 | 1.78 | 39.66 |
| | 4. | Pant Rai 18 | 1.73 | 39.77 | 1.27 | 28.25 |
| | 5. | RLM 619 | 1.41 | 32.24 | 1.90 | 42.38 |
| Checks (Heat Tolerant) | | RH 1566 | 0.46 | 10.56 | 0.42 | 9.45 |
| | | RH 1499-30 | 0.44 | 10.12 | 0.50 | 11.13 |
| Checks (Heat Susceptible) | | RH 749 | 1.03 | 23.69 | 1.28 | 28.55 |
| | | RH 8812 | 0.99 | 22.60 | 1.13 | 25.23 |

These genotypes have an average less than 0.5 HSI value and 10 % seed yield reduction in *Rabi* 2020-21 and *Rabi* 2021-22. The heat susceptible genotypes recorded the high HSI value (>1) and high per cent yield reduction (~ 40 %). Based on these criteria, top five genotypes *viz*; Radhika, Varuna, RGN 145, Pant Rai 18 and RLM 619 identified as terminal heat susceptible. The mean HSI value was 1.04 and 1.02 during *Rabi* 2020-21 and 2021-22, respectively. The HSI value was ranged from 0.18 (RH 2041, RH 1935) to 1.77 (Radhika) during *Rabi* 2020-21 and 0.21 (RH 2020) to 2.18 (Pusa Vijay) during *Rabi* 2021-22. The per cent seed yield reduction was ranged from 4.12 % (RH 2041) to 40.66 % (Radhika) during *Rabi* 2020-21 and 4.73 % (RH 2020) to 48.57 % (Pusa Vijay) during *Rabi* 2021-22 (Table 4.7).

**Table 4.7: Heat Susceptibility Index (HSI) and per cent seed yield reduction (%) of different genotypes for seed yield/plant**

| Sr. No. | Genotype | 2020-21 | | 2021-22 | |
|---|---|---|---|---|---|
| | | HSI | YD% | HSI | YD% |
| 1 | RH 30 | 1.12 | 25.77 | 1.00 | 22.22 |
| 2 | RH 0119 | 1.07 | 24.54 | 1.19 | 26.37 |
| 3 | RH 406 | 0.54 | 12.30 | 0.61 | 13.52 |

| | | | | | |
|---|---|---|---|---|---|
| 4 | RH 725 | 0.37 | 8.47 | 0.71 | 15.90 |
| 5 | PBR 91 | 0.99 | 22.73 | 0.56 | 12.45 |
| 6 | RH 761 | 0.66 | 15.12 | 0.85 | 18.83 |
| 7 | RH 8113 | 0.45 | 10.34 | 1.12 | 25.00 |
| 8 | PBR 97 | 1.66 | 38.18 | 1.30 | 28.86 |
| 9 | RH 9304 | 0.92 | 21.21 | 0.81 | 18.10 |
| 10 | RH 9801 | 0.89 | 20.44 | 0.91 | 20.15 |
| 11 | RB 50 | 0.65 | 14.88 | 0.91 | 20.29 |
| 12 | RL 1359 | 1.38 | 31.58 | 1.47 | 32.67 |
| 13 | PM 21 | 0.79 | 18.18 | 0.78 | 17.32 |
| 14 | PM 22 | 0.48 | 11.11 | 0.90 | 20.00 |
| 15 | PM 24 | 0.90 | 20.55 | 0.77 | 17.19 |
| 16 | PM 25 | 1.06 | 24.33 | 1.24 | 27.60 |
| 17 | PM 26 | 0.35 | 7.93 | 0.40 | 8.85 |
| 18 | PM 28 | 1.09 | 25.01 | 0.84 | 18.68 |
| 19 | PDZM 31 | 1.18 | 26.98 | 1.29 | 28.67 |
| 20 | Pusa Vijay | 1.34 | 30.71 | 2.18 | 48.57 |
| 21 | Pusa Jai Kisan | 1.26 | 28.84 | 1.72 | 38.28 |
| 22 | Pusa Karishma | 1.30 | 29.79 | 1.36 | 30.26 |
| 23 | Pusa Bold | 1.63 | 37.34 | 0.82 | 18.21 |
| 24 | Pusa Jagannath | 0.99 | 22.75 | 0.97 | 21.48 |
| 25 | BPR 349-9 | 0.45 | 10.39 | 0.31 | 6.81 |
| 26 | BPR 540-6 | 0.97 | 22.24 | 0.47 | 10.35 |
| 27 | BPR 543-2 | 0.64 | 14.64 | 0.77 | 17.10 |
| 28 | BPR 549-9 | 1.07 | 24.64 | 1.01 | 22.44 |
| 29 | DRMR 150-35 | 1.10 | 25.16 | 1.12 | 25.01 |
| 30 | DRMR 1165-40 | 1.08 | 24.74 | 1.41 | 31.48 |
| 31 | NRCDR 2 | 1.02 | 23.46 | 0.56 | 12.50 |
| 32 | NRCDR 601 | 1.10 | 25.30 | 0.85 | 18.88 |
| 33 | RGN 48 | 1.27 | 29.02 | 1.22 | 27.04 |
| 34 | RGN 145 | 1.71 | 39.13 | 1.78 | 39.66 |
| 35 | RGN 229 | 0.78 | 17.87 | 1.36 | 30.30 |
| 36 | RGN 236 | 1.65 | 37.78 | 1.85 | 41.17 |
| 37 | RGN 298 | 1.32 | 30.28 | 1.04 | 23.09 |
| 38 | CS 56 | 0.70 | 16.13 | 1.25 | 27.91 |
| 39 | Radhika | 1.77 | 40.66 | 1.97 | 43.88 |
| 40 | Shivani | 1.08 | 24.78 | 0.75 | 16.67 |
| 41 | Aravali | 1.16 | 26.55 | 1.59 | 35.42 |
| 42 | Varuna | 1.62 | 37.10 | 2.05 | 45.60 |
| 43 | Pant Rai 18 | 1.73 | 39.77 | 1.27 | 28.25 |
| 44 | Pant Rai 19 | 1.66 | 38.17 | 1.39 | 31.00 |
| 45 | Pant Rai 20 | 1.14 | 26.14 | 0.91 | 20.15 |
| 46 | RH 781 | 0.83 | 19.08 | 0.96 | 21.27 |
| 47 | Giriraj | 1.42 | 32.45 | 1.62 | 36.06 |
| 48 | RH 819 | 1.27 | 29.17 | 1.07 | 23.73 |
| 49 | RVM 2 | 1.57 | 35.90 | 1.54 | 34.36 |
| 50 | CS 52 | 1.73 | 39.76 | 1.48 | 32.91 |
| 51 | IC 122287 | 1.10 | 25.16 | 1.04 | 23.03 |
| 52 | IC 333591 | 1.45 | 33.33 | 1.08 | 24.04 |
| 53 | IC 470135 | 1.28 | 29.41 | 1.25 | 27.78 |
| 54 | IC 491390 | 1.59 | 36.50 | 1.15 | 25.49 |
| 55 | RH 847 | 1.22 | 28.01 | 1.28 | 28.58 |
| 56 | RH 1400-2 | 0.53 | 12.26 | 0.43 | 9.52 |

| | | | | | |
|---|---|---|---|---|---|
| 57 | RC 2 | 1.56 | 35.71 | 1.40 | 31.25 |
| 58 | RC 5 | 1.19 | 27.19 | 1.43 | 31.79 |
| 59 | RC 12 | 1.22 | 27.93 | 1.46 | 32.40 |
| 60 | RC 48 | 1.72 | 39.55 | 1.18 | 26.24 |
| 61 | RC 81 | 1.64 | 37.53 | 0.76 | 16.90 |
| 62 | RC 91 | 0.77 | 17.65 | 1.22 | 27.08 |
| 63 | RC 104 | 1.65 | 37.80 | 1.22 | 27.10 |
| 64 | RC 106 | 1.50 | 34.44 | 1.57 | 34.96 |
| 65 | RC 108 | 1.28 | 29.44 | 0.68 | 15.22 |
| 66 | RC 118 | 1.38 | 31.55 | 1.20 | 26.72 |
| 67 | RC 134 | 1.50 | 34.31 | 1.93 | 42.86 |
| 68 | RC 162 | 1.54 | 35.38 | 1.70 | 37.79 |
| 69 | RC 280 | 1.65 | 37.76 | 1.38 | 30.78 |
| 70 | RC 330 | 1.61 | 36.84 | 1.45 | 32.21 |
| 71 | RC 448 | 1.00 | 23.00 | 1.52 | 33.81 |
| 72 | RC 449 | 0.98 | 22.53 | 0.84 | 18.75 |
| 73 | RC 587 | 0.76 | 17.35 | 0.58 | 12.90 |
| 74 | RC 713 | 1.26 | 28.95 | 0.85 | 18.88 |
| 75 | RC 734 | 0.90 | 20.63 | 1.55 | 34.51 |
| 76 | RC 806 | 1.11 | 25.54 | 1.00 | 22.22 |
| 77 | RC 840 | 1.47 | 33.77 | 0.40 | 8.97 |
| 78 | RC 904 | 1.19 | 27.20 | 0.93 | 20.74 |
| 79 | RLC 1 | 1.73 | 39.65 | 0.84 | 18.64 |
| 80 | RLC 2 | 1.27 | 29.23 | 0.79 | 17.65 |
| 81 | RLC 3 | 1.33 | 30.43 | 1.10 | 24.51 |
| 82 | RLM 619 | 1.41 | 32.24 | 1.90 | 42.38 |
| 83 | PBR 357 | 1.09 | 25.02 | 2.14 | 47.61 |
| 84 | RH 1916 | 1.71 | 39.32 | 0.77 | 17.14 |
| 85 | RH 1917 | 0.71 | 16.20 | 0.78 | 17.27 |
| 86 | RH 1918 | 1.25 | 28.70 | 0.91 | 20.33 |
| 87 | RH 1919 | 1.74 | 40.00 | 0.90 | 20.13 |
| 88 | RH 1922 | 1.71 | 39.25 | 1.21 | 26.95 |
| 89 | RH 1923 | 1.29 | 29.63 | 0.75 | 16.67 |
| 90 | RH 1924 | 1.30 | 29.86 | 0.68 | 15.07 |
| 91 | RH 1927 | 1.60 | 36.69 | 0.90 | 20.12 |
| 92 | RH 1928 | 0.80 | 18.27 | 1.21 | 26.98 |
| 93 | RH 1929 | 0.89 | 20.50 | 0.31 | 6.91 |
| 94 | RH 1930 | 0.64 | 14.76 | 1.13 | 25.05 |
| 95 | RH 1931 | 1.15 | 26.38 | 1.46 | 32.39 |
| 96 | RH 1932 | 0.96 | 21.94 | 0.85 | 18.94 |
| 97 | RH 1934 | 0.55 | 12.61 | 0.85 | 18.93 |
| 98 | RH 1935 | 0.18 | 4.18 | 0.76 | 16.81 |
| 99 | RH 1936 | 1.14 | 26.06 | 0.46 | 10.22 |
| 100 | RH 1937 | 0.86 | 19.63 | 0.71 | 15.89 |
| 101 | RH 1938 | 0.53 | 12.15 | 0.89 | 19.72 |
| 102 | RH 1939 | 0.89 | 20.51 | 0.79 | 17.48 |
| 103 | RH 1940 | 0.53 | 12.25 | 1.13 | 25.16 |
| 104 | RH 2001 | 0.95 | 21.78 | 0.25 | 5.62 |
| 105 | RH 2002 | 1.01 | 23.09 | 0.99 | 21.94 |
| 106 | RH 2003 | 0.51 | 11.64 | 0.89 | 19.83 |
| 107 | RH 2004 | 0.67 | 15.44 | 0.68 | 15.17 |
| 108 | RH 2005 | 0.32 | 7.24 | 0.74 | 16.48 |
| 109 | RH 2006 | 1.25 | 28.56 | 1.14 | 25.26 |

| | | | | | |
|---|---|---|---|---|---|
| 110 | RH 2007 | 0.81 | 18.55 | 0.34 | 7.58 |
| 111 | RH 2008 | 0.67 | 15.46 | 0.99 | 21.93 |
| 112 | RH 2012 | 0.62 | 14.29 | 0.91 | 20.26 |
| 113 | RH 2013 | 0.98 | 22.45 | 0.55 | 12.17 |
| 114 | RH 2014 | 0.65 | 14.93 | 0.39 | 8.68 |
| 115 | RH 2015 | 0.44 | 10.09 | 0.58 | 12.85 |
| 116 | RH 2016 | 0.68 | 15.53 | 0.92 | 20.42 |
| 117 | RH 2017 | 1.29 | 29.64 | 1.16 | 25.75 |
| 118 | RH 2018 | 0.95 | 21.86 | 0.92 | 20.46 |
| 119 | RH 2019 | 0.61 | 14.07 | 0.76 | 16.88 |
| 120 | RH 2020 | 0.33 | 7.56 | 0.21 | 4.73 |
| 121 | RH 2021 | 0.62 | 14.24 | 1.16 | 25.72 |
| 122 | RH 2022 | 0.95 | 21.79 | 0.91 | 20.25 |
| 123 | RH 2023 | 0.66 | 15.16 | 0.82 | 18.22 |
| 124 | RH 2024 | 1.02 | 23.33 | 0.68 | 15.19 |
| 125 | RH 2025 | 1.31 | 29.97 | 1.12 | 24.83 |
| 126 | RH 2026 | 0.73 | 16.66 | 1.25 | 27.87 |
| 127 | RH 2027 | 0.84 | 19.15 | 1.17 | 26.09 |
| 128 | RH 2028 | 1.05 | 24.06 | 1.74 | 38.62 |
| 129 | RH 2029 | 0.88 | 20.09 | 1.06 | 23.50 |
| 130 | RH 2030 | 0.85 | 19.43 | 0.54 | 11.97 |
| 131 | RH 2031 | 0.80 | 18.29 | 0.73 | 16.18 |
| 132 | RH 2032 | 1.07 | 24.52 | 1.17 | 26.05 |
| 133 | RH 2033 | 0.70 | 16.11 | 0.67 | 14.91 |
| 134 | RH 2034 | 0.53 | 12.07 | 0.41 | 9.13 |
| 135 | RH 2035 | 0.86 | 19.68 | 0.80 | 17.87 |
| 136 | RH 2036 | 1.62 | 37.19 | 1.38 | 30.72 |
| 137 | RH 2038 | 1.48 | 33.89 | 1.33 | 29.70 |
| 138 | RH 2039 | 1.39 | 31.92 | 1.39 | 31.00 |
| 139 | RH 2040 | 0.81 | 18.54 | 0.77 | 17.04 |
| 140 | RH 2041 | 0.18 | 4.12 | 0.41 | 9.18 |
| 141 | RH 2042 | 0.91 | 20.95 | 1.16 | 25.82 |
| 142 | RH 2043 | 1.65 | 37.76 | 0.69 | 15.29 |
| 143 | RH 2044 | 1.17 | 26.88 | 1.18 | 26.24 |
| 144 | RH 2045 | 0.78 | 17.87 | 1.17 | 26.03 |
| 145 | RH 2046 | 0.65 | 14.85 | 1.02 | 22.80 |
| 146 | RH 2047 | 0.83 | 19.10 | 0.96 | 21.31 |
| 147 | RH 2048 | 0.72 | 16.61 | 0.82 | 18.18 |
| 148 | RH 2049 | 0.35 | 8.05 | 0.36 | 8.04 |
| 149 | RH 2050 | 0.19 | 4.35 | 0.29 | 6.38 |
| 150 | RH 2051 | 0.61 | 13.99 | 0.83 | 18.53 |
| 151 | RH 1566 | 0.46 | 10.56 | 0.42 | 9.45 |
| 152 | RH 1499-30 | 0.44 | 10.12 | 0.50 | 11.13 |
| 153 | RH 749 | 1.03 | 23.69 | 1.28 | 28.55 |
| 154 | RH 8812 | 0.99 | 22.60 | 1.13 | 25.23 |
| **Mean** | | **1.04** | **23.84** | **1.02** | **22.64** |
| **Range** | **Min.** | **0.18** | **4.12** | **0.21** | **4.73** |
| | **Max.** | **1.77** | **40.66** | **2.18** | **48.57** |

### 4.6 Cluster analysis

Cluster analysis is a highly promising and efficient technology for categorizing genotypes according to genetic similarities. Plant breeders and geneticists use clustering

analysis to organise the genotype and select diverse parents who would produce prolific offspring. Cluster analysis could be performed on the basis of similarity as well as dissimilarity among the genotypes.

A dendrogram was plotted for the studied Indian mustard genotypes on the basis of related magnitude of various morphological and physiological traits in Euclidian distance form. All the genotypes were grouped into nine clusters. The clusters are separated by different colour combinations in the dendrogram. The magnitude of variation of each morphological and physiological trait has been depicted by variation in strength of colour‘s shade in two-way dendrogram (Figure 4.19). Cluster 3 was the largest with 41 genotypes, followed by cluster 4 with 26 genotypes, cluster 8 with 21 genotypes, cluster 1 with 19 genotypes, cluster 7 with 16 genotypes, cluster 5 with 15 genotypes, cluster 9 with seven genotypes, cluster 2 with five genotypes while cluster 6 was the smallest and included only four genotypes (Table 4.8).

Mean of morphological and physiological traits was also estimated for each cluster (Table 4.9). Cluster 1 grouped the genotypes having average performance for all of the studied traits. Cluster 2 grouped the genotypes having shortest crop duration (lowest days to 50% flowering and days to maturity), lowest plant height, highest main shoot length, lowest number of siliquae on main shoot and highest transpiration rate with a cluster mean value of 54 days, 123 days, 186.32 cm, 82.62 cm, 45.27 and 6.13 mmol $H_2O/m^2/s$, respectively. Cluster 3 included the genotypes with longest crop duration (maximum days to maturity) with cluster mean value of 130 days. Cluster 4 had the genotypes having maximum 1000-seed weight with an average cluster mean value of 5.84 g. Cluster 5 included the genotypes with maximum plant height (204.03 cm) and lowest oil content (34.48 %). Cluster 6 included the genotypes having the highest number of primary branches/plant, number of secondary branches/plant, number of siliquae on main shoot, siliqua length, number of seeds/siliqua, seed yield/plant, photosynthetic rate, stomatal conductance, total chlorophyll content, and carotenoid content with cluster mean value of 6.39, 13.15, 54.35, 5.31 cm, 14.66, 27.98 g, 20.94 µmol $CO_2/m^2/s$, 0.55 mmol/$m^2/s$, 1.31 mg and 2.58 mg, respectively, while minimum cluster mean value was estimated for transpiration rate (3.73 mmol $H_2O/m^2/s$). Cluster 7 was having the genotypes with maximum cluster mean value for number of days to 50% flowering (62 days), while minimum cluster mean value for number of primary branches/plant (3.95), main shoot length (68.04 cm), number of seeds/siliqua (12.50) and total chlorophyll content (0.92 mg). Cluster 8 grouped the genotypes having minimum number of primary branches/plant (7.93), seed yield/plant (14.81 g), photosynthetic rate (10.76 µmol $CO_2/m^2/s$) and carotenoid content (2.01 mg). Cluster 8 included the genotypes having maximum cluster mean value for oil content (38.81 %) while, minimum cluster meanmean value for 1000-seed weight (3.63 g) and stomatal conductance (0.26 mmol/$m^2/s$).

**Table 4.8 Details of genotypes distributed in nine clusters by Ward's method**

| Cluster | Number of genotypes | Genotypes |
|---|---|---|
| **1** | 19 | RH 30, RH 9304, RH 9801, Pusa Jagannath, RH 2036, BPR 349-9, BPR 543-2, Giriraj, RH 2048, NRCDR 2, RH 1936, RH 1939, RH 2014, RH 1937, RH 1938, RH 1940, RH 2002, RH 2015, RH 2001 |
| **2** | 5 | PM 25, PM 28, BPR 540-6, RH 1400-2, Pant Rai 19 |
| **3** | 41 | RH 0119, RH 2034, RH 406, RH 761, RH 2033, RH 2003, RH 2029, RH 2004, RH 2020, RH 2041, RB 50, Varuna, RH 781, RH 2016, RH 2017, RH 2018, RH 2032, RH 1566, RH 1499-30, RH 1930, RH 1932, RH 2006, RH 1935, RH 2025, PM 21, RH 2028, RH 1916, RH 2035, RH 2042, RH 2046, NRCDR 601, RH 2008, RH 1929, RH 1934, RH 2040, RH 2043, RH 2044, RH 1917, RH 2031, RH 2030, RH 2051 |
| **4** | 26 | PM 22, RH 2026, RH 2038, RH 2039, RH 2007, RH 2021, RH 749, RH 8812, RH 2019, RH 2045, RH 2047, RH 2005, Pusa Vijay, RH 1922, RH 1928, RH 2027, BPR 549-9, Radhika, RH 1918, RH 1924, RH 2024, RH 2012, RH 2023, RH 1931, RH 2013, RH 2022 |
| **5** | 15 | PM 24, PBR 357, RH 847, RH 1923, RH 1927, Pusa Jai Kisan, RGN 48, RGN 236, RGN 229, RVM 2, RGN 298, CS 56, CS 52 |
| **6** | 4 | RH 725, PM 26, RH 2049, RH 2050 |
| **7** | 16 | PBR 91, RH 8113, PDZM 31, Shivani, RC 48, IC 122287, RC 5, RLM 619, RC 904, RH 1919, RH 819, RC 134, RLC 1, RLC 2, RC 713, RLC 3 |
| **8** | 21 | PBR 97, RL 1359, Pant Rai 18, IC 491390, RC 2, RC 12, RC 806, RC 840, Pant Rai 20, RC 162, DRMR 1165-40, IC 470135, RGN 145, Aravali, IC 333591, RC 81, RC 104, RC 106, Rc 91, RC 108 |
| **9** | 7 | RC 118, RC 280, RC 330, RC 448, RC 449, RC 734, RC 587 |

**Table 4.9 Cluster mean for various morphological and physiological traits**

| Cluster | DF | DM | PH | NPB | NSB | MSL | NSMS | SL | NSS | OC | TSW | SYP | A | Gs | E | Chl | Caro |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 57.38 | 126.80 | 196.67 | 5.44 | 11.02 | 78.53 | 48.88 | 4.92 | 13.39 | 38.60 | 5.25 | 23.16 | 14.61 | 0.36 | 5.41 | 1.12 | 2.43 |
| 2 | **53.65** | **123.10** | **186.32** | 4.72 | 9.60 | **82.62** | **45.27** | 4.39 | 12.96 | 38.62 | 4.31 | 18.60 | 12.74 | 0.36 | **6.13** | 0.99 | 2.07 |
| 3 | 60.73 | **130.49** | 203.11 | 5.33 | 10.94 | 78.19 | 49.89 | 4.84 | 13.42 | 38.75 | 5.70 | 23.30 | 13.51 | 0.36 | 5.21 | 1.13 | 2.35 |
| 4 | 60.03 | 129.94 | 196.63 | 4.88 | 9.69 | 78.47 | 47.18 | 4.95 | 12.88 | 38.61 | **5.84** | 21.34 | 12.62 | 0.34 | 5.94 | 1.02 | 2.26 |
| 5 | 61.13 | 129.65 | **204.03** | 4.69 | 9.33 | 78.52 | 49.53 | 4.24 | 13.00 | **38.48** | 4.50 | 18.51 | 13.02 | 0.36 | 6.11 | 1.02 | 2.10 |
| 6 | 57.19 | 126.06 | 199.69 | **6.39** | **13.15** | 77.02 | **54.35** | **5.31** | **14.66** | 38.56 | 5.75 | **27.98** | **20.94** | **0.55** | **3.73** | **1.31** | **2.58** |
| 7 | **61.97** | 130.25 | 203.62 | **3.95** | 8.92 | **68.04** | 47.65 | 4.05 | **12.50** | 38.80 | 4.03 | 15.33 | 11.32 | 0.31 | 5.36 | **0.92** | 2.21 |
| 8 | 59.08 | 128.31 | 192.71 | 4.06 | **7.93** | 73.91 | 46.20 | 4.19 | 12.74 | 38.58 | 4.04 | **14.81** | **10.76** | 0.28 | 5.56 | 0.94 | **2.01** |
| 9 | 57.21 | 126.50 | 198.38 | 4.25 | 12.99 | 71.85 | 52.40 | **3.93** | 13.22 | **38.81** | **3.63** | 16.35 | 10.77 | **0.26** | 4.60 | 1.01 | 2.02 |

DF-Days to 50% flowering; DM-Days to maturity; PH-Plant height (cm); NPB-Number of primary branches/plant; NSB-Number of secondary branches/plant; MSL-Main shoot length (cm), NSMS-Number of siliquae on main shoot; SL-Siliqua length (cm); NSS-Number of seeds/siliqua; OC-Oil content (%); TSW-1000-seed weight (g); A– Photosynthetic rate (µmol $CO^2/m^2/s$); Gs – Stomatal conductance (mmol/$m^2$/s); E – Transpiration rate (mmol $H_2O/m^2/s$); Chl – Total chlorophyll content (mg/g); Caro – Carotenoid content (mg/g).

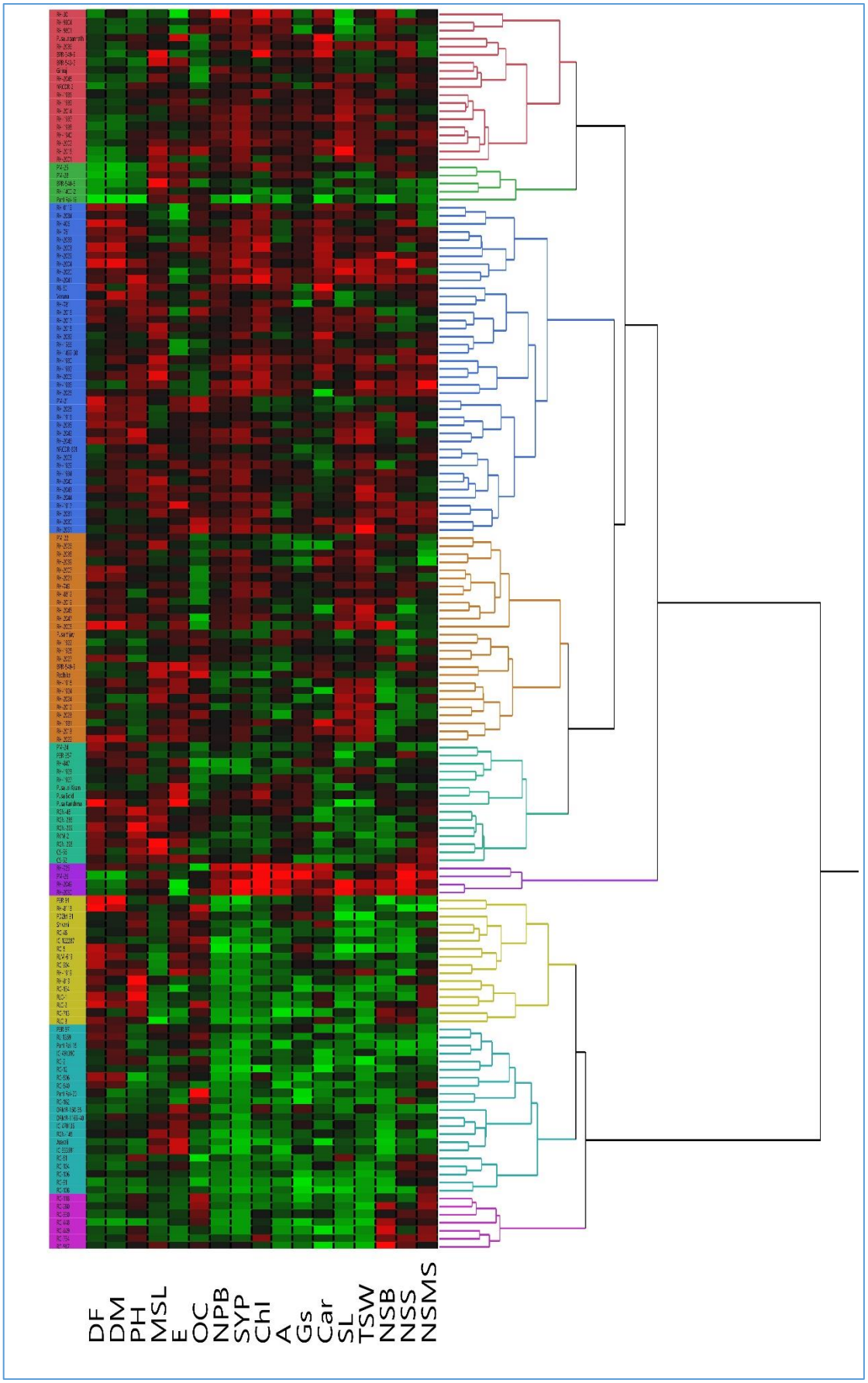


**Figure 4.19 Two-way dendrogram showing clustering pattern of 154 Indian mustard genotypes by WARD's method for morpho-physiological traits**

### 4.7 Molecular characterization:

To examine the molecular diversity of the genotypes, DNA was isolated from fresh leaves of the seedlings by the modified CTAB method. A total of 237 SSR markers distributed all over the genome were screened to find the genetic polymorphism in 154 genotypes of Indian mustard (**APPENDIX-I**). Out of the 237 SSR markers, 111 SSR markers were found polymorphic which were used in the final analyses (Table 4.10). These 111 primer pairs generated 312 polymorphic alleles with an average of 2.81 alleles per SSR marker and were further used to assess the genetic diversity in the present genotypes.

**Table 4.10 Major allele frequency, number of alleles, heterozygosity, gene diversity and PIC value of 111 polymorphic SSRs**

| Sr. No. | Marker | Major allele frequency | Number of alleles | Heterozygosity | Gene diversity | PIC |
|---|---|---|---|---|---|---|
| 1 | BG1 | 0.964 | 2 | 0.069 | 0.071 | 0.067 |
| 2 | BG4 | 0.497 | 3 | 0.513 | 0.981 | 0.394 |
| 3 | BG6 | 0.386 | 3 | 0.650 | 0.773 | 0.574 |
| 4 | BG7 | 0.633 | 3 | 0.474 | 0.617 | 0.373 |
| 5 | BG8 | 0.981 | 3 | 0.038 | 0.000 | 0.038 |
| 6 | BG9 | 0.987 | 2 | 0.026 | 0.026 | 0.025 |
| 7 | BG15 | 0.649 | 3 | 0.460 | 0.688 | 0.360 |
| 8 | BG17 | 0.714 | 3 | 0.415 | 0.545 | 0.339 |
| 9 | BG19 | 0.552 | 4 | 0.603 | 0.357 | 0.544 |
| 10 | BG31 | 0.964 | 3 | 0.069 | 0.006 | 0.067 |
| 11 | BG32 | 0.494 | 3 | 0.522 | 0.019 | 0.407 |
| 12 | BG35 | 0.727 | 3 | 0.420 | 0.442 | 0.365 |
| 13 | BG37 | 0.942 | 3 | 0.112 | 0.065 | 0.109 |
| 14 | BG44 | 0.971 | 3 | 0.057 | 0.006 | 0.056 |
| 15 | BG45 | 0.974 | 2 | 0.051 | 0.000 | 0.049 |
| 16 | BG48 | 0.948 | 2 | 0.098 | 0.000 | 0.094 |
| 17 | BG53 | 0.653 | 3 | 0.464 | 0.032 | 0.370 |
| 18 | BG65 | 0.974 | 2 | 0.051 | 0.000 | 0.049 |
| 19 | BG77 | 0.468 | 3 | 0.589 | 0.032 | 0.501 |
| 20 | BG79 | 0.958 | 2 | 0.081 | 0.084 | 0.078 |
| 21 | BG82 | 0.740 | 3 | 0.416 | 0.006 | 0.375 |
| 22 | BG83 | 0.958 | 3 | 0.081 | 0.071 | 0.079 |
| 23 | BG91 | 0.945 | 3 | 0.105 | 0.097 | 0.101 |
| 24 | BG101 | 0.552 | 6 | 0.582 | 0.688 | 0.511 |
| 25 | BG102 | 0.565 | 3 | 0.497 | 0.857 | 0.380 |
| 26 | BG105 | 0.373 | 5 | 0.688 | 0.214 | 0.627 |
| 27 | BG107 | 0.513 | 2 | 0.500 | 0.974 | 0.375 |
| 28 | BG109 | 0.393 | 4 | 0.679 | 0.565 | 0.614 |
| 29 | BG111 | 0.549 | 6 | 0.555 | 0.805 | 0.468 |
| 30 | BG115 | 0.565 | 3 | 0.497 | 0.857 | 0.380 |
| 31 | BG121 | 0.903 | 3 | 0.177 | 0.182 | 0.163 |
| 32 | BG123 | 0.627 | 3 | 0.482 | 0.695 | 0.383 |
| 33 | BG124 | 0.562 | 3 | 0.498 | 0.838 | 0.380 |
| 34 | BG125 | 0.458 | 3 | 0.643 | 0.461 | 0.571 |
| 35 | BG126 | 0.656 | 3 | 0.503 | 0.065 | 0.444 |
| 36 | BG127 | 0.672 | 3 | 0.456 | 0.604 | 0.373 |
| 37 | BG129 | 0.776 | 3 | 0.351 | 0.331 | 0.293 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 38 | BG130 | 0.692 | 3 | 0.443 | 0.058 | 0.367 |
| 39 | BG131 | 0.890 | 2 | 0.196 | 0.221 | 0.177 |
| 40 | BG133 | 0.487 | 3 | 0.525 | 0.974 | 0.412 |
| 41 | BG137 | 0.916 | 3 | 0.156 | 0.117 | 0.145 |
| 42 | BG138 | 0.812 | 2 | 0.306 | 0.338 | 0.259 |
| 43 | BG142 | 0.471 | 4 | 0.558 | 0.942 | 0.459 |
| 44 | BG144 | 0.503 | 3 | 0.519 | 0.955 | 0.403 |
| 45 | BG149 | 0.513 | 3 | 0.617 | 0.442 | 0.547 |
| 46 | BG150 | 0.994 | 2 | 0.013 | 0.013 | 0.013 |
| 47 | BG153 | 0.464 | 3 | 0.564 | 0.929 | 0.466 |
| 48 | BG156 | 0.571 | 2 | 0.490 | 0.857 | 0.370 |
| 49 | BG157 | 0.825 | 2 | 0.289 | 0.221 | 0.247 |
| 50 | BG158 | 0.808 | 2 | 0.310 | 0.305 | 0.262 |
| 51 | BG161 | 0.916 | 3 | 0.158 | 0.071 | 0.152 |
| 52 | BG165 | 0.893 | 3 | 0.197 | 0.006 | 0.188 |
| 53 | BG167 | 0.373 | 3 | 0.658 | 0.487 | 0.583 |
| 54 | BG171 | 0.571 | 2 | 0.490 | 0.000 | 0.370 |
| 55 | BG172 | 0.968 | 2 | 0.063 | 0.065 | 0.061 |
| 56 | BG175 | 0.523 | 4 | 0.630 | 0.812 | 0.574 |
| 57 | BG180 | 0.506 | 4 | 0.527 | 0.974 | 0.416 |
| 58 | BG183 | 0.951 | 2 | 0.093 | 0.097 | 0.088 |
| 59 | BG186 | 0.682 | 2 | 0.434 | 0.000 | 0.340 |
| 60 | BG190 | 0.987 | 2 | 0.026 | 0.026 | 0.025 |
| 61 | BG193 | 0.481 | 3 | 0.537 | 0.948 | 0.429 |
| 62 | BG194 | 0.916 | 3 | 0.156 | 0.000 | 0.148 |
| 63 | BG195 | 0.987 | 2 | 0.026 | 0.000 | 0.025 |
| 64 | BG196 | 0.344 | 3 | 0.666 | 0.636 | 0.592 |
| 65 | BG198 | 0.883 | 3 | 0.211 | 0.006 | 0.196 |
| 66 | BG200 | 0.442 | 3 | 0.596 | 0.883 | 0.510 |
| 67 | BG201 | 0.682 | 3 | 0.484 | 0.221 | 0.435 |
| 68 | BG204 | 0.448 | 3 | 0.647 | 0.545 | 0.574 |
| 69 | BG206 | 0.714 | 3 | 0.442 | 0.117 | 0.393 |
| 70 | BG207 | 0.503 | 3 | 0.551 | 0.877 | 0.451 |
| 71 | BG208 | 0.961 | 2 | 0.075 | 0.078 | 0.072 |
| 72 | BG209 | 0.481 | 3 | 0.537 | 0.961 | 0.429 |
| 73 | BG210 | 0.497 | 3 | 0.506 | 0.994 | 0.385 |
| 74 | BG212 | 0.763 | 3 | 0.365 | 0.370 | 0.303 |
| 75 | BG214 | 0.506 | 3 | 0.512 | 0.870 | 0.394 |
| 76 | BG220 | 0.766 | 2 | 0.358 | 0.000 | 0.294 |
| 77 | BG222 | 0.503 | 3 | 0.513 | 0.955 | 0.394 |
| 78 | BG228 | 0.799 | 4 | 0.345 | 0.331 | 0.322 |
| 79 | BG231 | 0.477 | 3 | 0.542 | 0.929 | 0.437 |
| 80 | BG233 | 0.750 | 3 | 0.387 | 0.448 | 0.329 |
| 81 | BG235 | 0.795 | 3 | 0.328 | 0.396 | 0.278 |
| 82 | BG237 | 0.961 | 2 | 0.075 | 0.078 | 0.072 |
| 83 | BG238 | 0.597 | 4 | 0.550 | 0.494 | 0.481 |
| 84 | BG240 | 0.503 | 2 | 0.500 | 0.994 | 0.375 |
| 85 | BG241 | 0.734 | 2 | 0.391 | 0.532 | 0.314 |
| 86 | BG244 | 0.688 | 3 | 0.459 | 0.104 | 0.394 |
| 87 | BG246 | 0.523 | 3 | 0.553 | 0.812 | 0.456 |
| 88 | BG247 | 0.987 | 2 | 0.026 | 0.000 | 0.025 |
| 89 | BG248 | 0.669 | 2 | 0.443 | 0.000 | 0.345 |
| 90 | BG249 | 0.526 | 2 | 0.499 | 0.000 | 0.374 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 91 | BG250 | 0.529 | 2 | 0.498 | 0.942 | 0.374 |
| 92 | BG251 | 0.646 | 3 | 0.462 | 0.695 | 0.361 |
| 93 | BG252 | 0.471 | 3 | 0.615 | 0.721 | 0.535 |
| 94 | BG254 | 0.987 | 2 | 0.026 | 0.000 | 0.025 |
| 95 | BG256 | 0.513 | 2 | 0.500 | 0.974 | 0.375 |
| 96 | BG257 | 0.545 | 3 | 0.502 | 0.896 | 0.382 |
| 97 | BG258 | 0.994 | 2 | 0.013 | 0.000 | 0.013 |
| 98 | BG259 | 0.503 | 2 | 0.500 | 0.994 | 0.375 |
| 99 | BG260 | 0.844 | 2 | 0.263 | 0.312 | 0.228 |
| 100 | BG261 | 0.523 | 3 | 0.505 | 0.942 | 0.384 |
| 101 | BG263 | 0.529 | 3 | 0.575 | 0.734 | 0.490 |
| 102 | BG264 | 0.643 | 4 | 0.468 | 0.688 | 0.370 |
| 103 | BG266 | 0.951 | 3 | 0.094 | 0.006 | 0.091 |
| 104 | BG269 | 0.792 | 2 | 0.329 | 0.416 | 0.275 |
| 105 | BG270 | 0.942 | 2 | 0.110 | 0.000 | 0.104 |
| 106 | BG271 | 0.571 | 3 | 0.582 | 0.429 | 0.517 |
| 107 | BG272 | 0.494 | 3 | 0.513 | 0.987 | 0.394 |
| 108 | BG276 | 0.503 | 2 | 0.500 | 0.994 | 0.375 |
| 109 | BG277 | 0.773 | 4 | 0.360 | 0.039 | 0.308 |
| 110 | BG278 | 0.649 | 2 | 0.455 | 0.701 | 0.352 |
| 111 | BG280 | 0.532 | 2 | 0.498 | 0.935 | 0.374 |
| **Mean** | | **0.688** | **2.81** | **0.382** | **0.432** | **0.317** |
| **Range** | | **0.344 - 0.994** | **2 - 6** | **0.013 - 0.688** | **0 - 0.994** | **0.013 - 0.627** |

The average major allele frequency for SSR markers was 0.688, with a range of 0.344 (BG196) to 0.994 (BG150/BG258). The average gene diversity was 0.432 with a range of 0 to 0.994. The PIC (Polymorphic Information Content) value for SSR markers ranged from 0.013 (BG 150/BG258) to 0.627 (BG 105) with an average PIC value of 0.317. The SSR markers used in the present study were distributed throughout the 18 chromosomes (10 chromosome – A genome, 8 chromosome – B genome) of amphidiploid Indian mustard (Figure 4.20). The chromosome-wise average of PIC values revealed that chromosome 10 has the highest mean value of PIC (0.546) followed by chromosome 14 (0.506) and chromosome 4 (0.436). The lowest PIC value was recorded in chromosome 6 followed by 7 and 16. Higher PIC value of SSR showed higher information efficiency of markers. Distribution of alleles among SSR in the current study revealed that more than 90% of polymorphic markers showed 2 or 3 number of alleles and the rest had 4, 5 or 6 alleles per marker (Table 4.11).

**Table 4.11 Distribution of alleles amplified by 111 SSR markers**

| Sr. No. | Number of SSR markers | Number of alleles | Total alleles |
|---|---|---|---|
| 1. | 38 | 2 | 76 |
| 2. | 61 | 3 | 183 |
| 3. | 9 | 4 | 36 |
| 4. | 1 | 5 | 5 |
| 5. | 2 | 6 | 12 |
| **Total** | **111** | **-** | **312** |

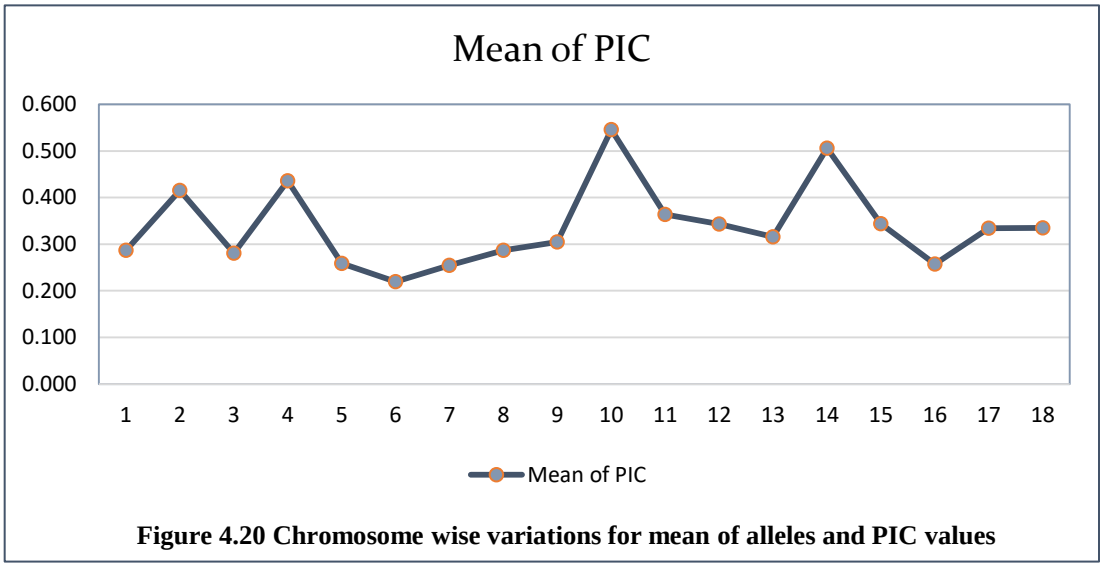


**Figure 4.20 Chromosome wise variations for mean of alleles and PIC values**

Based on genotypic data of 111 polymorphic markers, genetic distance was estimated among the 154 genotypes of Indian mustard using Nei's method implemented in POWER MARKER v 3.25 software. A dendrogram constructed using this distance matrix (Figure 4.21) shows the evidence of three major clusters among the test genotypes. Major cluster C could be further grouped into two sub-clusters *viz*. C1 and C2. The sub-cluster C2 could be divided into two minor clusters *viz*. C21 and C22 and further C22 minor clusters into C22a and C22b. Cluster A, B, sub-cluster C1 and minor cluster C21 occupied all the genotypes of CCS HAU, Hisar and minor cluster C22 occupied the mixed genotypes from all of the institutions.

## 4.8 Population structure and AMOVA

### 4.8.1. Population structure

STRUCTURE software was used for the analysis of population structure in the 154 genotypes of Indian mustard. The results of STRUCTURE software were subjected to STRUCTURE HARVESTER to estimate the delta K (Figure 4.22) value by Evanno table (Table 4.12) which is used to find the significant number of sub-populations in the whole test genotypes at the molecular level. A cut-off of 70% membership probability was used as a threshold value for placing a genotype into a particular cluster using the admixture model. A total of two clusters were identified each having 83 (red cluster) and 54 (green cluster) genotypes of Indian mustard (Figure 4.23). Seventeen genotypes could not match the cut-off membership probability of any of the clusters and were considered admixture. The clustering of Indian mustard genotypes by population structure had been similar to the ancestral history of the genotypes. The reason for less number of significant clusters might be the same geographical origin of most of the genotypes in the present study.

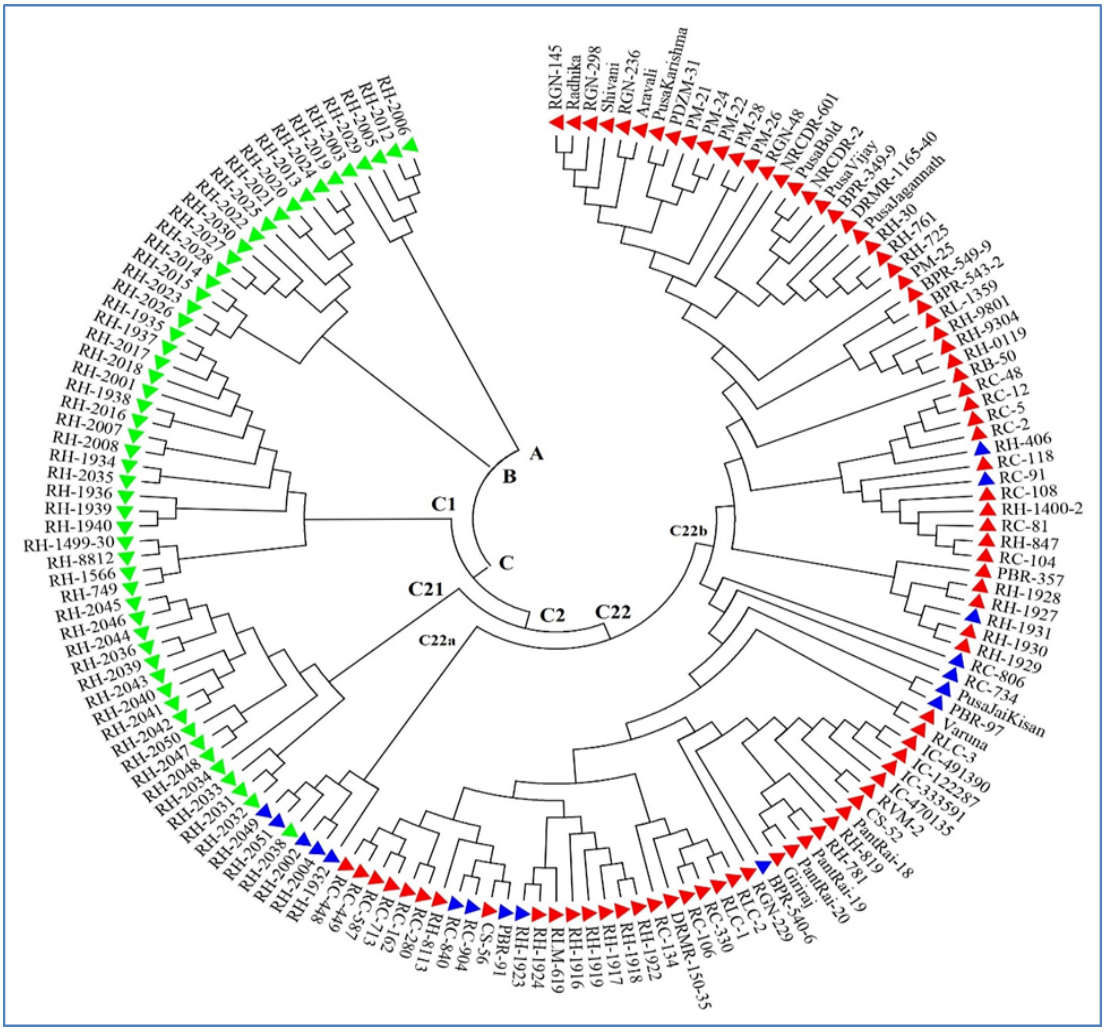


**Figure 4.21 Dendrogram representing genetic relationships among 154 Indian mustard genotypes**

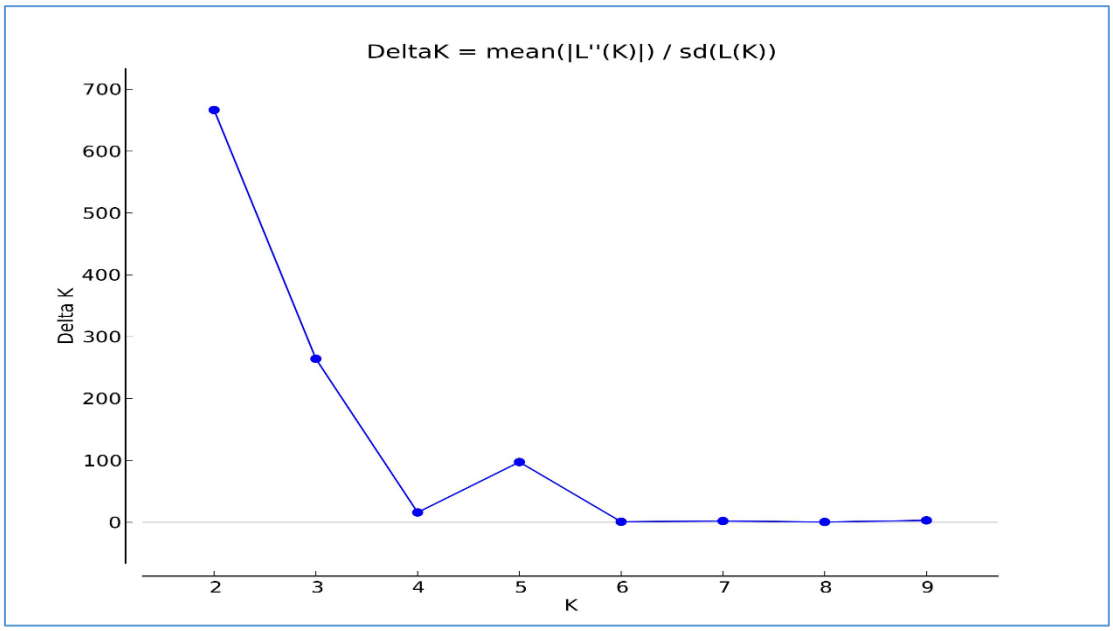


**Figure 4.22 Probability of delta K for estimating the number of clusters for K ranging from 2 to 10**

**Table 4.12 Evanno table to validate the delta K graph to estimate the significant number of clusters in the complete population**

| K | Reps | Mean LnP(K) | Stdev LnP(K) | Ln'(K) | \|Ln''(K)\| | DeltaK |
|---|---|---|---|---|---|---|
| **1** | 10 | -10608.167 | 0.416 | NA | NA | NA |
| **2** | 10 | -9280.767 | 0.710 | 1327.400 | 472.800 | 666.422 |
| **3** | 10 | -8426.167 | 2.566 | 854.600 | 678.067 | 264.271 |
| **4** | 10 | -8249.633 | 4.809 | 176.533 | 76.433 | 15.895 |
| **5** | 10 | -8149.533 | 9.361 | 100.100 | 911.633 | 97.389 |
| **6** | 10 | -8961.067 | 1521.964 | -811.533 | 1239.100 | 0.814 |
| **7** | 10 | -8533.500 | 492.043 | 427.567 | 1030.333 | 2.094 |
| **8** | 10 | -9136.267 | 1590.265 | -602.767 | 725.633 | 0.456 |
| **9** | 10 | -9013.400 | 839.000 | 122.867 | 2603.433 | 3.103 |

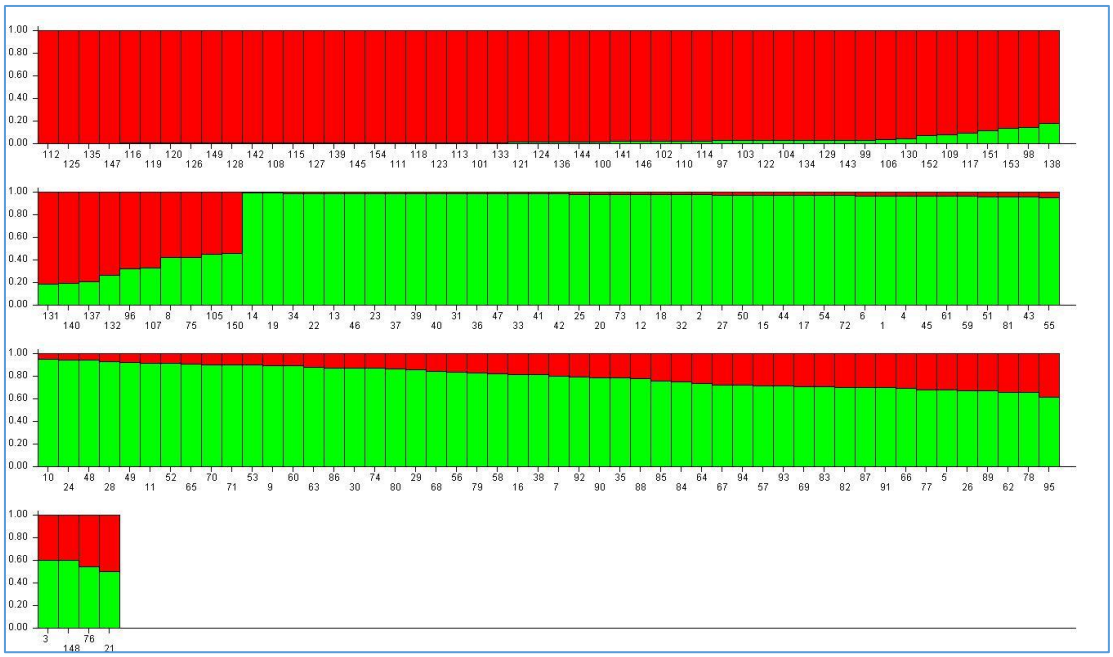

**Figure 4.23 Bar graph for population structure of Indian mustard genotypes**

**Analysis of Molecular Variance (AMOVA)**

AMOVA (Analysis of molecular variance) was estimated by GenAlEx version 6.5 and revealed that differences between populations obtained from STRUCTURE analysis were 22% of the total variation contributed by whole genotypes. However, 78% variation was attributed to diversity between individuals within populations (Table 4.13).

**Table 4.13 Analysis of molecular variance**

| Source | Df | SS | MS | Est. Var. | Var. % |
|---|---|---|---|---|---|
| **Between populations** | 2 | 505.453 | 252.727 | 5.299 | 22% |
| **Within populations** | 151 | 2774.014 | 18.371 | 18.371 | 78% |
| **Total** | 153 | 3279.468 | - | 23.670 | 100% |

df= degree of freedom, SS=Sum of Square, MS= Mean Sum of Square, Est. Var. = Estimated Variance, Var. % = per cent of variance

In the present study, the number of different alleles per locus (Na) were 1.874, 1.444 and 1.475 for sub- population 1, 2 and 3 (admixture) of Indian mustard genotypes, respectively. While, number of private alleles were 12 for sub-population 1 and 2 for sub-population 2. Mean value of I (Shannon's Information Index) was 0.312 for sub-population 1, 0.178 for sub-population 2 and 0.261 for sub-population 3 of Indian mustard genotypes. Gene diversity (h) value was 0.195 for sub-population 1, 0.108 for sub-population 2 and 0.168 for sub-population 3 in whole Indian mustard genotypes. Unbiased diversity (uh) value was slightly higher for each sub-population as compared to gene diversity (h) *viz*. 0.197, 0.110 and 0.179 for sub-population 1, sub-population 2 and sub-population 3, respectively (Table 4.14, Figure 4.24).

**Table 4.14 Genetic diversity and mean allelic pattern across sub-populations**

| Population | Population1 | | Population2 | | Population3 | |
|---|---|---|---|---|---|---|
| | Mean | SE | Mean | SE | Mean | SE |
| **Na** | 1.874 | 0.025 | 1.444 | 0.044 | 1.475 | 0.044 |
| **Ne** | 1.318 | 0.024 | 1.165 | 0.018 | 1.274 | 0.022 |
| **NP** | 12.000 | - | 2.000 | - | 0.000 | - |
| **I** | 0.312 | 0.016 | 0.178 | 0.015 | 0.261 | 0.018 |
| **No. L Com. Alleles (<=25%)** | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| **No. L Com. Alleles (<=50%)** | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| **h (Diversity)** | 0.195 | 0.012 | 0.108 | 0.010 | 0.168 | 0.012 |
| **uh (Unbaised diversity)** | 0.197 | 0.012 | 0.110 | 0.010 | 0.179 | 0.013 |

Na = No. of Different Alleles per locus, Ne = No. of Effective Alleles per locus, NP= No. Private Alleles per locus, I = Shannon's Information Index, h = Gene Diversity, uh = Unbiased Diversity

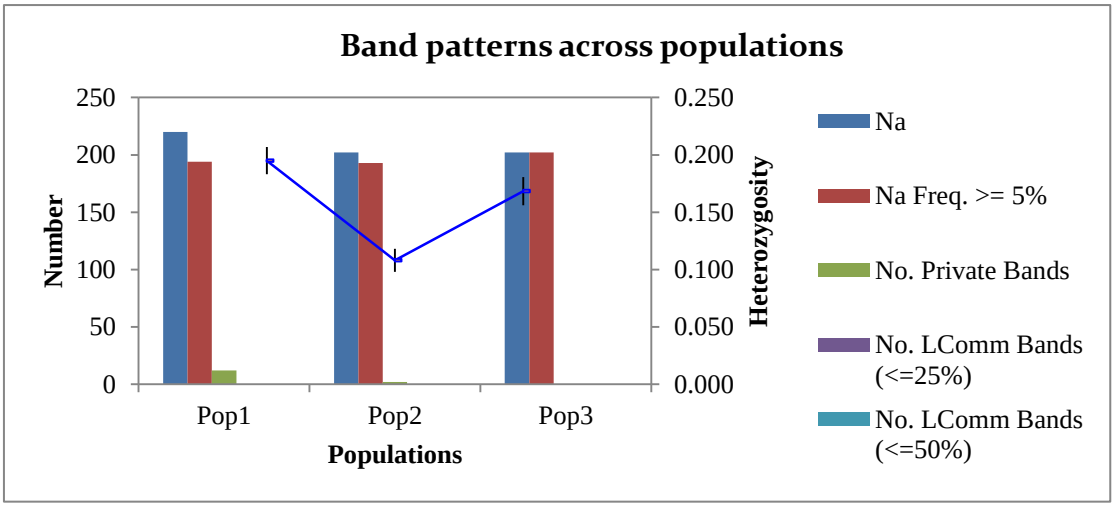


**Figure 4.24 Graphical presentation of mean allelic pattern across the sub-populations**

### 4.9 Linkage disequilibrium (LD)

Linkage disequilibrium is the non-random co-segregation of alleles at two or more loci. In other words, it is the difference between observed and expected allelic frequencies (assuming random distribution due to independent assortment). This non-random co-segregation could be between loci on the same chromosome or between loci on different chromosomes. LD can differ

greatly, extending from a few hundred base pairs to kilo bases, between different crops, as well as different genomic regions and genes within a crop. LD is vital to association mapping as the rate of LD decay in a particular species/crop normalizes the number and density of the molecular markers required to execute or Genome Wide Association mapping (GWAS) association mapping.

Tight linkage between two alleles on same chromosome can be translated in high LD. Therefore, LD can be measured as allele frequency correlation ($r^2$) between the pairs of markers located on same chromosome. TASSEL software was used to study LD and LD decay in this study. Total 312 SSR marker-based alleles were used to calculate the extent of LD that led pairwise LD detection in 24754 locus pairs in total 154 Indian mustard genotypes. Out of 24754 locus pairs, a total 580 SSR marker pairs (2.3%) showed significant LD at threshold (i.e. $r^2 \geq 0.1$). Out of 580 SSR markers pairs significant LD, 24 significant LD were collinear i.e. markers on the same chromosome and 556 significant LD were inter-chromosomal. (Table 4.15).

LD blocks were observed as demonstrated by triangle plots for pairwise LD between SSRs (Figure 4.25). Sizes of intra-chromosomal LD blocks were also calculated; at $r^2 \geq 0.1$ in 18 chromosomes, the longest LD block (26.87 Mbp) was observed on chromosome 5 between BG 269 and BG 196 (Table 4.16). The size of significant collinear LD block was calculated in Mega base pairs (bp). Darker shade of red colour in triangle plot depicts the significant collinear LD block on the different Indian mustard chromosomes. Out of total 24 significant collinear LD block, ten (five each) were on the chromosome 3 and 5, four were on chromosome 13, three were on chromosome 4 and 8, and rests of four were on chromosome 2, chromosome 11, chromosome 14 and chromosome 15.

**Table 4.15 Extent of linkage disequilibrium (LD) between SSR loci at whole genome level**

| **Parameters** | **$r^2 \geq 0.1$** |
|---|---|
| Sample size | 154 |
| Collinear LD % (LD/non-LD) | 0.48 (24/50) |
| Inter-chromosomal LD % (LD/non-LD) | 0.022 (556/24704) |
| Total LD% (LD/non-LD) | 0.023 (580/24754) |

**Table 4.16 Size of collinear linkage disequilibrium blocks in Indian mustard loci at significant threshold of $r^2 \geq 0.1$**

| **Sr. No.** | **Chr** | **Locus1** | | **Locus2** | | **Distance (Mb)** | **$R^2$** | **D'** |
|---|---|---|---|---|---|---|---|---|
| | | **Primer** | **Location (Mb)** | **Primer** | **Location (Mb)** | | | |
| **1.** | 2 | BG15 | 21.45 | BG17 | 2.38 | 19.07 | 0.167 | 0.495 |
| **2.** | 3 | BG115 | 22.71 | BG101 | 19.76 | 2.95 | 0.311 | 0.573 |
| **3.** | 3 | BG238 | 22.71 | BG123 | 0.15 | 22.56 | 0.297 | 0.719 |
| **4.** | 3 | BG204 | 19.76 | BG125 | 0.15 | 19.61 | 0.196 | 0.623 |
| **5.** | 3 | BG195 | 19.76 | BG144 | 14.27 | 5.49 | 0.191 | 0.789 |
| **6.** | 3 | BG149 | 14.27 | BG130 | 14.01 | 0.26 | 0.123 | 1 |
| **7.** | 4 | BG4 | 4.57 | BG123 | 2.72 | 1.85 | 0.263 | 0.55 |
| **8.** | 4 | BG32 | 19.75 | BG204 | 12.42 | 7.33 | 0.193 | 0.476 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **9.** | 4 | BG249 | 20.11 | BG235 | 2.72 | 17.39 | 0.131 | 0.593 |
| **10.** | 5 | BG127 | 31.26 | BG15 | 23.11 | 8.15 | 0.159 | 0.49 |
| **11.** | 5 | BG209 | 23.11 | BG193 | 0.08 | 23.04 | 0.152 | 0.648 |
| **12.** | 5 | BG269 | 33.85 | BG196 | 6.98 | 26.87 | 0.152 | 0.536 |
| **13.** | 5 | BG130 | 35.49 | BG266 | 31.26 | 4.23 | 0.117 | 0.487 |
| **14.** | 5 | BG171 | 35.49 | BG53 | 33.85 | 1.64 | 0.32 | 0.662 |
| **15.** | 8 | BG6 | 22.27 | BG102 | 18.49 | 3.78 | 0.22 | 0.552 |
| **16.** | 8 | BG144 | 18.49 | BG212 | 16.50 | 1.98 | 0.186 | 0.615 |
| **17.** | 8 | BG249 | 21.03 | BG149 | 18.49 | 2.54 | 0.132 | 0.398 |
| **18.** | 11 | BG257 | 52.82 | BG123 | 38.80 | 14.02 | 0.293 | 0.759 |
| **19.** | 13 | BG257 | 15.57 | BG238 | 8.23 | 7.34 | 0.224 | 0.656 |
| **20.** | 13 | BG7 | 25.72 | BG204 | 8.23 | 17.49 | 0.208 | 0.912 |
| **21.** | 13 | BG167 | 27.05 | BG196 | 8.23 | 18.82 | 0.152 | 0.648 |
| **22.** | 13 | BG149 | 25.72 | BG235 | 15.57 | 10.15 | 0.131 | 1 |
| **23.** | 14 | BG127 | 34.88 | BG195 | 21.79 | 13.09 | 0.16 | 1 |
| **24.** | 15 | BG109 | 17.35 | BG251 | 11.77 | 5.58 | 0.32 | 0.662 |

Where, chr – chromosome; bp – base pairs; $R^2$ - squared coefficient of correlation; D' - a relative measure of disequilibrium (D) compared to its maximum (D'= D/Dmax)

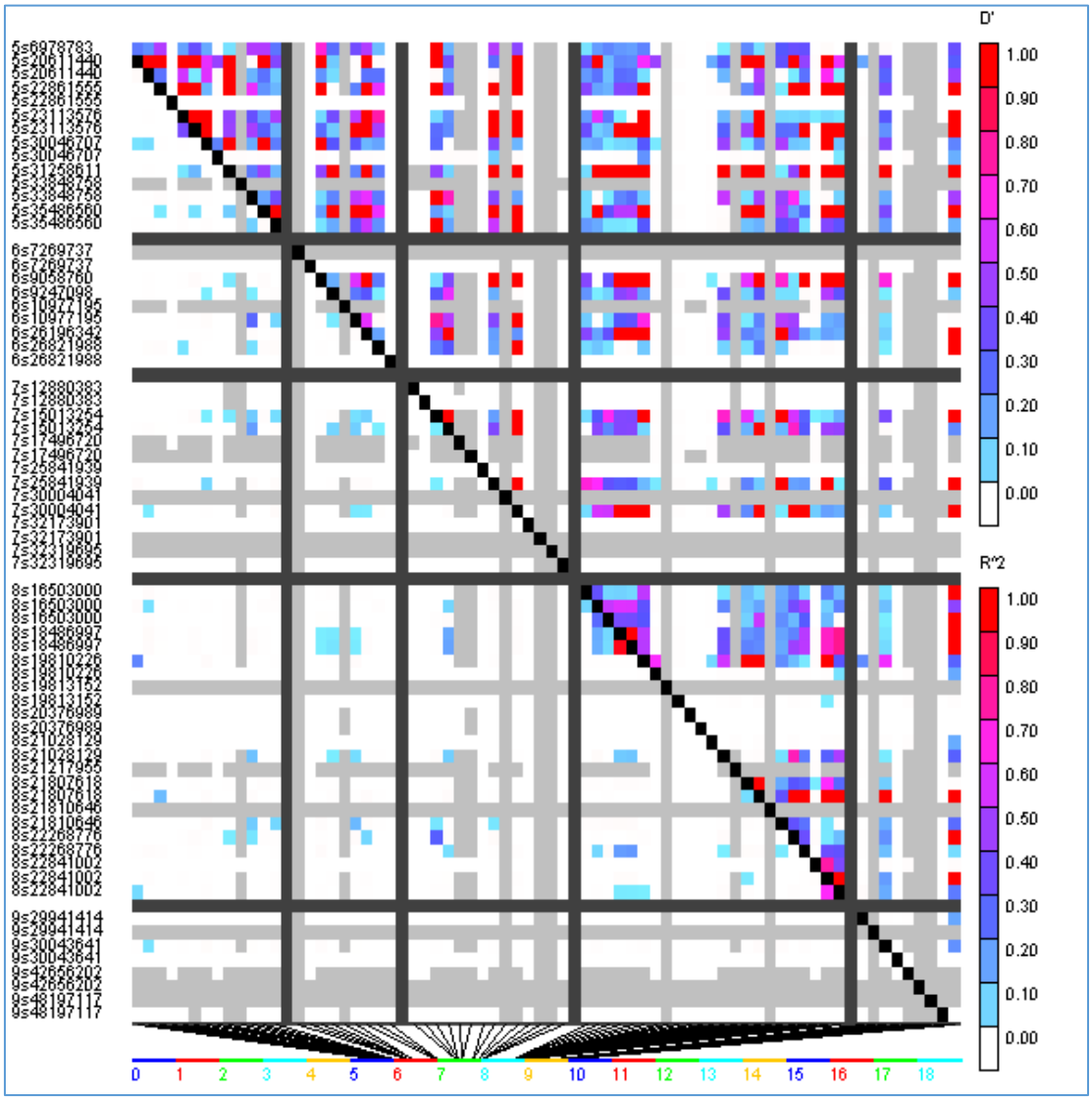


**Figure 4.25 Triangle heat plot showing pairwise locus combinations in 18 chromosomes of Indian mustard in 154 genotypes**

### 4.10 Association mapping:

Analysis of linkage disequilibrium and moderately sized LD blocks showed that numbers of markers were sufficient enough to conduct the association mapping in the present group of 154 Indian mustard genotypes with 111 polymorphic SSR makers. In order to have significant marker trait association a general linear model (GLM) and a mixed linear model (MLM) - based approach implemented in TASSEL v 5.2.85 (http://www.maizegenetics.net/) (Bradbury, 2007). In the TASSEL, GLM model takes into account only the population structure (Q Matrix generated through STRUCTURE) while, MLM model takes into account both i.e. the population structure (Q matrix generated through STRUCTURE) and the familial relatedness i.e. Kinship matrix (K matrix generated from genotypic data) information in the model henceforth named as Q + K model. Quantitative data from *Rabi* 2020-21 and *Rabi* 2021-22 was pooled for both timely and late sown environments. The results for association mapping of timely and late sown environment are as given under-

#### 4.10.1 Association mapping for timely sown environment by GLM (Q):

In timely sown environment, a total of 51 marker-trait associations were found significant for 11 morphological and 4 physiological traits by GLM (Q) method using TASSEL. A p-value $\leq 0.005$ threshold was used to identify significant marker-trait associations (Table 4.17). Out of these 51 marker trait association, one for photosynthetic rate (A), three for carotenoids content, two for chlorophyll content, two for days to 50% flowering, three for days to maturity, four for stomatal conductance (Gs), 12 for main shoot length, five for number of primary branches/plant, three for number of secondary branches/plant, two for number of siliqua on main shoot, two for oil content, two for plant height, one for siliqua length, two for seed yield/plant and seven for 1000-seed weight were found with significant association.

Thirty six SSR markers were involved in 51 marker-trait association because few of the markers were significantly associated with two or more traits. SSR marker BG 133 was found significantly associated with days to 50% flowering, main shoot length and carotenoid content. BG 204 was found significantly associated with seed yield/plant and number of primary branches/plant. BG 212 was found significantly associated with number of primary branches/plant, main shoot length and 1000-seed weight. BG 65 was found significantly associated with main shoot length and plant height. BG 198 was found significantly associated with main shoot length and 1000-seed weight. BG 111 was found significantly associated with main shoot length and number of secondary branches/plant. BG 246 was found significantly associated with days to maturity and photosynthetic rate.

Out of these significantly associated markers, maximum markers were on the chromosome 8 (9 markers) followed by chromosome 2 (7 markers), chromosome 5 (6 markers), chromosome 7 (5 markers) and chromosome 9, 16 and 18 had three markers on each.

Chromosome 6 and 12 had two association on each and chromosome 1, 4, 10, 11, 14 and 15 had single marker on each.

**Table 4.17 Significant marker-trait association identified at threshold p ≤ 0.005 in GLM (Q) method in timely sown experiment**

| Sr. No. | Trait | Marker | Chromosome | Position (bp) | p value |
|---|---|---|---|---|---|
| 1. | A | BG246-170 | 0* | 0 | 0.00466 |
| 2. | Caro | BG222-125 | 9 | 48907017 | 1.71E-04 |
| 3. | Caro | BG133-250 | 2 | 13814497 | 4.14E-04 |
| 4. | Caro | BG133-340 | 2 | 13814497 | 4.14E-04 |
| 5. | Chl | BG249-300 | 3 | 22708276 | 5.88E-04 |
| 6. | Chl | BG175-160 | 10 | 16547276 | 0.00312 |
| 7. | DF | BG133-250 | 2 | 13814497 | 0.00336 |
| 8. | DF | BG133-340 | 2 | 13814497 | 0.00336 |
| 9. | DM | BG246-430 | 0* | 0 | 0.00263 |
| 10. | DM | BG129-285 | 4 | 14806946 | 0.00307 |
| 11. | DM | BG79-150 | 7 | 32319695 | 0.00447 |
| 12. | Gs | BG201-310 | 7 | 12880383 | 4.46E-04 |
| 13. | Gs | BG264-225 | 6 | 10977195 | 0.00108 |
| 14. | Gs | BG247-275 | 16 | 31977603 | 0.00255 |
| 15. | Gs | BG31-200 | 7 | 25841939 | 0.0036 |
| 16. | MSL | BG7-215 | 8 | 22268776 | 2.76E-05 |
| 17. | MSL | BG266-280 | 3 | 14010676 | 8.60E-05 |
| 18. | MSL | BG8-235 | 8 | 19810226 | 1.24E-04 |
| 19. | MSL | BG250-125 | 11 | 35988657 | 2.07E-04 |
| 20. | MSL | BG198-480 | 16 | 57694668 | 3.89E-04 |
| 21. | MSL | BG133-250 | 2 | 13814497 | 8.60E-04 |
| 22. | MSL | BG133-340 | 2 | 13814497 | 8.60E-04 |
| 23. | MSL | BG111-190 | 12 | 60453633 | 0.00138 |
| 24. | MSL | BG190-100 | 9 | 29941414 | 0.00191 |
| 25. | MSL | BG277-220 | 18 | 60426956 | 0.00287 |
| 26. | MSL | BG212-240 | 5 | 76113 | 0.0036 |
| 27. | MSL | BG65-200 | 18 | 56206550 | 0.00381 |
| 28. | NPB | BG204-290 | 8 | 18486997 | 6.04E-04 |
| 29. | NPB | BG212-270 | 5 | 76113 | 0.00107 |
| 30. | NPB | BG144-370 | 5 | 23113576 | 0.00201 |
| 31. | NPB | BG204-230 | 8 | 18486997 | 0.00228 |
| 32. | NPB | BG48-215 | 6 | 9058760 | 0.00399 |
| 33. | NSB | BG256-250 | 7 | 30004041 | 9.28E-04 |
| 34. | NSB | BG111-190 | 12 | 60453633 | 0.00193 |
| 35. | NSB | BG8-235 | 8 | 19810226 | 0.00349 |
| 36. | NSMS | BG17-550 | 5 | 6978783 | 5.58E-04 |
| 37. | NSMS | BG260-215 | 9 | 48197117 | 5.71E-04 |
| 38. | OC | BG138-200 | 5 | 20611440 | 0.00174 |
| 39. | OC | BG180-160 | 8 | 22841002 | 0.00354 |

| | | | | | |
|---|---|---|---|---|---|
| 40. | PH | BG65-200 | 18 | 56206550 | 2.93E-04 |
| 41. | PH | BG101-145 | 14 | 21787834 | 0.00266 |
| 42. | SL | BG158-220 | 8 | 21807618 | 0.0015 |
| 43. | SYP | BG204-290 | 8 | 18486997 | 9.64E-05 |
| 44. | SYP | BG204-230 | 8 | 18486997 | 6.72E-04 |
| 45. | TSW | BG212-240 | 5 | 76113 | 1.72E-04 |
| 46. | TSW | BG35-600 | 7 | 15013254 | 2.50E-04 |
| 47. | TSW | BG124-140 | 1 | 8370403 | 4.08E-04 |
| 48. | TSW | BG198-480 | 16 | 57694668 | 4.68E-04 |
| 49. | TSW | BG251-290 | 15 | 11768714 | 5.63E-04 |
| 50. | TSW | BG149-225 | 3 | 19759971 | 0.00196 |
| 51. | TSW | BG171-175 | 2 | 21449511 | 0.00244 |

*Position of marker is unknown

### 4.10.2 Association mapping for timely sown environment by MLM (Q + K):

A total of 29 marker-trait associations were found significant for 14 morpho-physiological traits using MLM (Q + K) model using TASSEL software in timely sown environment. A p-value $\leq$ 0.005 threshold was used to identify significant marker-trait associations (Table 4.18). Out of these 29 significant marker-trait association, four significant marker-trait associations were for main shoot length, three for stomatal conductance, number of primary branches/plant, number of secondary branches/plant and plant height, two for photosynthetic rate, chlorophyll content, days to 50% flowering and seed yield/plant and one for carotenoid content, number of siliqua on main shoot, number of seeds/siliqua, oil content and 1000-seed weight. In timely sown environment, no marker found significantly associated with siliqua length, days to maturity and transpiration rate.

Nineteen SSR markers were involved in 29 significant marker-trait association because few of the markers were significantly associated with two or more traits. BG 204 was found significantly associated with seed yield/plant, number of primary branches/plant, photosynthetic rate and chlorophyll content. BG 198 was found significantly associated with main shoot length and 1000-seed weight. BG 44 was found significantly associated with plant height and photosynthetic rate.

Out of these 29 significant marker trait association, maximum associations were on the chromosome 8 (9 associations) and chromosome 5, 15 and 16 had three associations on each.

**Table 4.18 Significant marker-trait association identified at threshold p ≤ 0.005 in MLM (Q + K) method in Timely sown environment**

| Sr. No. | Trait | Marker | Chromosome | Position (bp) | p value |
|---|---|---|---|---|---|
| 1. | A | BG204-290 | 8 | 18486997 | 0.00383 |
| 2. | A | BG44-200 | 15 | 50573568 | 0.00249 |
| 3. | Caro | BG222-125 | 9 | 48907017 | 0.00215 |
| 4. | Chl | BG204-230 | 8 | 18486997 | 8.48E-04 |
| 5. | Chl | BG204-290 | 8 | 18486997 | 0.00238 |

| | | | | | |
|---|---|---|---|---|---|
| 6. | DF | BG133-250 | 2 | 13814497 | 0.00498 |
| 7. | DF | BG133-340 | 2 | 13814497 | 0.00498 |
| 8. | Gs | BG247-275 | 16 | 31977603 | 0.00234 |
| 9. | Gs | BG264-225 | 6 | 10977195 | 0.00173 |
| 10. | Gs | BG31-200 | 7 | 25841939 | 0.00491 |
| 11. | MSL | BG7-215 | 8 | 22268776 | 7.60E-05 |
| 12. | MSL | BG198-480 | 16 | 57694668 | 0.00183 |
| 13. | MSL | BG266-280 | 3 | 14010676 | 0.00252 |
| 14. | MSL | BG8-235 | 8 | 19810226 | 0.00381 |
| 15. | NPB | BG144-370 | 5 | 23113576 | 0.0046 |
| 16. | NPB | BG204-230 | 8 | 18486997 | 0.00424 |
| 17. | NPB | BG204-290 | 8 | 18486997 | 7.04E-04 |
| 18. | NSB | BG193-280 | 4 | 12423289 | 0.00472 |
| 19. | NSB | BG256-250 | 7 | 30004041 | 0.00175 |
| 20. | NSB | BG277-600 | 18 | 60426956 | 0.00449 |
| 21. | NSMS | BG17-550 | 5 | 6978783 | 0.00404 |
| 22. | NSS | BG175-160 | 10 | 16547276 | 0.00323 |
| 23. | OC | BG138-200 | 5 | 20611440 | 0.00201 |
| 24. | PH | BG44-200 | 15 | 50573568 | 0.0039 |
| 25. | PH | BG44-235 | 15 | 50573568 | 0.00486 |
| 26. | PH | BG65-200 | 18 | 56206550 | 3.49E-04 |
| 27. | SYP | BG204-230 | 8 | 18486997 | 5.28E-04 |
| 28. | SYP | BG204-290 | 8 | 18486997 | 9.55E-05 |
| 29. | TSW | BG198-480 | 16 | 57694668 | 0.00103 |

Results of significant marker-trait associations presented in Table 4.18 can be better visualized using Manhattan plot (Figure 4.26 to 4.30), which shows the distribution of markers over the chromosome for traits under consideration. The dark red line is a threshold line (at $p \leq 0.005$ or –log10 value = 2.30) marker whose value go above this threshold line are considered as significant marker-trait associations. Further quantile-quantile (QQ) plots was drawn to examine the MLM (Q + K) model fitness for all morpho-physiological traits, as QQ plots (Figure 4.31) is one of the most common ways of determining how well observed value fits with expected. In the QQ plots marker dot which got value on right side above the normal straight line depict the markers have true and significant association with the trait.

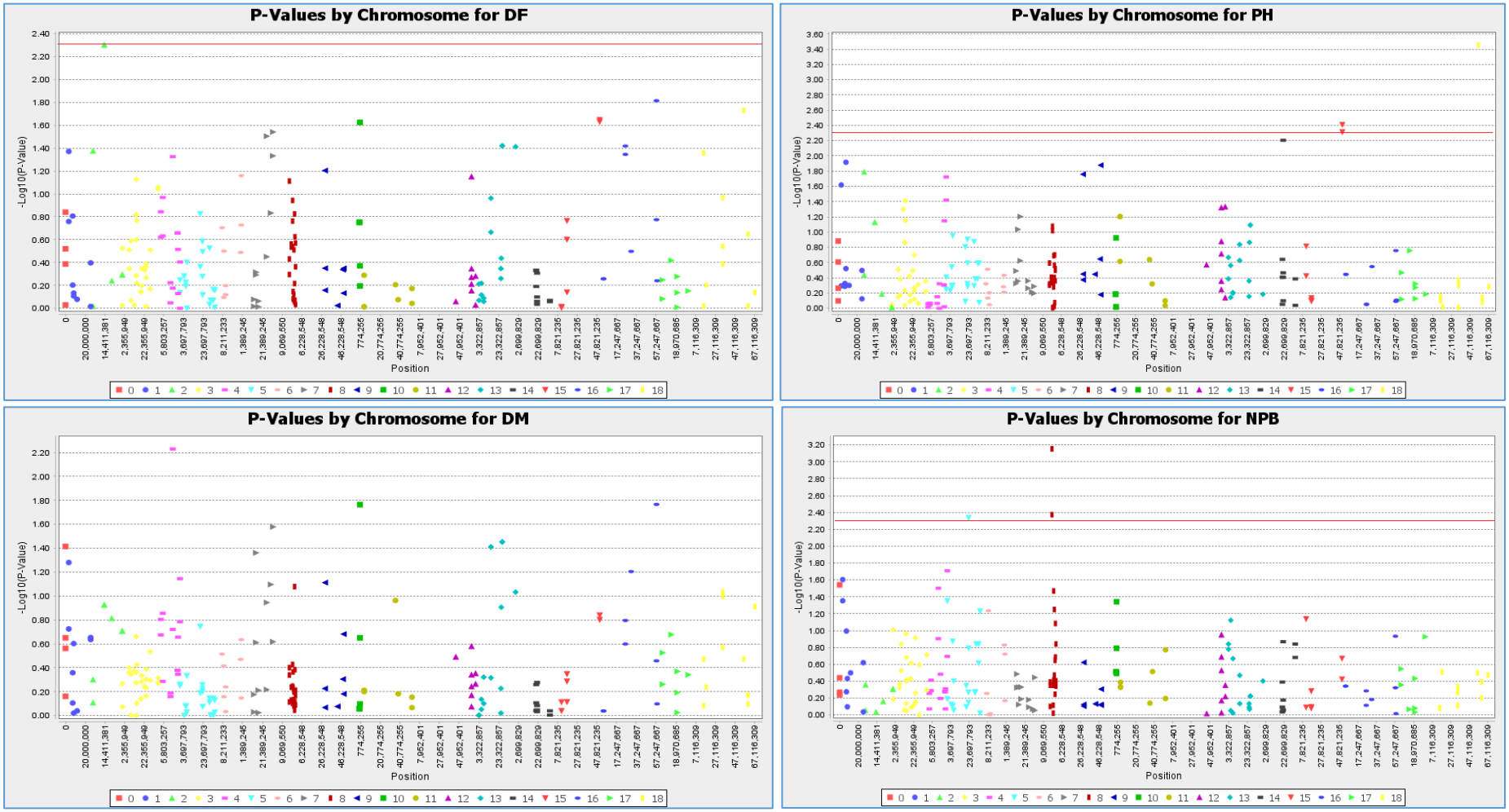


**Figure 4.26 Manhattan plots for plant height, days to 50% flowering, days to maturity and number of primary branches/plant under timely sown environment by MLM (Q+K) model. Here a threshold line at {- log10 (0.005) = 2.30} is drawn to highlight significant markers**

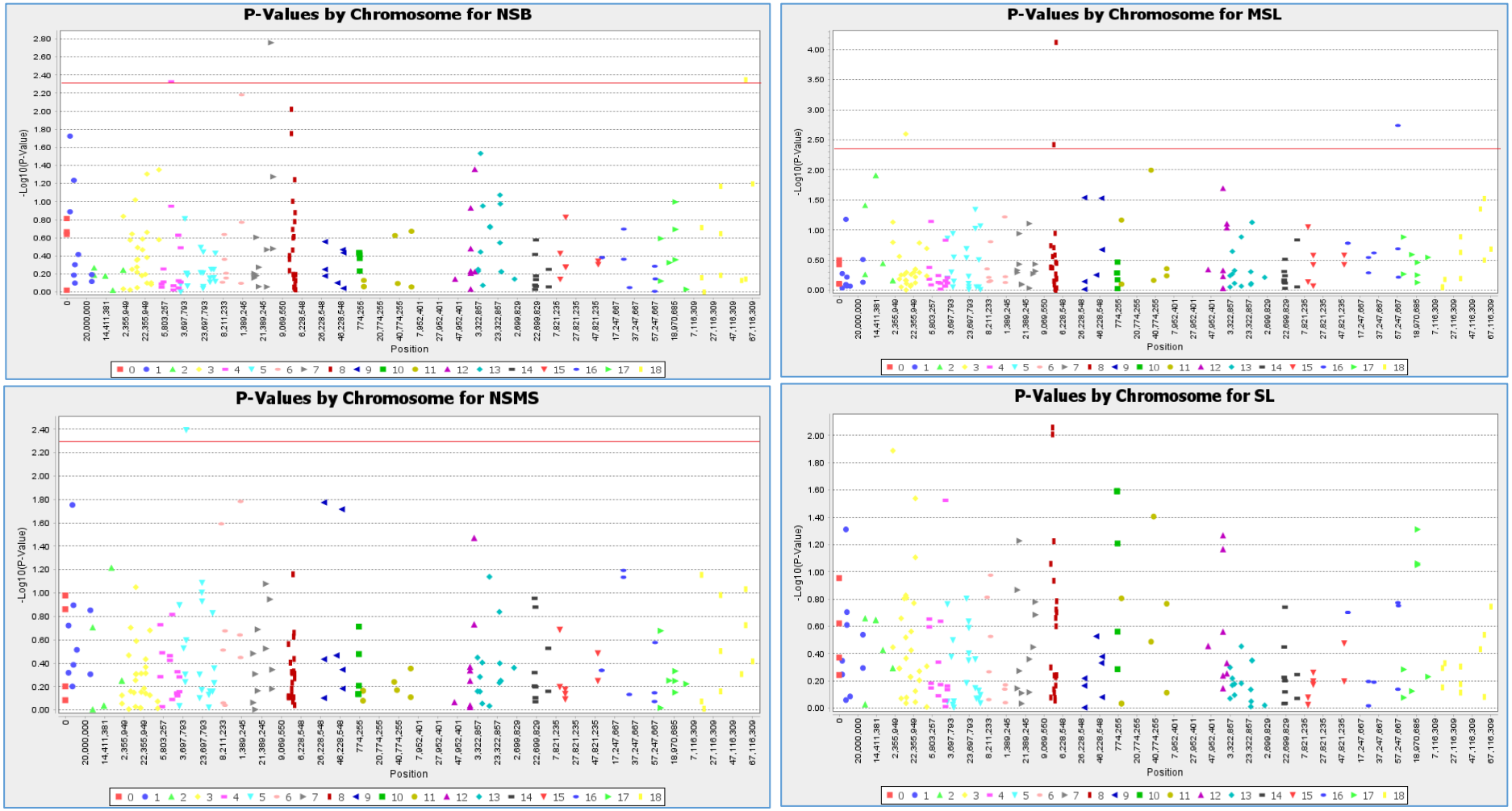


**Figure 4.27 Manhattan plots for number of secondary branches/plant, main shoot length, number of siliquae on main shoot and siliqua length under timely sown environment by MLM (Q+K) model. Here a threshold line at {- log10 (0.005) = 2.30} is drawn to highlight significant markers**

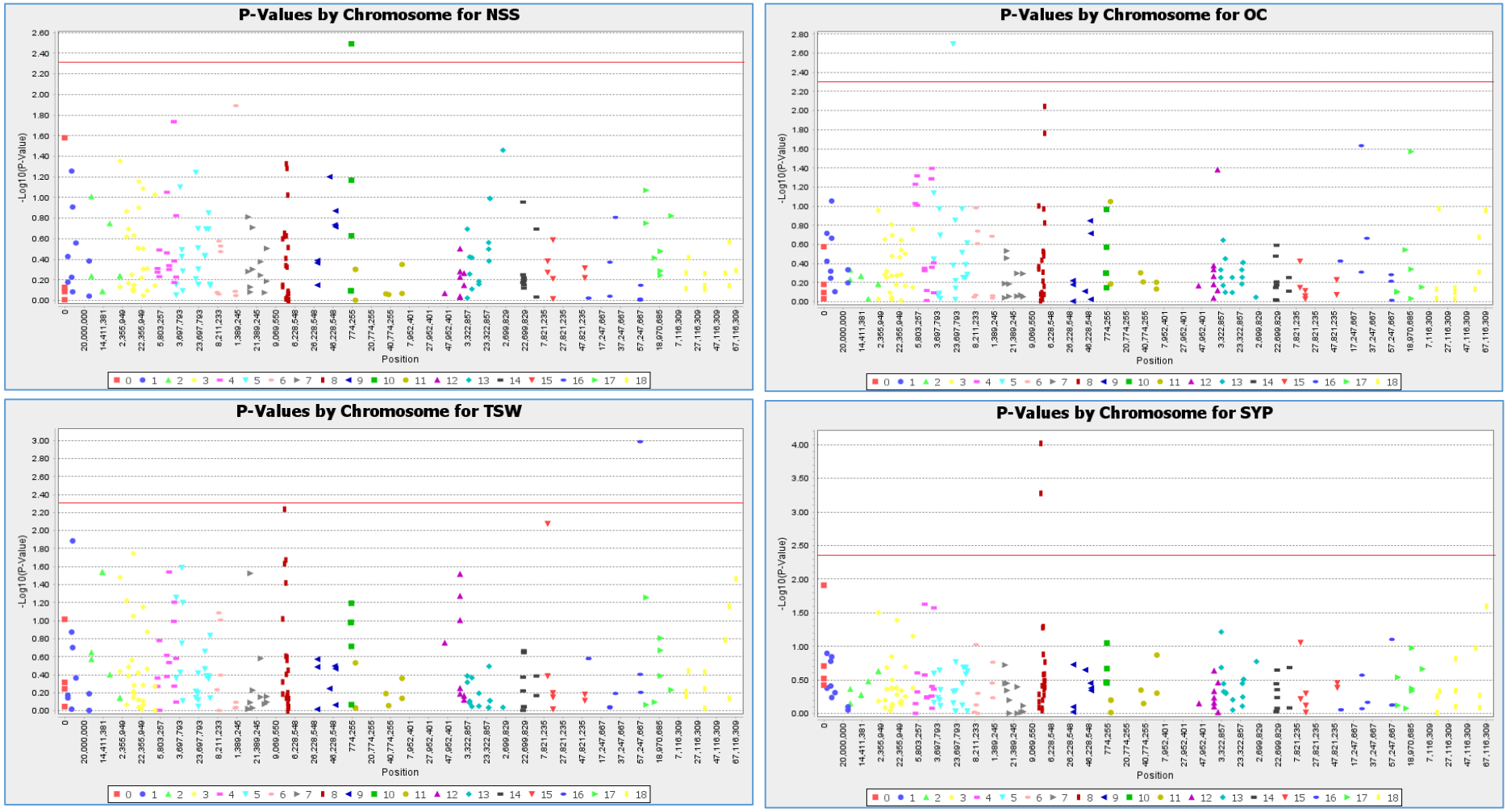


**Figure 4.28 Manhattan plots for number of seeds/siliqua, oil content, 1000-seed weight and seed yield/plant under timely sown environment by MLM (Q+K) model. Here a threshold line at {- log10 (0.005) = 2.30} is drawn to highlight significant markers**


**Figure 4.29 Manhattan plots for photosynthetic rate, stomatal conductance, transpiration rate and chlorophyll content under timely sown environment by MLM (Q+K) model. Here a threshold line at {- log10 (0.005) = 2.30} is drawn to highlight significant markers**

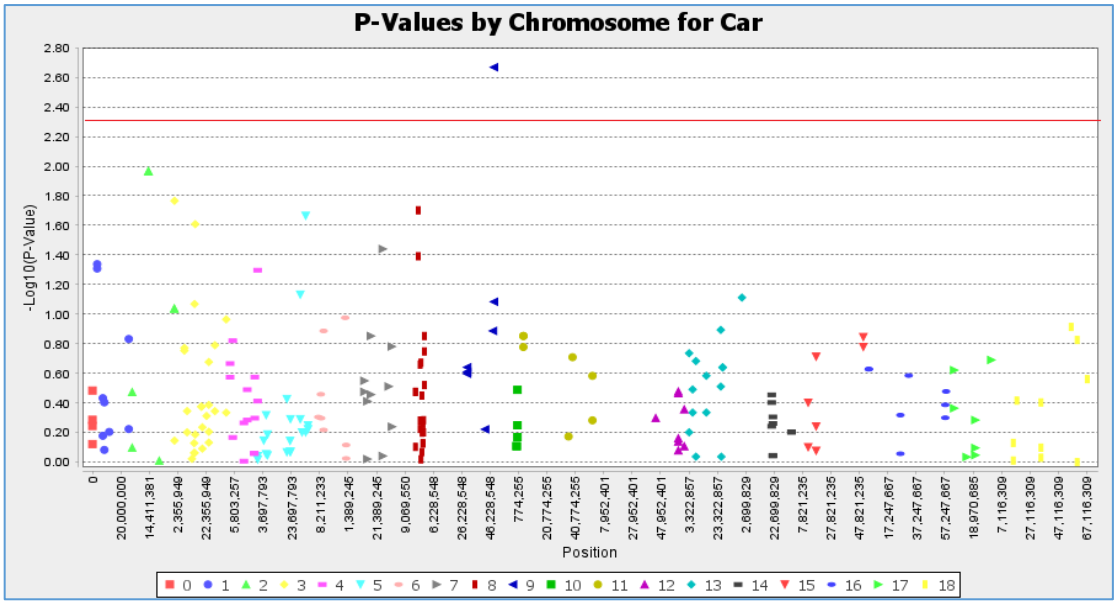


**Fig 4.30 Manhattan plot representing the significant marker-trait association in MLM (Q+K) method for carotenoid content (timely sown environment)**

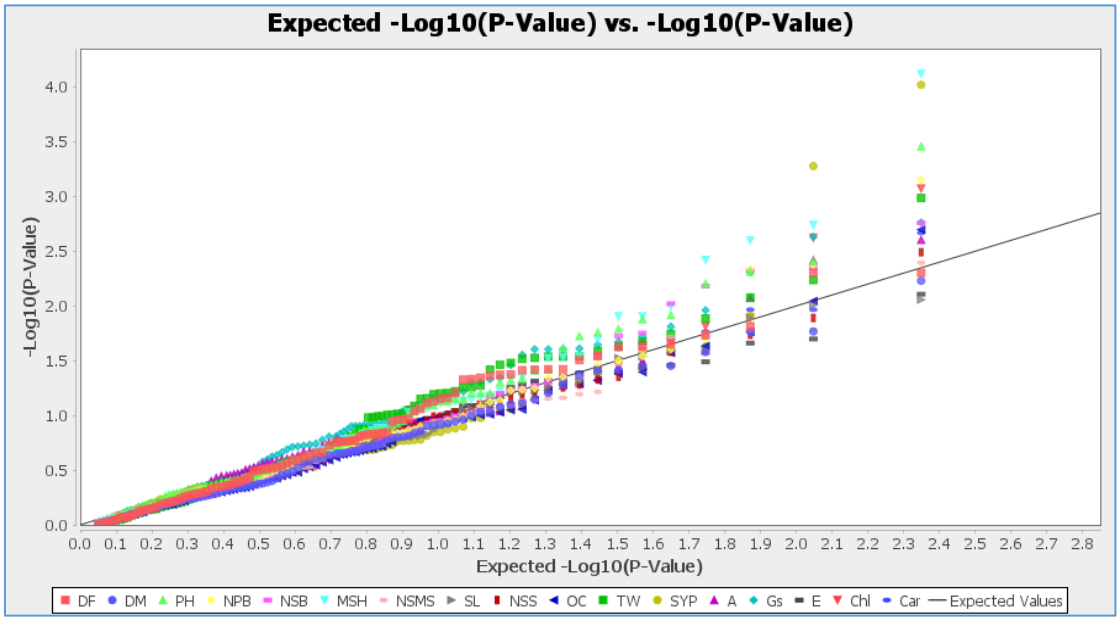


**Figure 4.31 Quartile-Quartile (QQ) plots representing the expected log10 and observed log10 value of p for morpho-physiological traits in timely sown environment**

### 4.10.3 Association mapping for late sown environment by GLM (Q):

A total of 50 marker-trait associations were found significant for 16 morpho-physiological traits using GLM (Q) model in late sown environment. A p-value $\leq$ 0.005 threshold was used to identify significant marker-trait associations (Table 4.19). Out of these 50 marker-trait associations, 13 association were found significant for main shoot length, five associations for seed yield/plant, five associations for 1000-seed weight, four associations for number of primary branches/plant, three associations for photosynthetic rate, chlorophyll content, number of secondary branches/plant and siliqua length, two associations for carotenoid content, days to maturity, and stomatal conductance and one association for days to 50%

flowering, transpiration rate, number of siliquae on main shoot, number of seeds/siliqua and plant height.

Thirty three SSR markers were involved in 50 significant marker-trait associations because few of the markers were associated with two or more traits significantly. The SSR marker BG 204 was found significantly associated with seed yield/plant, number of primary branches/plant and chlorophyll content. BG 247 was found significantly associated with number of seeds/siliqua, stomatal conductance, days to maturity, chlorophyll content, carotenoid content and photosynthetic rate. BG 250 was found significantly associated with seed yield/plant and number of secondary branches/plant. The SSR marker BG 35 was observed significantly associated with siliqua length and 1000-seed weight whereas, BG 48 was found significantly associated with number of primary branches/plant and 1000-seed weight. Similarly, BG 175 was found significantly associated with number of primary branches/plant, seed yield/plant and stomatal conductance. The SSR marker BG 198 was found significantly associated with main shoot length and 1000-seed weight.

Out of these 50 significantly marker-trait associations, maximum associations were on the chromosome 16 (11 associations) followed by chromosome 8 (eight associations), chromosome 7 (five associations), chromosome 3 (four associations) and chromosome 2, 10 and 11 had three associations on each.

**Table 4.19 Significant marker-trait association identified at threshold p ≤ 0.005 in GLM (Q) method in late sown experiment**

| Sr. No. | Trait | Marker | Chromosome | Position (bp) | p value |
|---|---|---|---|---|---|
| 1. | A | BG247-275 | 16 | 31977603 | 2.76E-06 |
| 2. | A | BG252-190 | 3 | 28596818 | 2.34E-05 |
| 3. | A | BG263-400 | 17 | 5727804 | 0.00289 |
| 4. | Caro | BG247-275 | 16 | 31977603 | 2.75E-04 |
| 5. | Caro | BG31-160 | 7 | 25841939 | 0.00426 |
| 6. | Chl | BG247-275 | 16 | 31977603 | 7.46E-07 |
| 7. | Chl | BG204-230 | 8 | 18486997 | 0.00333 |
| 8. | Chl | BG278-200 | 16 | 58276982 | 0.00413 |
| 9. | DF | BG157-220 | 16 | 26103509 | 9.51E-04 |
| 10. | DM | BG258-225 | 13 | 26096134 | 0.00206 |
| 11. | DM | BG247-275 | 16 | 31977603 | 0.00271 |
| 12. | E | BG278-200 | 16 | 58276982 | 0.00257 |
| 13. | Gs | BG247-275 | 16 | 31977603 | 1.49E-10 |
| 14. | Gs | BG175-210 | 10 | 16547276 | 0.00409 |
| 15. | MSL | BG133-250 | 2 | 13814497 | 7.85E-05 |
| 16. | MSL | BG133-340 | 2 | 13814497 | 7.85E-05 |
| 17. | MSL | BG198-480 | 16 | 57694668 | 1.49E-04 |
| 18. | MSL | BG260-215 | 9 | 48197117 | 2.05E-04 |
| 19. | MSL | BG8-235 | 8 | 19810226 | 0.00118 |
| 20. | MSL | BG115-110 | 14 | 34878594 | 0.0016 |
| 21. | MSL | BG44-200 | 15 | 50573568 | 0.00205 |
| 22. | MSL | BG65-200 | 18 | 56206550 | 0.00236 |
| 23. | MSL | BG270-240 | 11 | 4175750 | 0.00284 |
| 24. | MSL | BG138-170 | 5 | 20611440 | 0.00286 |
| 25. | MSL | BG7-215 | 8 | 22268776 | 0.00385 |
| 26. | MSL | BG266-280 | 3 | 14010676 | 0.00421 |

| | | | | | |
|---|---|---|---|---|---|
| 27. | MSL | BG111-190 | 12 | 60453633 | 0.00431 |
| 28. | NPB | BG175-160 | 10 | 16547276 | 6.38E-04 |
| 29. | NPB | BG204-290 | 8 | 18486997 | 0.00193 |
| 30. | NPB | BG48-215 | 6 | 9058760 | 0.00229 |
| 31. | NPB | BG204-230 | 8 | 18486997 | 0.00276 |
| 32. | NSB | BG256-250 | 7 | 30004041 | 2.55E-05 |
| 33. | NSB | BG201-310 | 7 | 12880383 | 0.00142 |
| 34. | NSB | BG250-125 | 11 | 35988657 | 0.00262 |
| 35. | NSMS | BG121-630 | 1 | 7333407 | 0.00426 |
| 36. | NSS | BG247-275 | 16 | 31977603 | 3.51E-04 |
| 37. | PH | BG149-160 | 3 | 19759971 | 0.00364 |
| 38. | SL | BG35-600 | 7 | 15013254 | 2.41E-04 |
| 39. | SL | BG158-220 | 8 | 21807618 | 0.00304 |
| 40. | SL | BG228-240 | 3 | 24310231 | 0.00405 |
| 41. | SYP | BG204-230 | 8 | 18486997 | 2.83E-05 |
| 42. | SYP | BG204-290 | 8 | 18486997 | 3.15E-05 |
| 43. | SYP | BG175-160 | 10 | 16547276 | 2.56E-04 |
| 44. | SYP | BG280-185 | 17 | 31854376 | 4.82E-04 |
| 45. | SYP | BG250-125 | 11 | 35988657 | 0.00145 |
| 46. | TSW | BG35-600 | 7 | 15013254 | 4.45E-04 |
| 47. | TSW | BG156-200 | 18 | 67551398 | 0.00136 |
| 48. | TSW | BG198-480 | 16 | 57694668 | 0.00143 |
| 49. | TSW | BG48-215 | 6 | 9058760 | 0.00346 |
| 50. | TSW | BG171-175 | 2 | 21449511 | 0.00486 |

### 4.10.4 Association mapping for late sown environment by MLM (Q + K)

A total of 33 marker-trait associations were found significant for 15 morpho-physiological traits using MLM (Q + K) model in late sown environment. A p-value $\leq 0.005$ threshold was used to identify significant marker-trait associations (Table 4.20). Out of these 33 significant marker-trait associations, six for main shoot length, two for seed yield/plant, photosynthetic rate, days to maturity, stomatal conductance, number of seeds/siliqua, number of secondary branches/plant and plant height, three for carotenoid content, chlorophyll content and 1000-seed weight were found with significant associations. No significant marker trait association was found for oil content and transpiration rate in late sown environment.

Nineteen SSR markers were involved in 33 significant marker-trait associations because some markers were associated with two or more traits significantly. The SSR marker BG 204 was found significantly associated with seed yield/plant, number of primary branches/plant, chlorophyll content and 1000-seed weight. The SSR marker BG 247 was found significantly associated with carotenoid content, chlorophyll content, photosynthetic rate, stomatal conductance, days to maturity and number of seeds/siliqua. BG 121 was found significantly associated with number of siliquae on main shoot and number of seeds/siliqua, while BG 198 was found significantly associated with main shoot length and 1000-seed weight.

Out of these 33 significant marker-trait associations, maximum association were on chromosome 16 (nine associations) followed by chromosome 8 (eight associations),

chromosome 2 (three association), chromosome 7 (three associations) and chromosome 1 and 3 had two association on each.

**Table 4.20 Significant marker-trait association identified at threshold p ≤ 0.005 in MLM (Q + K) method in late sown experiment**

| Sr. No. | Trait | Marker | Chromosome | Position (bp) | p value |
|---|---|---|---|---|---|
| 1. | A | BG247-275 | 16 | 31977603 | 1.33E-05 |
| 2. | A | BG252-190 | 3 | 28596818 | 0.0031 |
| 3. | Caro | BG247-275 | 16 | 31977603 | 2.20E-04 |
| 4. | Caro | BG31-160 | 7 | 25841939 | 0.00415 |
| 5. | Caro | BG204-230 | 8 | 18486997 | 0.00419 |
| 6. | Chl | BG247-275 | 16 | 31977603 | 2.34E-06 |
| 7. | Chl | BG204-230 | 8 | 18486997 | 9.38E-05 |
| 8. | Chl | BG204-290 | 8 | 18486997 | 0.00341 |
| 9. | DF | BG157-220 | 16 | 26103509 | 0.002 |
| 10. | DM | BG258-225 | 13 | 26096134 | 0.00149 |
| 11. | DM | BG247-275 | 16 | 31977603 | 0.00393 |
| 12. | Gs | BG247-275 | 16 | 31977603 | 1.67E-08 |
| 13. | Gs | BG175-210 | 10 | 16547276 | 0.00291 |
| 14. | MSL | BG198-480 | 16 | 57694668 | 9.61E-04 |
| 15. | MSL | BG133-250 | 2 | 13814497 | 0.00166 |
| 16. | MSL | BG133-340 | 2 | 13814497 | 0.00166 |
| 17. | MSL | BG260-215 | 9 | 48197117 | 0.00236 |
| 18. | MSL | BG53-400 | 2 | 2382107 | 0.0032 |
| 19. | MSL | BG7-215 | 8 | 22268776 | 0.00444 |
| 20. | NPB | BG204-290 | 8 | 18486997 | 0.00234 |
| 21. | NSB | BG256-250 | 7 | 30004041 | 1.56E-04 |
| 22. | NSB | BG193-280 | 4 | 12423289 | 0.00455 |
| 23. | NSMS | BG121-630 | 1 | 7333407 | 0.00439 |
| 24. | NSS | BG247-275 | 16 | 31977603 | 5.60E-04 |
| 25. | NSS | BG121-630 | 1 | 7333407 | 0.00478 |
| 26. | PH | BG82-200 | 5 | 6001487 | 0.0031 |
| 27. | PH | BG149-160 | 3 | 19759971 | 0.00439 |
| 28. | SL | BG35-600 | 7 | 15013254 | 0.00299 |
| 29. | SYP | BG204-230 | 8 | 18486997 | 8.64E-05 |
| 30. | SYP | BG204-290 | 8 | 18486997 | 9.65E-05 |
| 31. | TSW | BG156-200 | 18 | 67551398 | 0.00211 |
| 32. | TSW | BG198-480 | 16 | 57694668 | 0.00276 |
| 33. | TSW | BG204-290 | 8 | 18486997 | 0.00362 |

Results of significant marker-trait associations presented in Table 4.20 can be better visualized using Manhattan plot (Figure 4.32 to 4.36), which shows the distribution of markers over the chromosome for traits under consideration. The dark red line is a threshold line (at $p \leq 0.005$ or $-\log10$ value = 2.30), marker whose value go above this threshold line are considered as significant marker-trait associations. Further quantile-quantile (QQ) plots was drawn to examine the MLM (Q + K) model fitness for all morpho-physiological traits, as QQ plots (Figure 4.37) is one of the most common ways of determining how well observed value fits with expected. In the QQ plots marker dot which got value on right side above the normal straight line depict the markers have true and significant association with the trait.

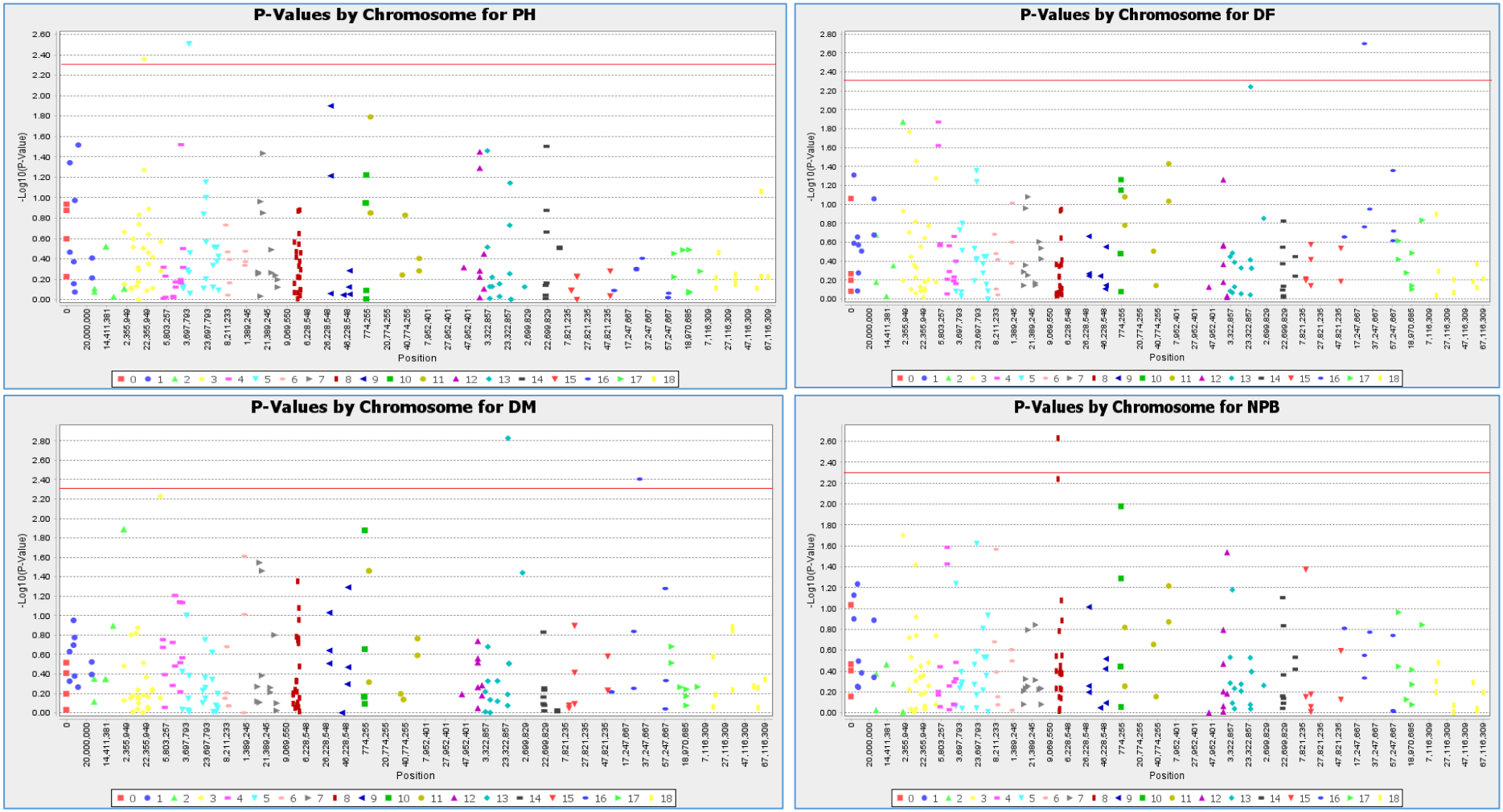


**Figure 4.32 Manhattan plots for plant height, days to 50% flowering, days to maturity and number of primary branches/plant under late sown environment by MLM (Q+K) model. Here a threshold line at {- log10 (0.005) = 2.30} is drawn to highlight significant markers**

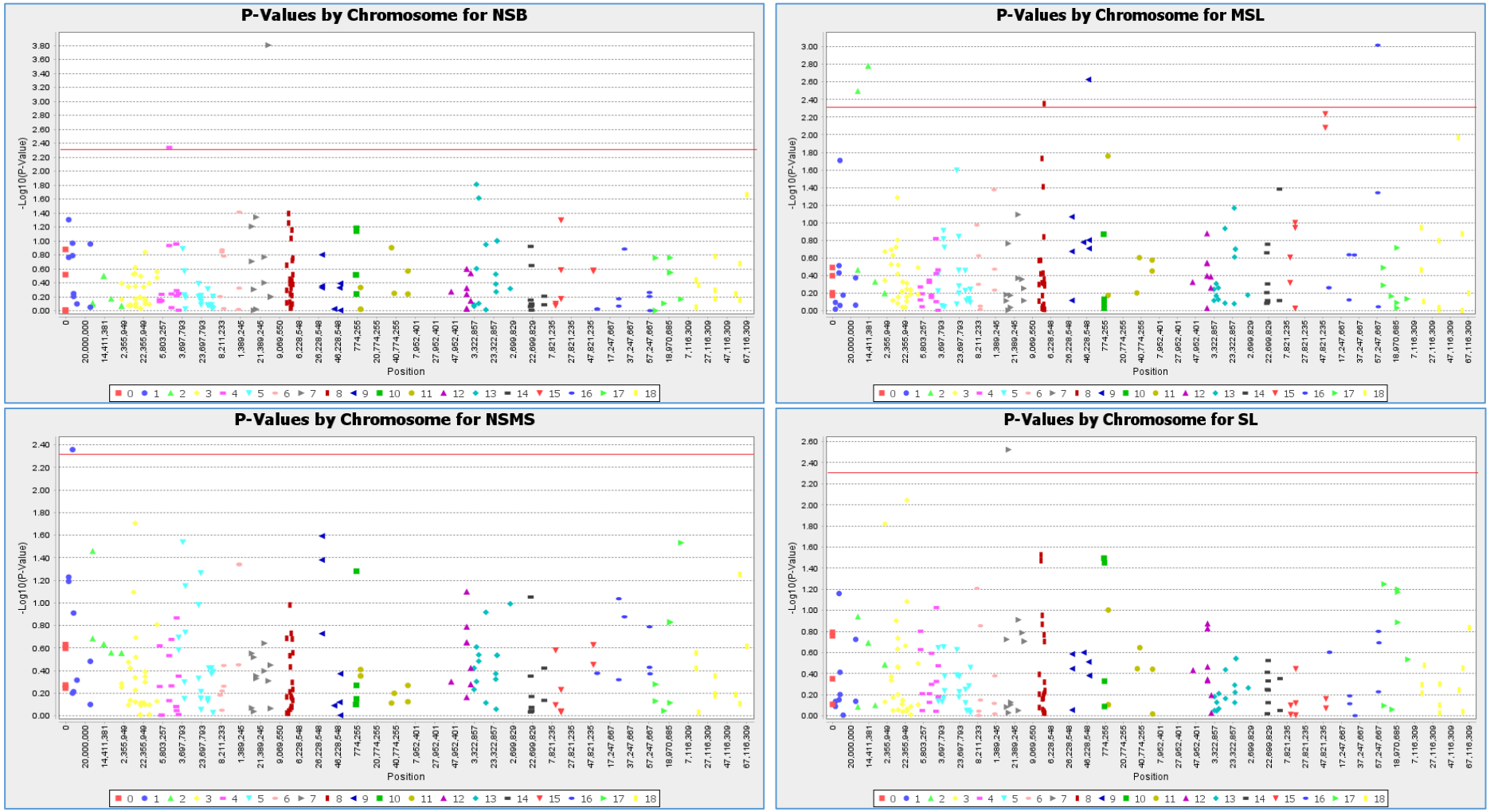


**Figure 4.33 Manhattan plots for number of secondary branches/plant, main shoot length, number of siliquae on main shoot and siliqua length under late sown environment by MLM (Q+K) model. Here a threshold line at {- log10 (0.005) = 2.30} is drawn to highlight significant markers**


**Figure 4.34 Manhattan plots for number of seeds/siliqua, oil content, 1000-seed weight and seed yield/plant under late sown environment by MLM (Q+K) model. Here a threshold line at {- log10 (0.005) = 2.30} is drawn to highlight significant markers**

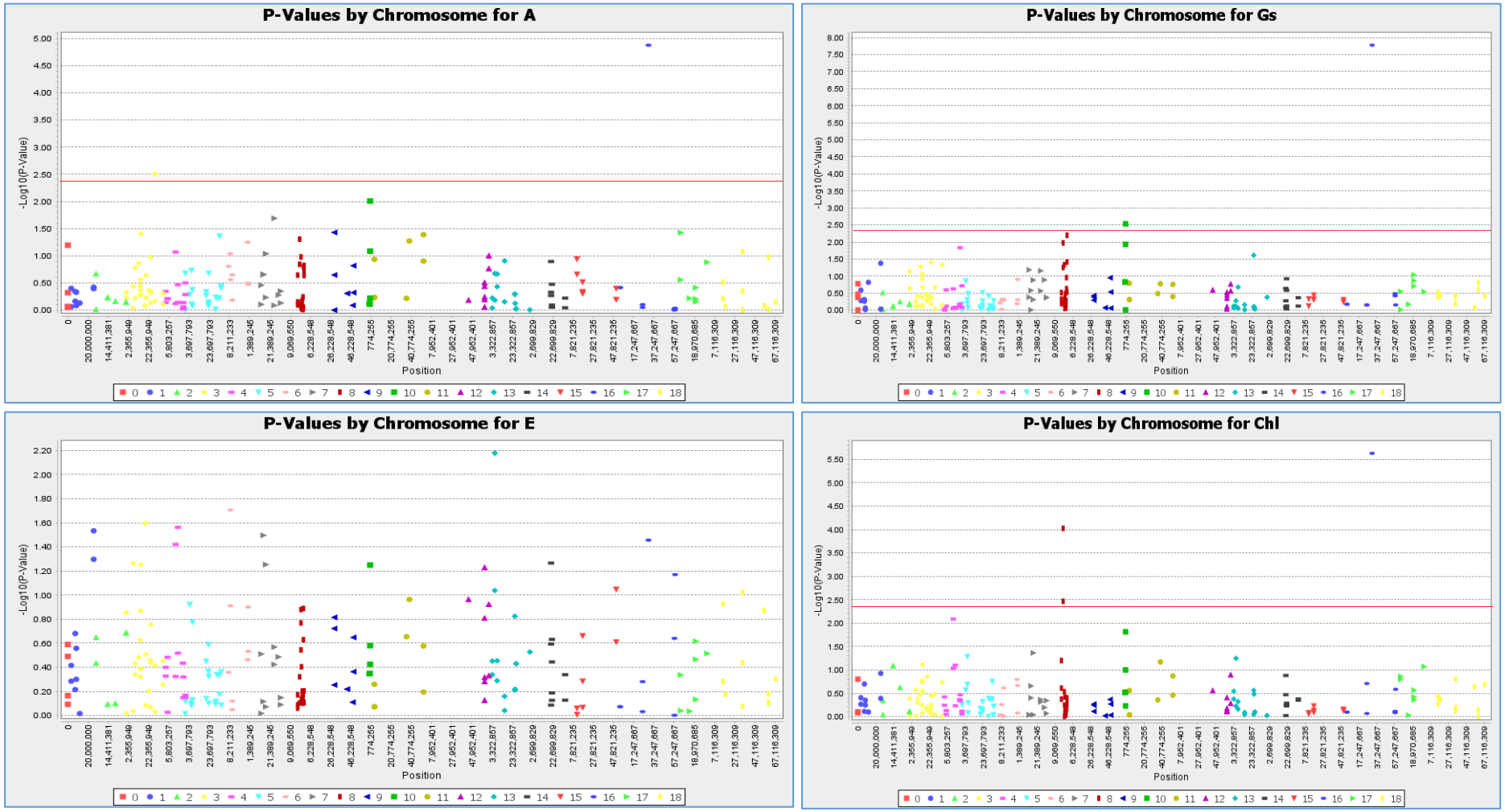


**Figure 4.35 Manhattan plots for photosynthetic rate, stomatal conductance, transpiration rate and chlorophyll content under late sown environment by MLM (Q+K) model. Here a threshold line at {- log10 (0.005) = 2.30} is drawn to highlight significant markers**

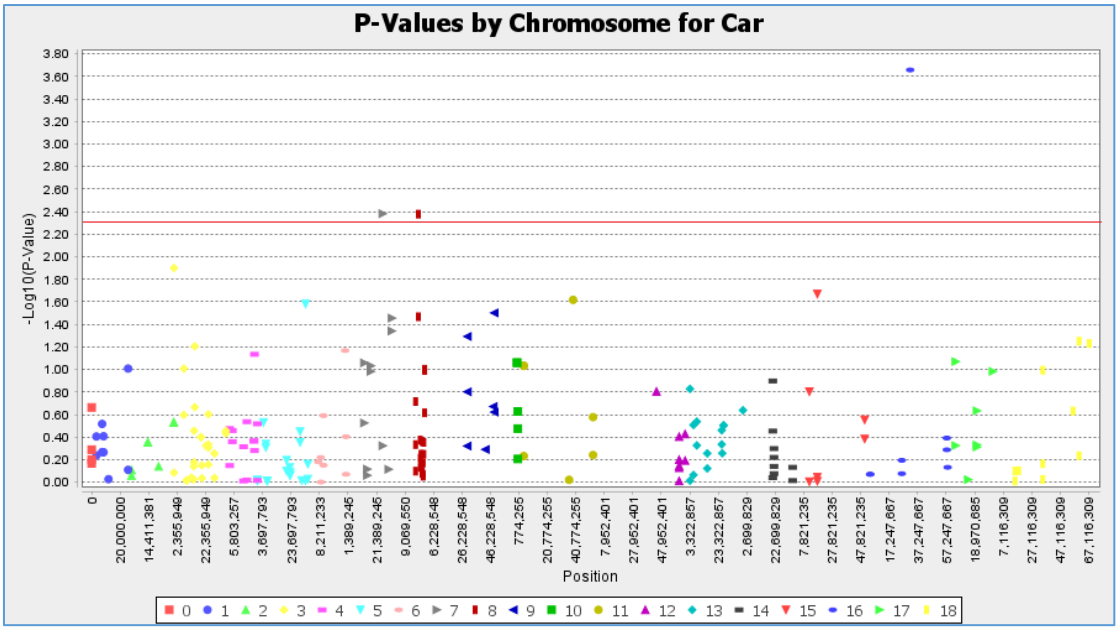


**Fig 4.36: Manhattan plot representing the significant marker-trait association in MLM (Q+K) method for carotenoid content (late sown environment)**

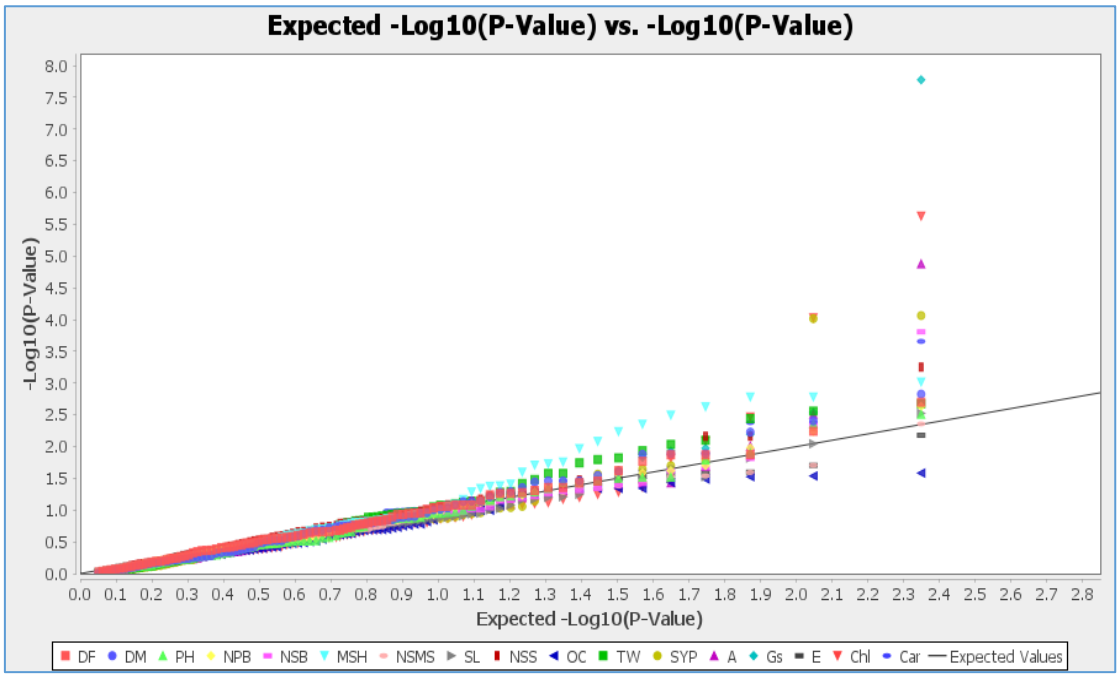


**Figure 4.37 Quartile-Quartile (QQ) plots representing the expected log10 and observed log10 value of p for morpho-physiological traits in late sown environment**

## *CHAPTER*-V

## *DISCUSSION*

The released cultivars, advanced breeding lines, registered genetic stocks, and germplasm lines available at the Oilseeds Section of the Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar; ICAR-Directorate of Rapeseed and Mustard Research, Bharatpur; the Punjab Agricultural University, Ludhiana; and the ICAR-Indian Agricultural Research Institute, New Delhi—all served as the experimental plant materials in this study for association mapping of terminal heat stress tolerance in Indian mustard [*Brassica juncea* (L.) Czern & Coss.]. The main objective was to determine the QTL/genes responsible for physiological characteristics related to terminal heat stress tolerance, as well as seed yield and the factors that contribute to it. The genotypes under investigation had wide differences in physiological as well as morphological traits. Thus, the availability of considerable variation allowed us to apply an association mapping approach for studying the genetic basis of phenotypic variation for traits recorded under a wide range of environmental conditions. The experimental plant material was evaluated under normal sown and late sown environmental conditions during *Rabi* seasons of 2020–21 and 2021–22. The time of sowing in Indian mustard is critical to crop production, from germination to harvesting. Due to the late harvest of rice and cotton, mustard planting is often delayed, resulting in heat stress at the terminal stage, causing variable expression of different yield and yield contributing traits. The measured physiological and morphological traits responded differently to varying environments. These variations were subjected to molecular marker (SSR) screening, and after measuring the linkage disequilibrium (LD) and LD decay, significant marker-trait associations were identified, which are discussed in this chapter under the following headings:

5.1 Analysis of variance

5.2 Studies of genetic variability components

5.3 Correlation and path coefficient analysis

5.4 Heat susceptibility index and per cent seed yield reduction

5.5 Genetic diversity

5.6 Molecular characterization

5.7 Population structure and AMOVA

5.8 Linkage disequilibrium

5.9 Association mapping

### 5.1. Analysis of variance:

The analysis of variance results for all the morphological and physiological traits revealed considerable differences for sowing dates (S), showing substantial environmental differences during different dates of sowing. The mean sum of squares due to genotype revealed significant differences for all morpho-physiological traits in both timely and late sown plants, indicating adequate genetic variation among genotypes, whereas the mean sum of squares due to year revealed significant differences for all traits except number of primary branches, main shoot length, seed yield/plant, transpiration rate, chlorophyll content, and carotenoid content. The interaction effects of genotypes, sowing date, and year also showed significant differences for days to 50% flowering, days to maturity, number of secondary branches, main shoot length, number of seeds per siliqua, and stomatal conductance, indicating sufficient environmental and genotypic differences present in the test environment and genotypes, respectively. Results are in agreement with previous studies reported by Gupta *et al.* (2021).

### 5.2. Studies of genetic variability components

In plant breeding, genetic variability is the basis for selection and the desired improvement of any target trait. In the present study, the extent of variability was calculated in terms of the coefficient of variation. For the traits under study, PCV was slightly higher than GCV, which indicates that all traits were little affected by environmental factors. The high PCV and GCV recorded for photosynthetic rate and stomatal conductance indicate a high possibility of selection on these traits. Primary branches/plant, secondary branches/plant, siliqua length, 1000-seed weight, seed yield/plant, transpiration rate, total chlorophyll content, and carotenoid content had moderate PCV and GCV. The low GCV was observed for days to 50% flowering, days to maturity, plant height, main shoot length, number of siliquae on the main shoot, number of seeds/siliqua and oil content. Low PCV and GCV estimates for these traits show a constricted range of variability and restricted possibility for selection. The results are in agreement with previous studies reported by Chauhan *et al.* (2009), Yadava *et al.* (2011), and Singh *et al.* (2017).

Trait heritability refers to the proportion of phenotypic variance explained by heritable genetic components. Trait heritability assessment is essential to maximize the selection response (Falconer and Mackay, 1996). The term "broad-sense heritability" refers to the estimation of the total heritable component of a trait, which includes all potential sources of heritable variation (additive, dominance, epistatic, and maternal effects). The high (> 60%) broad-sense heritability for all of the studied traits except transpiration rate indicates little environmental effect on the expression of these traits, and most of the variation is genetic. Thus, phenotype-based selection will be effective for improving these traits. Similar to our findings, Yadava *et al.* (2011) and Patel *et al.* (2021) in Indian mustard also reported high broad-sense

heritability for most of the yield contributing traits.

The degree of improvement in a character under a specific selection pressure is explained by genetic advance. The extent of genetic advance under selection for morphological and physiological traits is expressed as a percentage of the 5% of mean. High heritability estimates combined with high genetic advance are more consistent and expressive than the former alone and provide more opportunities for selection during early segregating generations, which improves character significantly. In the present study, high (> 60%) broad sense heritability along with high (> 20%) genetic advance was estimated for primary branches, number of secondary branches, siliqua length, 1000-seed weight, seed yield/plant, photosynthetic rate, stomatal conductance, total chlorophyll content, and carotenoid content, while, moderate (10-20%) genetic advance was estimated for main shoot length and number of siliquae on main shoot. Nearly similar results for one or more characters were previously reported by Yadava *et al.* (2011), Kumar *et al.* (2020), Chaurasiya *et al.* (2019), and Singh *et al.* (2022d). Although the five traits, *viz*. days to 50% flowering, days to maturity, plant height, number of seeds/siliqua and oil content, showed high broad-sense heritability but low genetic advance, suggesting that these traits were predominantly controlled by non-additive gene action and that selection for these traits would not give a good response in early segregating generations. Therefore, selection should be delayed until heterozygosity is reduced in these populations. Higher estimates of heritability and genetic advance for variables that contribute to yield provide evidence that indirect selection for yield through these traits may be more efficient than direct selection for seed yield per se.

### 5.3. Correlation and path coefficient analysis

The main goal of any crop breeding programme is to increase seed yield. Yield is a complex trait that depends on a number of yield-contributing traits. As a result, different traits should be taken into account to increase yield. Studies of correlation offer a more accurate knowledge of yield components that aid plant breeders in selection (Robinson *et al.,* 1951). In this study, seed yield per plant was significantly and positively correlated with plant height, number of primary branches/plant, number of secondary branches/plant, main shoot length, number of siliquae on the main shoot, siliqua length, number of seeds/siliqua, 1000-seed weight, photosynthetic rate, stomatal conductance, total chlorophyll content, and carotenoid content. These results indicate that most of the morpho-physiological traits have a highly significant positive association with seed yield/plant. Similar results were reported in previous studies by Singh *et al.* (2011), Singh *et al.* (2013b), Shekhawat *et al.* (2014), Kumar *et al.* (2018), Rout *et al.* (2019), Sandhu *et al.* (2019), Tiwari (2019), Singh *et al.* (2020), Choudhary *et al.* (2022), and Yadav *et al.* (2022). The number of days to 50% flowering and the rate of transpiration were found to be inversely related to seed yield/plant. The high-yielding genotypes

flower in the fewest number of days and reach the siliqua filling stage in the fewest number of days. Because Indian mustard has an indeterminate inflorescence, the number of days between the first flowering and the siliqua filling stage exceeds the number of flowers produced, resulting in siliquae formation. This increased siliqua numbers has a direct impact on seed yield/plant. Similarly, higher transpiration rates result in water loss from the plant, and plants struggle to maintain cell turgidity and plant water-use efficiency, resulting in a yield penalty (Akter and Islam, 2017).

1000-seed weight was significantly and positively correlated with days to maturity, number of primary branches/plant, number of secondary branches/plant, main shoot length, siliqua length, number of seeds/siliqua, seed yield/plant, photosynthetic rate, stomatal conductance, total chlorophyll content, and total carotenoid content. The oil content was found to be negatively correlated with plant height, number of primary branches/plant, main shoot length, siliqua length, number of seeds/siliqua, 1000-seed weight, and seed yield/plant. Previously, Singh *et al.* (2013b) found oil content negatively correlated with days to 50% flowering, main shoot length, siliqua length, and 1000-seed weight. Similarly, Singh *et al.* (2020) also reported a negative correlation of oil content with the number of primary branches/plant, number of secondary branches/plant, siliqua length, and 1000-seed weight. The photosynthetic rate had a significant positive association with number of primary branches/plant, number of secondary branches/plant, main shoot length, number of siliquae on main shoot, siliqua length, number of seeds/siliqua, 1000-seed weight, seed yield/plant, stomatal conductance, total chlorophyll content, and carotenoid content, although it had a significant negative association with days to 50% flowering and transpiration rate. These findings are in agreement with a previous study by Junjariya (2014).

When more characters are included in the correlation analysis investigation, correlation alone is insufficient. A mutual link, whether positive or negative, between several of the traits makes their correlation with one another obvious. The indirect associations between the variables get more complicated and less clear as additional factors are included in the correlation analysis. In these situations, the path coefficient analysis offers a useful way to distinguish between the direct and indirect causes of association, permits critical examination of the specific forces acting to produce a given correlation, and measures the relative importance of each causal factor. Path analysis revealed that the direct contribution towards seed yield/plant is determined by the number of primary branches/plant, 1000-seed weight, number of secondary branches/plant, siliqua length, and number of seeds/siliqua. Hence, these characters are important during selection for improving seed yield/plant. It indicates that these traits can be used for indirect selection of seed yield. Previously, Lodhi *et al.* (2014) reported that the number of primary branches/plant, main shoot length, and number of seeds/siliqua

showed a positive direct effect on seed yield/plant, suggesting that selection for the number of primary branches/plant, main shoot length, and number of seeds/siliqua would be effective in improving seed yield in Indian mustard. Similarly, Singh *et al.* (2013b) reported that days to 50% flowering had the maximum direct effects on seed yield/plant, followed by number of seeds/siliqua, siliquae on the main shoot, 1000-seed weight, and oil content. Through path analysis, Patel *et al.* (2021) discovered that the number of siliquae/plant and seeds/siliqua had a positive direct effect on seed yield/plant. Therefore, considering these characters, like number of siliquae/plant and number of seeds/siliqua, as selection criteria will be helpful in improving seed yield in Indian mustard.

### 5.4. Heat susceptibility index and per cent seed yield reduction

Angadi *et al.* (2000) studied high temperature tolerance at the terminal stage of Indian mustard and revealed that reductions in fertile siliquae, 1000-seed weight, and seeds/siliquae were responsible for the reduced seed yield. In our study, the mean performance for all the characters studied showed reduced performance under the late sown crop for all the genotypes as compared to the timely sown crop. The concept of heat susceptibility index (HSI) and per cent seed yield reduction was given by Fischer and Maurer (1978) and Sandhu *et al.* (2019), respectively, to determine the effect of heat stress in terms of a reduction in the seed yield. In this study, the HSI was estimated for seed yield/plant to determine the effect of terminal heat stress in the 154 genotypes screened in an augmented design. Heat susceptibility index (an indicator of the susceptibility of a genotype) and per cent seed yield reduction enabled the identification of potential terminal heat stress donors for validation and utilization in commercial breeding (Sandhu *et al.* 2019). Out of 150 genotypes tested, the top 10 lines (Table 4.7), which expressed lower YD (less than 10%) and low HSI (below 0.5), were marked for their potency as terminal heat stress donor lines and hence recommended for exploitation in commercial breeding after evaluation in larger plots. Among these identified donors, PM 26 was a released variety from IARI, New Delhi; BPR 349-9 was a germplasm line from DRMR, Bharatpur; RH 2041, RH 2020, RH 2050, RH 2049, RH 2034, RH 1400-2, RH 2015, and RH 1935 were pre-breeding lines from CCS HAU, Hisar. Previously, Meena *et al.* (2013) evaluated 22 genotypes of Indian mustard under late-sown conditions and reported that genotype BPR 538-10 showed terminal heat tolerance for seed yield/plant. Similar to our study, Sharma *et al*. (2022b) evaluated 145 genotypes of Indian mustard under late-sown conditions, and five genotypes, *viz*., DRMR 59, DRMR 2094, DRMR 2129, DRMR 2071, and DRMR 2136, were identified as high yielding as well as heat stress tolerant lines.

The late-sown crop was negatively impacted throughout their growth and development phase (vegetative growth stage) due to harsh winter, foggy, and frost conditions, while during the terminal siliqua filling stage, it influenced the movement of photosynthetic

products to the developing sink and resulted in lowering seed weight (size) and ultimately seed yield (Bhuller and Jenner, 1985). The per cent seed yield reduction ranged from 4.12% to 40.66% during *Rabi* season 2020–21 and from 4.73% to 48.57% during 2021–22. Previously, Chauhan *et al.* (2009) studied 18 advanced lines and four varieties of Indian mustard under terminal heat stress and reported a 22.2% reduction for number of seeds/siliqua and a 69.2% reduction for seed yield/plant.

### 5.5. Genetic diversity

Cluster analysis is a statistical method for grouping genotypes in such a way that genotypes that belong to the same group are similarly compared to genotypes in other groups, or clusters. It helps to discover the relationship between genotypes from various regions or origins and helps in the identification of genotypes in the various groups with useful traits for hybridization. In the present study, all 154 genotypes of Indian mustard were grouped into nine clusters on the basis of morpho-physiological traits. Previously, Singh *et al.* (2018) grouped 31 varieties of Indian mustard into five clusters, Sandhu *et al*. (2019) clustered the 96 genotypes into three clusters, Sharma *et al.* (2022a) divided 145 germplasm accessions of Indian mustard into two major groups based on agro-morphological traits, and Yadav *et al.* (2022) grouped 76 genotypes into five clusters based on their dissimilarity matrix. Out of these nine clusters, cluster 3 had the maximum number of genotypes (41 genotypes), and cluster 6 had the minimum number of genotypes (four) (Table 4.9). All four genotypes (RH 725, PM 26, RH 2049, and RH 2050) in cluster 6 were high-yielding genotypes. Clusters 1, 2, 3, and 6 had the heat-tolerant test and check genotypes, while cluster 4 had the heat-susceptible check genotypes (RH 749 and RH 8812).

The mean performance of morphological traits in each cluster showed that cluster 2 had the most promising genotypes, having the highest main shoot length and transpiration rate while having the minimum days to maturity, days to 50% flowering, and plant height. These genotypes are desirable under terminal heat conditions because early-maturing genotypes escape the terminal heat and give a good yield. Similarly, reduced plant height makes Indian mustard varieties preferred by the farmer's community due to lodging resistance. These genotypes could be used in a future heat-tolerant variety or hybrid development program. Cluster 3 had classified the genotypes based on their maximum days to maturity. These genotypes could be used for the development of high yielding varieties through pedigree breeding. Cluster 4 had the genotypes with the highest test weight. These bold-seeded genotypes are desirable for the development of bold-seeded hybrids because seed size is a major concern in the heterosis breeding of Indian mustard. Cluster 6 had four genotypes with the highest number of primary branches/plant, number of secondary branches/plant, number of siliquae on the main shoot, siliqua length, number of seeds/siliqua, seed yield/plant,

photosynthetic rate, stomatal conductance, chlorophyll content, and carotenoid content (two released and popular varieties, RH 725 and PM 26; two pre-breeding lines, RH 2049 and RH 2050). Three of the four genotypes were both high yielding and heat tolerant (PM 26, RH 2049, and RH 2050). These genotypes are suitable for late-sowing conditions with a minimum yield penalty under terminal heat conditions. These findings are in agreement with a previous study conducted by Yadav *et al.* (2022).

### 5.6. Molecular characterization

The present experiment examined the genetic diversity for heat stress tolerance in 154 genotypes (with different tolerance levels to heat) using SSR markers. The discovery of PCR based molecular marker techniques (like SSRs) led to the possibility of performing extensive molecular diversity studies in an important oil yielding crop like Indian mustard to find genotypes with desirable agronomic traits. In the present study, a total of 312 polymorphic alleles were generated by 111 polymorphic SSR primers in 154 genotypes tested, with an average of 2.81 alleles per primer, ranging from 2 to 6 alleles. The number of polymorphic SSR alleles determined (312) in the present study is significantly higher than the 157 alleles detected by Singh *et al.* (2021c); the 114 alleles reported by Singh *et al.* (2021b); the 109 alleles reported by Shyam *et al.* (2020); and the 47 alleles reported by Avtar *et al.* (2016). This difference might be due to different sampling sizes, different genotypes used in previous studies, and different SSR primers taken for the study.

The concept of polymorphism is used to determine the genetic variability in the population. The measure or value of the "polymorphism information content" (PIC) is determined by the ability of a SSR marker to establish polymorphism in the population depending on the number of alleles detected and their distribution frequency (Botstein, 1980). On the basis of PIC value, Botstein (1980) classified the markers as highly informative (PIC > 0.5), informative (0.5 > PIC > 0.25), and slightly informative (PIC < 0.25). In the present study, we found 15 SSR markers were highly informative, 63 SSR markers were informative, and 33 SSR markers were slightly informative. The PIC value ranged from 0.013 (BG 150/BG 258) to 0.627 (BG 105) with an average PIC value of 0.31, while Sharma *et al.* (2022a) and Singh *et al.* (2021b) reported slightly higher mean PIC values (0.39 and 0.43, respectively) for Indian mustard germplasm evaluated for yield traits and Sclerotinia stem rot. The higher PIC values in the above studies could be due to the diverse germplasm, the greater number of makers, or the resolution systems used. The gene diversity ranged from 0 to 0.994, with an average value of 0.432. The average major allele frequency for SSR markers was 0.688, with a range of 0.344 (BG196) to 0.994 (BG150/BG258). These findings are in agreement with previous studies by Yu *et al.* (2010), Shyam *et al.* (2020), Thakur *et al.* (2021), Singh *et al.* (2021a), Sharma *et al.* (2022a), and Yadav *et al.* (2022).

A dendrogram was constructed using Nei's distance matrix, and a UNJ tree was constructed using this distance matrix, which clustered 154 Indian mustard genotypes into three main clusters (A, B, and C). Our findings are consistent with preceding studies where Singh *et al.* (2021b) also obtained three major clusters while evaluating 16 Indian mustard genotypes for Sclerotinia stem rot resistance. Similarly, Shyam *et al.* (2020) also obtained three major clusters while evaluating 48 genotypes to identify genotypes with low and high erucic acid content on the basis of SSR markers. Major cluster C could be further grouped into two sub-clusters, *viz*., C1 and C2. The sub-cluster C2 could be divided into two minor clusters, *viz*., C21 and C22, and further C22 minor clusters into C22a and C22b. The clusters A and B, the sub-cluster C1, and the minor cluster C21 contain all the genotypes, germplasm lines, and pre-breeding lines from CCS HAU, Hisar. Seven of the top ten heat-tolerant genotypes belonged to one of these clusters. Because of the lower dissimilarity between individual genotypes from the same research station, these all fell into sub-cluster 2 (green color) of the population structure. The minor cluster C22 occupies the genotypes from CCS HAU, Hisar; DRMR, Bharatpur; IARI, New Delhi; and PAU, Ludhiana. The similar pattern of grouping matched the findings of Sharma *et al*. (2022a), where, out of 145 total genotypes, 136 accessions of Indian mustard germplasm, comprising both Indian and exotic accessions from countries including Australia, Canada, Germany, and the UK, showed grouping in sub-subgroup IIb2.

### 5.7. Population structure and AMOVA

Two clusters were identified, consisting of 83 (the red cluster) and 54 (the green cluster) genotypes. Seventeen genotypes did not match the cut-off membership probability of 70% and were considered mixtures. The reason for the smaller number of significant clusters might be due to the same geographical origin and close ancestral history of the test genotypes in the present study. Our findings are consistent with preceding studies where Zhang *et al.* (2017) obtained the highest likelihood score at delta K = 2 and grouped the 81 accessions of *B. carinata* into two sub-populations. Similarly, Singh *et al*. (2022d) grouped the 87 varieties of Indian mustard into two sub-populations. However, Singh *et al*. (2021b), Thakur *et al*. (2021), Rahman *et al.* (2022), and Sharma *et al.* (2022a) grouped the genotypes into three clusters (sub-populations). The greater the number of sub-populations, the more diverse the genotypes in the panel, which are drawn from different geographical regions. The two sub-populations exhibited by structure analysis were exposed to AMOVA to depict the patterns of variation among and within sub-populations. The AMOVA results showed that higher variation (78%) was observed among the genotypes (within populations) as compared to between the groups (populations), indicating that the genotypes used in the present study were diverse and could be used in Brassica improvement programme. A similar pattern of variation was reported in previous studies by Sharma *et al.* (2022a), where they obtained 88% of the total variation within the

groups during assessing 145 Indian mustard germplasm accessions. Similarly, 76.5% of the total variation within the groups reported by Rahman *et al.* (2022) during examining 383 rapeseed germplasm accessions.

### 5.8. Linkage disequilibrium

Linkage disequilibrium can be defined as the correlation among polymorphisms in a given population (Goode, 2011). The strength of association mapping relies on the degree of LD between the SSR marker and the functional variant. A total of 312 SSR marker-based alleles were used to calculate the extent of LD that led to pairwise LD detection in 24754 locus pairs in 154 Indian mustard genotypes. Out of 24754 locus pairs, a total of 580 SSR marker pairs (2.3%) showed significant LD at threshold (i.e., $r^2 \geq 0.1$). Out of 580 SSR marker pairs with significant LD, 24 significant LD were collinear, i.e., marker pairs on the same chromosome, and 556 significant LD were inter-chromosomal. In the entire collection considering both the A and B genomes, the mean linked LD and mean unlinked LD were 0.48 and 0.02, respectively; and the loci pair under linked LD and unlinked LD was 4.14% and 95.86%, respectively. The same pattern of LD was reported by Rahman *et al.* (2022) and Akhatar *et al*. (2021) in *Brassica napus* and *Brassica juncea* germplasm, respectively.

The extent of LD can provide information for the required marker density and mapping resolution in an association mapping study (Gupta *et al.,* 2005). In many self-pollinated and often cross-pollinated crops, LD extends over long chromosomal distances (Malysheva-Otto *et al.,* 2006), so a relatively small number of markers are needed to cover the entire genome, whereas a higher number of markers per linkage group is necessary where LD decays very rapidly in cross pollinated species like maize (Remington *et al.,* 2001). Hence, with relatively few markers per chromosome, the Indian mustard genome may be efficient for LD-mapping of seed yield and yield-related traits. The size of intra-chromosomal LD blocks was also calculated; at $r\,2 \geq 0.1$ on 18 chromosomes, the longest LD block (26.86 Mb) was observed on chromosome 5 between BG 269 and BG 196. The average LD block size of the A genome was 9.93 Mb and that of the B genome was 12.36 Mb, so the few SSR markers per chromosome for the B genome may be efficient for association mapping. These findings are in agreement with previous studies by Korber *et al.* (2016) and Rahman *et al.* (2022). The presence of long LD blocks indicates the high level of gene conservation, which could have resulted from the exchange of large chromosomal segments during evolution or from limited natural recombination (Rahman *et al.,* 2022). The average LD (D' value) of the A genome (0.60) was lower as compared to the B genome (0.80). Though the low LD of an A genome requires more markers to pinpoint the location of various QTL.

### 5.9. Association mapping

TASSEL 5.0 (http://www.maizegenetics.net/) was used to implement a general linear

model and a mixed linear model-based approach in order to have significant marker-trait associations (Bradbury, 2007). Population structure and genetic relatedness are the main obstacles to the successful application of association mapping in plants, since they may produce false marker-trait associations that make it difficult to identify loci that actually influence the desired traits (Gupta *et al*., 2005; Myles *et al.,* 2009). To address this population structure and relatedness issue, several statistical techniques and models have been developed (Yu *et al.*, 2006). MLM (mixed linear model) is a straightforward and effective technique that simultaneously takes into consideration many degrees of relatedness and can enhance control over both type I and type II error rates to reduce false results (Yu *et al.*, 2006). In the present study, both GLM (Q) and MLM (Q + K) methods were used to compare the results and remove false marker-trait associations. Previously, Cai *et al.* (2014), Luo *et al.* (2015), Akhtar *et al.* (2021), and Wassan *et al.* (2021) used similar methods of marker-trait association for mapping various traits in different Brassica species.

Significant marker-trait associations were found in the pooled *Rabi* 2020-21 and 2021-22 data, in both timely and late-sown data. A p-value threshold of $P \leq 0.005$ (–log10 value = 2.30) was used to identify significant marker-trait associations. Under a timely-sown environment, a total of 51 and 29 significant marker trait associations were identified by GLM (Q) and MLM (Q + K) methods, respectively (Table 5.1). Out of these 80 associations, 20 significant marker-trait associations were common in both methods (Table 5.2). Under the late-sown environment, a total of 50 and 33 significant marker trait associations were identified by the GLM (Q) and MLM (Q + K) methods, respectively (Table 5.3). Out of these 83 associations, 26 significant marker-trait associations were common in both methods (Table 5.4).

**Table 5.1 Number of significant markers-trait associations identified in GLM (Q) and MLM (Q + K) method under timely sown environment**

| Method | Number of Significant Association |
|---|---|
| GLM (Q) | 51 |
| MLM (Q + K) | 29 |
| Common | 20 |

**Table 5.2: List of Significant marker-trait association common in GLM (Q) and MLM (Q + K) method under timely sown environment**

| Sr. No. | Trait | Marker | Chr | Position (Mb) | p value | Previous reports |
|---|---|---|---|---|---|---|
| **1.** | Caro | BG222-125 | A9 | 48.91 | 1.71E-04 | |
| **2.** | DF | BG133-250 | A2 | 13.81 | 0.00336 | Akhatar *et al.* (2021) |
| **3.** | DF | BG133-340 | A2 | 13.81 | 0.00336 | Akhatar *et al.* (2021) |
| **4.** | Gs | BG264-225 | A6 | 10.98 | 0.00108 | |
| **5.** | Gs | BG31-200 | A7 | 25.84 | 0.0036 | |
| **6.** | Gs | BG247-275 | B6 | 31.98 | 0.00255 | |

| | | | | | | |
|---|---|---|---|---|---|---|
| **7.** | MSL | BG266-280 | A3 | 14.01 | 8.60E-05 | |
| **8.** | MSL | BG7-215 | A8 | 22.27 | 2.76E-05 | Cai *et al.* (2014), Yadava *et al.* (2012) |
| **9.** | MSL | BG8-235 | A8 | 19.81 | 1.24E-04 | Cai *et al.* (2014), Yadava *et al.* (2012) |
| **10.** | MSL | BG198-480 | B6 | 57.69 | 3.89E-04 | |
| **11.** | NPB | BG144-370 | A5 | 23.11 | 0.00201 | |
| **12.** | NPB | BG204-290 | A8 | 18.49 | 6.04E-04 | |
| **13.** | NPB | BG204-230 | A8 | 18.49 | 0.00228 | |
| **14.** | NSB | BG256-250 | A7 | 30.00 | 9.28E-04 | Yadava *et al.* (2012) |
| **15.** | NSMS | BG17-550 | A5 | 6.98 | 5.58E-04 | |
| **16.** | OC | BG138-200 | A5 | 20.61 | 0.00174 | Yadava *et al.* (2012) |
| **17.** | PH | BG65-200 | B8 | 56.21 | 2.93E-04 | Akhatar *et al.* (2021), Sandhu *et al*. (2019) |
| **18.** | SYP | BG204-290 | A8 | 18.49 | 9.64E-05 | |
| **19.** | SYP | BG204-230 | A8 | 18.49 | 6.72E-04 | |
| **20.** | TW | BG198-480 | B6 | 57.69 | 4.68E-04 | |

Where, Caro – Carotenoid content (mg/g); DF-Days to 50% flowering; Gs – Stomatal conductance (mmol/$m^2$/S); MSL-Main shoot length (cm); NPB-Number of primary branches/plant; NSB-Number of secondary branches/plant; NSMS-Number of siliquae on main shoot; OC-Oil content (%); PH-Plant height (cm); SYP-Seed yield/plant; TSW-1000-seed weight (g)

**Table 5.3 Number of significant markers-trait associations identified in GLM (Q) and MLM (Q + K) method under late sown environment**

| Method | Number of Significant Association |
|---|---|
| GLM (Q) | 50 |
| MLM (Q + K) | 33 |
| Common | 26 |

**Table 5.4: List of Significant marker-trait association common in GLM (Q) and MLM (Q + K) method under late sown environment**

| Sr. No. | Trait | Marker | Chr | Position (Mb) | p value | Previous reports |
|---|---|---|---|---|---|---|
| **1.** | A | BG252-190 | A3 | 28.60 | 2.34E-05 | |
| **2.** | A | BG247-275 | B6 | 31.98 | 2.76E-06 | |
| **3.** | Caro | BG31-160 | A7 | 25.84 | 0.00426 | |
| **4.** | Caro | BG247-275 | B6 | 31.98 | 2.75E-04 | |
| **5.** | Chl | BG204-230 | A8 | 18.49 | 0.00333 | |
| **6.** | Chl | BG247-275 | B6 | 31.98 | 7.46E-07 | |
| **7.** | DF | BG157-220 | B6 | 26.10 | 9.51E-04 | Akhatar *et al.* (2021), Yadava *et al.* (2012) |
| **8.** | DM | BG258-225 | B3 | 26.10 | 0.00206 | Sandhu *et al*. (2019) |
| **9.** | DM | BG247-275 | B6 | 31.98 | 0.00271 | |
| **10.** | Gs | BG175-210 | A10 | 16.55 | 0.00409 | |
| **11.** | Gs | BG247-275 | B6 | 31.98 | 1.49E-10 | |
| **12.** | MSL | BG133-250 | A2 | 13.81 | 7.85E-05 | |
| **13.** | MSL | BG133-340 | A2 | 13.81 | 7.85E-05 | |
| **14.** | MSL | BG7-215 | A8 | 22.27 | 0.00385 | Cai *et al.* (2014), Yadava *et al.* (2012) |

| | | | | | | |
|---|---|---|---|---|---|---|
| **15.** | MSL | BG260-215 | A9 | 48.20 | 2.05E-04 | |
| **16.** | MSL | BG198-480 | B6 | 57.69 | 1.49E-04 | |
| **17.** | NPB | BG204-290 | A8 | 18.49 | 0.00193 | |
| **18.** | NSB | BG256-250 | A7 | 30.00 | 2.55E-05 | Yadava *et al.* (2012) |
| **19.** | NSMS | BG121-630 | A1 | 7.33 | 0.00426 | Sandhu *et al*. (2019) |
| **20.** | NSS | BG247-275 | B6 | 31.98 | 3.51E-04 | |
| **21.** | PH | BG149-160 | A3 | 19.76 | 0.00364 | Shi *et al.* (2009), Körber *et al*. (2016) |
| **22.** | SL | BG35-600 | A7 | 15.01 | 2.41E-04 | Yadava *et al.* (2012) |
| **23.** | SYP | BG204-230 | A8 | 18.49 | 2.83E-05 | |
| **24.** | SYP | BG204-290 | A8 | 18.49 | 3.15E-05 | |
| **25.** | TW | BG198-480 | B6 | 57.69 | 0.00143 | |
| **26.** | TW | BG156-200 | B8 | 67.55 | 0.00136 | Ramchiary *et al*.(2007), Yadava *et al.* (2012), Sandhu *et al*. (2019) |

Where, A–Photosynthetic rate (µmol $CO_2/m^2$ /S); Caro – Carotenoid content (mg/g); Chl – Total chlorophyll content (mg/g); DF-Days to 50% flowering; DM-Days to maturity; Gs – Stomatal conductance (mmol/$m^2$/S); MSL-Main shoot length (cm); NPB-Number of primary branches/plant; NSB-Number of secondary branches/plant; NSMS-Number of siliquae on main shoot; NSS-Number of seeds/siliqua; PH-Plant height (cm); SL-Siliqua length (cm); SYP-Seed yield/plant; TSW-1000-seed weight (g).

**Table 5.5 List of significant marker-trait association {MLM (Q + K)} identified at threshold p ≤ 0.005 only in late sown environment**

| Sr. No. | Trait | Marker | Chromosome | Position (Mb) | p value |
|---|---|---|---|---|---|
| 1 | A | BG252-190 | A3 | 28.6 | 0.0031 |
| 2 | A | BG247-275 | B6 | 31.98 | 1.33E-05 |
| 3 | Caro | BG31-160 | A7 | 25.84 | 0.00415 |
| 4 | Caro | BG204-230 | A8 | 18.49 | 0.00419 |
| 5 | Caro | BG247-275 | B6 | 31.98 | 2.20E-04 |
| 6 | Chl | BG247-275 | B6 | 31.98 | 2.34E-06 |
| 7 | DF | BG157-220 | B6 | 26.1 | 0.002 |
| 8 | DM | BG258-225 | B3 | 26.1 | 0.00149 |
| 9 | DM | BG247-275 | B6 | 31.98 | 0.00393 |
| 10 | Gs | BG175-210 | A10 | 16.55 | 0.00291 |
| 11 | MSL | BG53-400 | A2 | 2.38 | 0.0032 |
| 12 | MSL | BG260-215 | A9 | 48.2 | 0.00236 |
| 13 | NSMS | BG121-630 | A1 | 7.33 | 0.00439 |
| 14 | NSS | BG121-630 | A1 | 7.33 | 0.00478 |
| 15 | NSS | BG247-275 | B6 | 31.98 | 5.60E-04 |
| 16 | PH | BG149-160 | A3 | 19.76 | 0.00439 |
| 17 | PH | BG82-200 | A5 | 6 | 0.0031 |
| 18 | SL | BG35-600 | A7 | 15.01 | 0.00299 |
| 19 | TSW | BG204-290 | A8 | 18.49 | 0.00362 |
| 20 | TSW | BG156-200 | B8 | 67.55 | 0.00211 |

Where, A–Photosynthetic rate (µmol $CO_2/m^2$ /S); Caro – Carotenoid content (mg/g); Chl – Total chlorophyll content (mg/g); DF-Days to 50% flowering; DM-Days to maturity; Gs – Stomatal conductance (mmol/$m^2$/S); MSL-Main shoot length (cm); NSMS-Number of siliquae on main shoot; NSS-Number of seeds/siliqua; PH-Plant height (cm); SL-Siliqua length (cm); TSW-1000-seed weight (g).

The population structure (Q) and relative kinship (K) in the panel of different Brassica species lines have been evaluated previously by Ramchiary *et al*. (2007), Shi *et al*. (2009), Cai *et al.* (2014), Körber *et al.* (2016), Sandhu *et al.* (2019), and Akhatar *et al.* (2021), and their

effects on associations were evaluated with different statistical models.

Under late sown conditions, a total of 20 SSRs were specifically detected that were not associated under timely-sown conditions (Table 5.5). This meant that these specific genomic regions and QTLs were associated under terminal heat conditions, and it also meant that SSRs detected under timely sowing conditions might not be associated under late sowing conditions. These results are in agreement with the previous findings of Sandhu *et al.* (2019), where they found 24 SNPs only under terminal heat shock conditions.

The A genome contained 64% of the polymorphic SSR markers, the B genome contained 33%, and the remaining 3% were unknown. The chromosome wise distribution of polymorphic SSR markers is shown in Figure 5.2. The maximum number of markers (13) were found polymorphic on chromosome A3, followed by chromosomes A5 and A8. The average of six polymorphic SSR markers were found for per chromosome.

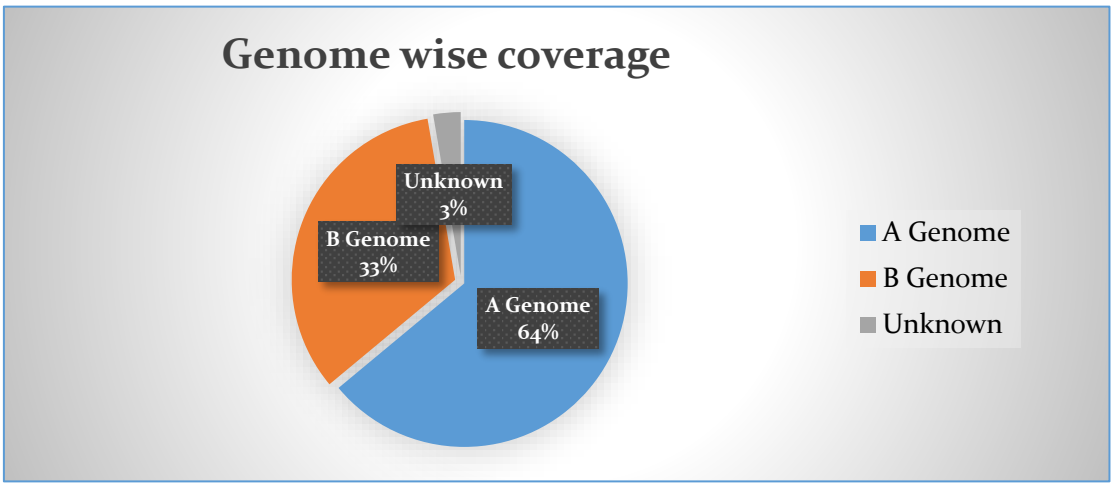


**Figure 5.1 Genome wise coverage of polymorphic SSR marker**

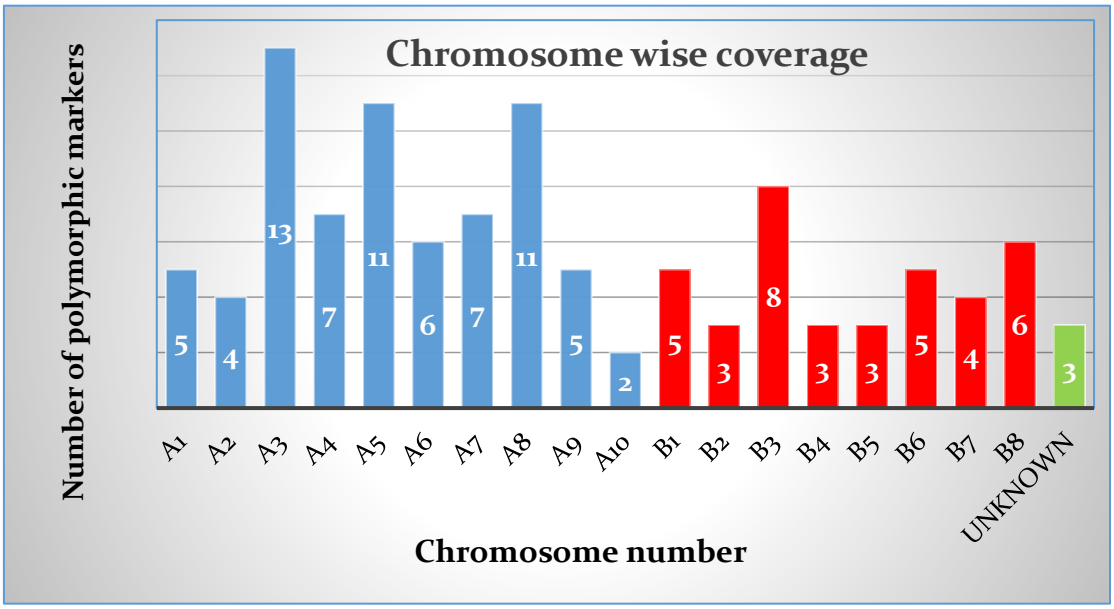


**Figure 5.2 Chromosome wise coverage of polymorphic SSR markers**

## *CHAPTER*-VI

## *SUMMARY AND CONCLUSION*

The present investigation entitled **"Population structure and genetic diversity studies for terminal heat stress tolerance in Indian mustard [*Brassica juncea* (L.) Czern & Coss.]"** was conducted at Research Farm of Oilseeds Section, Department of Genetics and Plant Breeding, during the *Rabi* seasons of 2020–21 and 2021–22. The main objectives have been:

- To investigate the morpho-physiological diversity among the genotypes of Indian mustard.
- To estimate the population structure and linkage disequilibrium (LD) using SSR markers for terminal heat tolerance in Indian mustard.
- To study correlation and path analysis among various yield-contributing and physiological traits.

The plant materials for the present study comprised 154 Indian mustard genotypes grown in an augmented design during *Rabi* 2020–21 and 2021–22 with two dates of sowing, i.e., timely (15$^{th}$ October) and late sown (15$^{th}$ November). Observations were recorded for 12 morphological traits, namely; days to 50% flowering, days to maturity, plant height, number of primary branches/plant, number of secondary branches/plant, main shoot length, number of siliquae on the main shoot, siliqua length, number of seeds/siliqua, 1000-seed weight, seed yield/plant and oil content. The observations for all the morphological traits were recorded on five randomly selected plants from each genotype, except for days to 50% flowering and days to maturity, where, observations were recorded on a whole plot basis. Three physiological traits *viz*; photosynthetic rate, stomatal conductance, and transpiration rate were measured with the help of an infrared gas analyser (CID 301, USA). During the siliqua filling stage, measurements were taken in triplicate between 11:00 a.m. and 1:00 p.m. Total chlorophyll content and carotenoid content were estimated using the DMSO method of pigment extraction. For both timely and late sown crops, a leaf sample (100 mg) was collected 20 days before crop maturity for pigment extraction. Total genomic DNA of 154 samples were isolated by CTAB extraction method of Saghai-Maroof (1984). A number of SSR markers have been developed for Indian mustard varying in their degree of polymorphism which in turn gives a molecular description of mustard cultivars. A total of 237 SSR markers were assayed for molecular analysis.

The whole study is summarized under the following four major experiments:

### 1. Morpho-physiological diversity

The mean sum of squares due to genotypes revealed significant differences for all the morpho-physiological traits in timely and late sown environments indicating the presence of adequate genetic variation among the genotypes, while, mean sum of squares due to year

showed significant differences for most traits except number of primary branches, main shoot length, seed yield/plant, transpiration rate, chlorophyll content, and carotenoid content. The quantitative data recorded were analysed for mean, range, GCV, PCV, heritability, genetic advance, heat susceptibility index (HSI) and per cent seed yield reduction (YD%). The genetic diversity analysis was carried out using Ward's method. A brief summary of the results obtained and the conclusions drawn from this study are furnished below:

- The high PCV and GCV estimates recorded for photosynthetic rate and stomatal conductance indicate a high possibility of selection on these traits. Primary branches/plant, secondary branches/plant, siliqua length, 1000-seed weight, seed yield/plant, transpiration rate, total chlorophyll content, and carotenoid content had moderate PCV and GCV. The low GCV was observed for days to 50% flowering, days to maturity, plant height, main shoot length, number of siliquae on main shoot, number of seeds/siliqua and oil content.
- High heritability (broad-sense) for all of the studied traits except transpiration rate. High heritability (broad sense) along with high genetic advance was estimated for number of primary branches/plant, number of secondary branches/plant, siliqua length, 1000-seed weight, seed yield/plant, photosynthetic rate, stomatal conductance, total chlorophyll content, and carotenoid content, while moderate genetic advance was estimated for main shoot length and number of siliquae on main shoot.
- Cluster analysis grouped 154 genotypes into nine clusters on the basis of morpho-physiological traits. Out of these nine clusters, cluster 3 had the maximum number of genotypes (41 genotypes), and cluster 6 had the minimum number of genotypes (4).
- Based on heat susceptibility index (HSI) and per cent seed yield reduction (YD%), ten genotypes, *viz.*, RH 2041, RH 2020, RH 2050, BPR 349-9, RH 2049, PM 26, RH 2034, RH 1400-2, RH 2015 and RH 1935 were observed heat tolerant at terminal stage.

**2. Correlation and path analysis**

The experimental material for this experiment comprised 154 genotypes (150 test genotypes and four checks). All the above mentioned genotypes were grown in an augmented design during *Rabi* 2020–21 and 2021–22 with two dates of sowing, i.e., timely and late sown. The pooled data from all four environments was subjected to statistical analysis. A brief summary of the results obtained and the conclusions drawn from this study are furnished below:

- Seed yield/plant was significantly and positively correlated with plant height, number of primary branches/plant, number of secondary branches/plant, main shoot length, number of siliquae on the main shoot, siliqua length, number of seeds/siliqua, 1000-seed weight, photosynthetic rate, stomatal conductance, total chlorophyll content, and carotenoid content.

- Path analysis revealed that traits *viz*; number of primary branches/plant, 1000-seed weight, number of secondary branches/plant, siliqua length, and number of seeds/siliqua had direct contribution towards seed yield/plant. Hence, these characters would be imperative during selection for improvement of seed yield/plant under terminal heat stress.

**3. Genetic diversity and population structure**

DNA was isolated from all 154 genotypes by the standard method and subjected to SSR marker analysis. A total of 237 SSR markers were run for molecular analysis. Out of these 237 SSR markers, 111 were polymorphic. The molecular data from these polymorphic markers were subjected to statistical analysis by the POWERMARKER version 3.25 and STRUCTURE version 2.3.4 software packages. The major findings are given below:

- The number of polymorphic alleles per SSR marker ranged from 2 to 6, with an average of 2.81 alleles.
- PIC values for all polymorphic SSR markers ranged from 0.013 to 0.627, with an average PIC value of 0.31.
- The chromosome-wise average of PIC values revealed that chromosome A10 has the highest mean value of PIC (0.546), followed by chromosome B4 (0.506), and chromosome A4 (0.436).
- All the 154 genotypes were categorized into three major clusters (A, B, and C) depending on Euclidean distance.
- Population structure analysis of the Indian mustard genotypes grouped the 154 genotypes into two clusters at a maximum likelihood value of delta K =2.
- AMOVA revealed that, major part of the total variance was among the genotypes present within a sub-population as compared to between different sub-populations.

**4. Linkage disequilibrium and association mapping**

Marker-trait associations were identified by GLM (Q) and MLM (Q + K) methods using the results of population structure and morpho-physiological traits. The morphological data (pooled data from timely and late environments separately), molecular data, and population structure (q matrix) were subjected to TASSEL version 5.2.85 statistical software. Significant marker-trait associations were identified for timely and late sown environments separately. On the basis of these results, the major findings of the present investigation are:

- A total of 580 SSR marker pairs (2.3%) showed significant LD at threshold (i.e., $r^2 \geq 0.1$). Out of 580 SSR marker pairs with significant low-density (LD), 24 significant LD were collinear, i.e., markers on the same chromosome, and 556 significant LD were inter-chromosomal.

- The longest LD block (26.87 Mbp) was observed on chromosome 5 between BG 269 and BG 196.
- A total of 51 and 50 significant marker-trait associations were identified by the GLM (Q) method at a significant threshold value of $P \leq 0.005$ in the timely and late sown environments, respectively.
- A total of 29 and 33 significant marker-trait associations were identified by the MLM (Q + K) method at a significant threshold value of $P \leq 0.005$ in timely and late-sown environments, respectively.
- For a timely planted crop, out of 80 {GLM (Q) + MLM (Q + K)} significant marker-trait associations, 20 marker-trait associations were common in the results of both methods.
- In the timely sown crop, out of 20 significant marker-trait associations common in both methods, 13 significant marker-trait associations identified were novel.
- For the late-planted crop, out of 83 {GLM (Q) + MLM (Q+K)} significant marker-trait associations, 26 marker-trait associations were common in the results of both methods.
- In the late-planted crop, out of the 26 significant marker-trait associations common to both methods, 18 significant marker-trait associations were novel.
- Twenty significant marker-trait associations detected only for late sown environment.

**Conclusion:**

Since Indian mustard is a heat-sensitive crop, high temperatures during the terminal stage might result in severe yield losses. Genotypes that are tolerant to terminal heat stress must be developed immediately in order to increase crop productivity under heat stress and expand the cultivation area. In order to do this, we tested 150 different genotypes of Indian mustard in the field, where heat stress was noted during the seed filling stage. The accessions found in this study had terminal heat stress tolerance related to a number of morpho-physiological traits, making them potential parental lines for breeding projects. As anticipated, several important marker-trait associations that are directly or indirectly connected to the terminal heat stress (THS) tolerance mechanism were found. These findings will help in the selection of donor lines or lines with favourable alleles for various THS tolerance traits. Under control and terminal heat stress conditions, the chromosomes B6 (12 marker-trait associations), A8 (11 marker-trait associations), and A7 (5 marker-trait associations) showed the highest number of marker-trait associations common in GLM (Q) and MLM (Q + K). Under terminal heat conditions, a total of 20 SSRs were specifically detected that were not associated under normal sown conditions. This meant that these specific genomic regions and QTLs were associated under terminal heat conditions. Before initiating a breeding programme for terminal heat stress tolerance in Indian mustard, significant marker-trait associations need further validation, and it will be a great help

in understanding complex genetic architecture traits under terminal heat stress. This study is a critical first step in understanding the genetics of terminal heat stress tolerance, which is crucial in the development of Indian mustard cultivars that can withstand high temperature stress.

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**APPENDIX-I: A brief description of 237 SSR primers used during the investigation**

| Sr. No. | Name of primer | Primer code | Forward Primer | Reverse Primer |
|---|---|---|---|---|
| 1 | cnu_m051a | BG 1 | GCTGGCTGCACAATAACAGA | GTACCACTGGAGGAGCTTCG |
| 2 | cnu_m054a | BG 2 | GGCCTTTGGAGGTGACTGTA | CAGGGATATGCGGTCTTTCT |
| 3 | cnu_m055a | BG 3 | AGGAACGTTGCAGAGCAGTT | GTCTGTGACGGAATCAGCAA |
| 4 | cnu_m056a | BG 4 | CTGGTTTGGTTCGGTTTGAT | CCTGACAAATAGCAAGAAGTCG |
| 5 | cnu_m057a | BG 5 | TCACATGTGGGAAACATTCCT | TGTCATTTTTACTGCATTTTCCGTAT |
| 6 | cnu_m058a | BG 6 | TGAGGGTGAGGATGGTGATG | GCACAGTACACCGACGCCTA |
| 7 | cnu_m059a | BG 7 | GGGATATTGAAGACCCGCAAA | TCTCCCGGTGGCTTAAAGAA |
| 8 | cnu_m060a | BG 8 | TTGGATCAATCAAACTAAACCCTGA | CCAAAATGCCAACAAAAGCA |
| 9 | cnu_m061a | BG 9 | GGTGACCACCTCCGTCTTCTT | CTGTATGGAGCCCCAAGCTC |
| 10 | cnu_m065a | BG 11 | AGCCCAAGGTTTACGGTGGT | CGAGTTTGGACCCTCGATATG |
| 11 | cnu_m066a | BG 12 | TTCGATTGAAACACTGAACATTGAA | GCGTTTTCTGTTTTCCCAATAA |
| 12 | cnu_m070a | BG 15 | CATAACCACACGGCCTCCTC | AAGTCATGCCCATTCGCCTA |
| 13 | cnu_m071a | BG 16 | CGAATCCGACGTGAATTTGA | ATTGAGAAGGTCCGCCATGA |
| 14 | cnu_m072a | BG 17 | TGTCATTGTTTCCGCCATTG | CTCCCTCCTCCGACAACAAC |
| 15 | cnu_m077a | BG 19 | CGTTGTGTGAAATCGCTCAAAT | TCCGAACTAGAAACCGAAAATATCC |
| 16 | cnu_m078a | BG 20 | TCACGTGGCAATATGCGAAC | CTCCGCCACTGGTTGAATCT |
| 17 | cnu_m081a | BG 23 | GAGGCAAAAGCGAAGGTGAA | AGCACCCAAACACTCCCAAA |
| 18 | cnu_m084a | BG 26 | GGTGGTGATCGTGATGGTAATG | TTGCCATACCACCAGTTTGCT |
| 19 | cnu_m085a | BG 27 | TCCATATGAATGGGTGGTAGTTG | GTTTGCCTCCAACCCCTTTC |
| 20 | cnu_m086a | BG 28 | TCACGCATGTCAGAGCCATT | AACCGCGCGTACGATACACT |
| 21 | cnu_m088a | BG 30 | GGATGTTCACGCCGTATGTG | CCATAAACTGCATTGTTTGAATTG |
| 22 | cnu_m089a | BG 31 | GCCAGTCGAAACAGATTAGCTAGG | CCACTTTGATTACCTTGCTTTTTCA |
| 23 | cnu_m091a | BG 32 | TCACCATGTGCGAGAGCCTA | CGGGCAGATCAAGAAACAGA |
| 24 | cnu_m092a | BG 33 | CGTGTGTCCTCTCGTGTCTCA | TGCTCAGCAGTCAGCAATCA |
| 25 | cnu_m094a | BG 35 | GAGAGAGAGAGAGAGAGAGAGAAAGA | CCGATACACAACCAGCCAAC |
| 26 | cnu_m096a | BG 37 | GCACCTAACCGAACCCCTTTAG | GAGAAGATCGTAGGGCACTGGA |
| 27 | cnu_m097a | BG 38 | AAATTCAGCGTTTTCGACCA | CTGAGGCGTGAGAGAAGAGAGA |
| 28 | cnu_m101a | BG 39 | TCAAACGCAAATTCAATAAGACAAA | ACTAGATTTCCACCCGCACAAC |
| 29 | cnu_m103a | BG 41 | TCCTCCGACAACAACAACTCAA | ATCTAACCCGTCTGCGAATCTG |
| 30 | cnu_m104a | BG 42 | CGTTTTTCCTTGGTTATTTGGA | TCGTTCAAATGTCGTATGGACAC |
| 31 | cnu_m106a | BG 43 | TTTTGTTTCGAAAAATTCATTCCAT | TGGTTGCAACGACTGACACA |
| 32 | cnu_m107a | BG 44 | TGGACGTAACACCCATCTTGAA | AGCTGAGGAAGTGGCTGAGG |
| 33 | cnu_m108a | BG 45 | TCCAAGAGACGAAACCACTTCC | GCTTGCTTATATCCTTCCTTGCC |
| 34 | cnu_m109a | BG 46 | AGAGAGGGAAGGCGAAAGTGAT | GGTTAAATGAAACAGAGGGACCAA |
| 35 | cnu_m111a | BG 48 | CACGAAAGCTGTAGAGGCATGA | TCTTTTCCTGTCCATGAGATTCAA |
| 36 | cnu_m112a | BG 49 | CGGAAACGCACATCTCTCACT | TCGATCCATTAAGCCAAACTCA |
| 37 | cnu_m113a | BG 50 | CGCCAAATCAAATTAGGGTTTA | CCACGAATTTAACAAGAGACATCC |
| 38 | cnu_m115a | BG 51 | GGCGGTCCATCAAACTGGTA | CTGTCCCACAAGCAAAGATTCA |

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|---|---|---|---|---|
| 39 | cnu_m117a | BG 52 | CAACAAAGGGTTTGAAAACTAAACTCA | GGCGCGGGTCTTAACCTAGT |
| 40 | cnu_m118a | BG 53 | TCCTTTTGCTTTCTCTCCATCC | AGACGCCGTCCAAGACAGAG |
| 41 | cnu_m121a | BG 54 | ACAGGAGAAACGCAACACCA | GATGCAAACGCTAGCCCAAT |
| 42 | cnu_m123a | BG 56 | ACTTGGGCGGTGAAACAGTAAA | GTTATGTGGTGGAGAGGCACAA |
| 43 | cnu_m124a | BG 57 | TGCTGGTTATGTTTGCTGATGG | TTCAATCCACGTTTTAGTGCCC |
| 44 | cnu_m128a | BG 60 | TCACATTGAAAGTAAAATAGGTTGA | ACACACACACATACACACACACA |
| 45 | cnu_m130a | BG 62 | GGAGAGTACGCGGAGAGGAA | TTGCAAACGCACCACCAC |
| 46 | cnu_m133a | BG 64 | ACATGAATTACGCCGTGCAT | TCAATTTCACTATTTTGAGATCCTTT |
| 47 | cnu_m134a | BG 65 | TCTCTTTGCCATCGTCGTTTC | CCCCTCAAACTGAGCAGTCAA |
| 48 | cnu_m135a | BG 66 | GAAAATACACCTCGCTTTTACACTCA | AAGAATTTAGGGTTCGAAAAGGAG |
| 49 | cnu_m136a | BG 67 | AAGCTTGCTTTCCCCGATTC | CCATATTAAGCTTCTATTTCTTTCACA |
| 50 | cnu_m137a | BG 68 | AACCTTCATTTCATATACATACACACA | TTCAATCATTTTTATTGGTCATCA |
| 51 | cnu_m140a | BG 70 | AGCTATAGCACATATTGAAACATATTG | AAGCGGGTACGTGTTGGAAG |
| 52 | cnu_m141a | BG 71 | TGGCAATGGTTTCAAGCTCA | ACTGCCTCGCAAGGAAAGAG |
| 53 | cnu_m144a | BG 73 | GCGTGCAGGGATTAGCTTGT | CCAACTCGCCCTTCTCTTCA |
| 54 | cnu_m147a | BG 75 | AAAAAGATACAAGACATGGATTTCTGC | AAACAATGTGTAATCGCAGCAGTAG |
| 55 | cnu_m151a | BG 76 | TGGACCACTTCCGTGGATCT | AGCATAATCGAAATGTCCCAAA |
| 56 | cnu_m152a | BG 77 | TCGAGAGAAGAAGATGGGATGA | CCGAACAAGTTGATAAAAAGTACAATG |
| 57 | cnu_m154a | BG 78 | TTTCAAAAATTTTAATTGCACAGA | TATTCGTACGCGGAAAGTCG |
| 58 | cnu_m155a | BG 79 | CGTTTCCTCAGCCTCCTTCA | TGCCTACATCCACCGGAGTT |
| 59 | cnu_m159a | BG 82 | CGTATCCATGGCCTTGAATTTT | GGCGAGAACCTTGATGATCC |
| 60 | cnu_m160a | BG 83 | TGCATGCCATTGAAGCCTTA | TATGTCCGCATCAGCTCCAC |
| 61 | cnu_m163a | BG 85 | GGGGGAAGGTTCTTTGTTACAT | GCATTTGGGGATGGTGAGAG |
| 62 | cnu_m166a | BG 88 | CTCCTCCTCCAGCGTCTTCA | CGCGTTTGAAGGAGATTTGG |
| 63 | cnu_m174a | BG 91 | ACGTGGCATTCATTAAACGG | GAAAGAGAGATCCTTCAGCCAA |
| 64 | cnu_m177a | BG 94 | CCTTCAAAAGAAAGGAGGGGAA | GAGAGAGAGAGAGGGCATAATAAAAGC |
| 65 | cnu_m208a | BG 101 | AGAGCGAGCTGCAAGAAAGC | CATTGCCGAACCTCACTTCC |
| 66 | cnu_m209a | BG 102 | GGACCGACTTTAGCAAGTCCA | GGGTAGCTTAGAAGATCATCTCTTTGG |
| 67 | cnu_m212a | BG 103 | TTTGTCCACCATTTCTTAAACATCT | TCAATGAAATTGTTAAAATACAGCAAA |
| 68 | cnu_m213a | BG 104 | CATGCGGAAACCCGTTAAAA | AAAGCAACCACCCCATTCAA |
| 69 | cnu_m214a | BG 105 | TCGATCTTTTTGCGGTGGAT | TTGCAATGGGCATTACATCCT |
| 70 | cnu_m216a | BG 106 | TTTTCCCTTTAAATTTCAATTGCTT | GGAAGTGAAGGTGAAGAGGAGTG |
| 71 | cnu_m217a | BG 107 | TCCGAATCGAGACAGGAACA | AGGGCTTACGAAGCCAAACC |
| 72 | cnu_m218a | BG 108 | TTTGGGCATCACGGATCTCT | CAAAAATAAGAAAGCGACAGCTGAA |
| 73 | cnu_m221a | BG 109 | AAGCCGGTTCTGCAAGTGTT | CATGGCATCCTACGTGGACA |
| 74 | cnu_m222a | BG 110 | GCATACTTCAATTCTTTGAGGACCA | GCAGCATTCCCCTATGTTGG |
| 75 | cnu_m223a | BG 111 | ACCCGAAAAGAGAATATGGCCT | ACAGTGGCGTTAGGTGGGG |
| 76 | cnu_m224a | BG 112 | TTTCAGCCATGGAGGACGTT | AGCATTGCACCAGTCTCAAAA |
| 77 | cnu_m226a | BG 113 | ATTGCTTCCGGAACTTGTCG | GCGTCACAGAGGCGGTTATT |
| 78 | cnu_m227a | BG 114 | GCAAAATCCATTGGTAATCAGGA | TGGGCAAGTCACACTCACTCA |
| 79 | cnu_m228a | BG 115 | GAGGAGGAGGAGAAGGAGGA | CCATCTTTGAAAAACCCCAAT |

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|---|---|---|---|---|
| 80 | cnu_m230a | BG 116 | ATGGGGGTTCTAGCGAAAAG | CTTTCAATTGAGTTTCTCGTAGTTCTT |
| 81 | cnu_m231a | BG 117 | CTGGAAAGCATACACTTTGGTG | CAAAGGATTTCCCTCGATCA |
| 82 | cnu_m232a | BG 118 | TGTGCTTGCGTTTTAAAGGA | GCAAAACCCACAGGTCAGAT |
| 83 | cnu_m233a | BG 119 | TTGTGAAATGGTGTCGAAGC | GCACGAGAATGCAAAGTTGA |
| 84 | cnu_m234a | BG 120 | TTTGAAACGACGATCAACACA | GCTTCATCTGCTTACTATGGTTTTT |
| 85 | cnu_m235a | BG 121 | CAACCACATGAGATTGGTTTAGTT | GAAATGGTTTTGGAGCGGTA |
| 86 | cnu_m236a | BG 122 | CCATTATTTGAAAATACCATTTGTTG | AAATATCAATGATGGATTGGTGA |
| 87 | cnu_m237a | BG 123 | CCAACGGAAGGGATGTTAAA | TTACCCTGCACACACACACA |
| 88 | cnu_m238a | BG 124 | TAGATCATTTCACACGGTGGAT | TCATAGCGACAAAAGTGACAGG |
| 89 | cnu_m239a | BG 125 | CGAACCGCGAACATAGTGTA | TAAGTGCCAGTCCATTGCAT |
| 90 | cnu_m240a | BG 126 | AGAACGAGTCGCGAGGATT | AGTGGGTGGAAGTTCGGTTA |
| 91 | cnu_m242a | BG 127 | GCGCCATCTAAACCGATATT | TACCGCGCCATTGATACATA |
| 92 | cnu_m243a | BG 128 | CCCTAGTCCGTTTGGGTTAGGT | CCTAATCGCTCTTTTGATTTTGGA |
| 93 | cnu_m244a | BG 129 | CGGAGATAACCGGAATGGAA | GGATGCTCTGAGACACCCAAA |
| 94 | cnu_m245a | BG 130 | TTGTCAGGGCGGATTTAGGA | TGAACCAGGAGACTTCCACAA |
| 95 | cnu_m247a | BG 131 | TTCGACAGATTATTTCCGTTGG | AAAGAGGGAGGAAGAAGAAGGAG |
| 96 | cnu_m248a | BG 132 | AATGCCCATCCCTCCTTGAT | TTTGAACACTCATTGTGGTGATGA |
| 97 | cnu_m249a | BG 133 | CGAACCAAACCGAACCAAAC | GCCGAACAAAAATCAAAAACC |
| 98 | cnu_m250a | BG 134 | CAGATTTTCGAAAGGTGGTTGG | CCATCACCCGAAAATCCAAA |
| 99 | cnu_m258a | BG 135 | TGATGAAGAATGGTGCATGG | TTCGAATCTCATCAGCTGCAC |
| 100 | cnu_m260a | BG 136 | TTGGAGAGGTCTGGGCTTTG | TCTCGCCTTGTTGTGAATCAA |
| 101 | cnu_m261a | BG 137 | TTGTTCGGTGTCCCCTTTGT | TGCTCCGGGAATGAACATCT |
| 102 | cnu_m262a | BG 138 | GGGTCCAAAGCTAGAGGCTGT | GAAGTAATCCCGCCCCATAGA |
| 103 | cnu_m264a | BG 139 | GTCGTCAGGCAAAGGTGGTT | GGCGGCAGAAAGAAAGAAAG |
| 104 | cnu_m265a | BG 140 | TTCGGTGCATTGATCTTTCA | TCCTTCTGATTCCCTTTTGCT |
| 105 | cnu_m267a | BG 141 | CGTGTTCCCTCTTCTTCCCTTT | TGATGCTTTTGCTTAAAGTGTTTGA |
| 106 | cnu_m270a | BG 142 | TCGATGATTAGTTTAGTTATTTCACG | CCTCAAACCAAGGAAGATTTCA |
| 107 | cnu_m272a | BG 143 | GGAATTGAAAGGAAAGCGAAAG | TCTTTGGTGCAAGAGTGAAGGT |
| 108 | cnu_m275a | BG 144 | TTGAATAACAACAATAACATTGGATAA | GAAAAGTTGTTGGACCCTTTCA |
| 109 | cnu_m278a | BG 145 | TTTCAATCCATCGATTTTTGTTCA | TCGCTCGCTCTCTCTCTCTG |
| 110 | cnu_m279a | BG 146 | CAGGAGAGCATCCATCACCA | TTGAGAATGCCCATGCCTTT |
| 111 | cnu_m281a | BG 147 | TGGCTTTCAGGAATTTTAATCATCTC | CAAAAGAAAGGAGGGGAAAATCA |
| 112 | cnu_m282a | BG 148 | TGGTGGAATAATTACGGAAAGAACA | TCCAACCAAGAACCACAGCA |
| 113 | cnu_m283a | BG 149 | TATGTGAACCTTGCCCTGGT | ATTCCGCAATCAAATTCCAG |
| 114 | cnu_m285a | BG 150 | TCCAAAGATTTCAACGGTCA | CATGCATGAGGTTTGGTCGT |
| 115 | cnu_m287a | BG 151 | TCGGGATTGCTATCAACAGT | AGCTCTCAAGCGAAATTCCA |
| 116 | cnu_m290a | BG 152 | CGATTTTGCCATTGTCTAAGC | TGAAGACACGTTGGTTGAACA |
| 117 | cnu_m291a | BG 153 | CCGTACAAAGATACGCACGA | ACAGAACTCGGGGCTACCTC |
| 118 | cnu_m292a | BG 154 | TGGATGATAAGGTCAACCACA | ATCGAACCTGGGTCTAAGCA |
| 119 | cnu_m294a | BG 155 | CAACGGTCCGTAACTGGTCT | AGCTTCTCAGGCTTGATTCG |
| 120 | cnu_m297a | BG 156 | CATTGATGAGGCAAGACTTTGA | CACCAAAGCTTCTCAACTTTCTAA |

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|---|---|---|---|---|
| 121 | cnu_m298a | BG 157 | GGAGAGGTGTTTCTCGAACCT | TTCAGTGTGCTATGCAGATCG |
| 122 | cnu_m299a | BG 158 | TGTGAGAATGCAGTCCAAAACT | TCTGGTCATGATGGTGGAAA |
| 123 | cnu_m301a | BG 159 | CAAAAATTCAAAAACCCGTGA | CAAGCACCAAGAAGTTGCAG |
| 124 | cnu_m303a | BG 161 | ATGCTCGTGCCCACAAAA | AAACGTTTTATTTGCTTTACCTATTT |
| 125 | cnu_m304a | BG 162 | TGCCACTGAGACTTCCTCCT | GAGGTTTGGGAGATGCAGAG |
| 126 | cnu_m305a | BG 163 | TGTGAAACAACAACAACCACCT | GCGCTAGCTAAACCCACTCC |
| 127 | cnu_m307a | BG 164 | GCTCGTTTCGATTTGGTCTC | GGCCATGGAGAGAGAGAGAG |
| 128 | cnu_m309a | BG 165 | TTCATTCTCGGCACAAAACA | ATGGCCCAAGAAACATCAAT |
| 129 | cnu_m311a | BG 166 | GCGTCATACTGTTGTTAGTTTATGG | GACATATTGCACATGCAAAGACT |
| 130 | cnu_m312a | BG 167 | CAAGTTAAGGAATTAAGTTTCTCCTTC | CCAAAATTAAACAGCTCAAGAACA |
| 131 | cnu_m313a | BG 168 | CAAAATCAATTTAACATTGGTCAAC | TTTCATTTTTGGACTTTCAAATCA |
| 132 | cnu_m314a | BG 169 | CTCCTTGCCGGAGATACAAA | CAGTTCGCTAGCAAGTCGTG |
| 133 | cnu_m315a | BG 170 | TTATCAGATATTTCATTAACACGGTTT | GAAGTGTGTGTTGGTGAGCTTT |
| 134 | cnu_m317a | BG 171 | TGTCTTCGACGATTCCATCA | CGATGTCGCTCTCAATCTCA |
| 135 | cnu_m319a | BG 172 | TGACTTGAAATTATATGTTGTTGAAAA | CCAAAGTTTGTAATTTTCGGTTG |
| 136 | cnu_m322a | BG 173 | TTCAATTCTTTGAGGACCAACA | AGCTGCAGCATTCCCCTAT |
| 137 | cnu_m323a | BG 174 | AATCACCGTCGTCGATTACC | CAGGCTTAAATCGCTTCTGG |
| 138 | cnu_m326a | BG 175 | GTTAGGTCTGGTGGCTCTGG | GGATGTTTCTAGGCCCGTTT |
| 139 | cnu_m329a | BG 176 | CACGACGTGCCCAATGATTA | TGCTTGACTGTAAATCTGAGCAAA |
| 140 | cnu_m330a | BG 177 | TGCATATGATAATTTTCCGCATGT | TCTGACTAATCCGATCTAAAAATTCG |
| 141 | cnu_m333a | BG 178 | GGCGTGAGGATTCGAGAGAG | CCCAACCCCACAGACAAAAA |
| 142 | cnu_m334a | BG 179 | TTGCATTTTGTTCAAAAAGTGGT | TCGCACGGGAACTCATTTTA |
| 143 | cnu_m335a | BG 180 | TGAAATGCTCACTTTGGGTTCA | TCCCAGCCCACTTTACTGACA |
| 144 | cnu_m336a | BG 181 | CAAAGTCCTTCCAAAACCAAAC | CTACCTCCATTAAAGACACGCC |
| 145 | cnu_m337a | BG 182 | TGTCCTCTTCTGGCTGTTGTGA | GGGTGTGATCTGAAACCTCTCG |
| 146 | cnu_m341a | BG 183 | GGTATTTGGCTTCTTTGGGCTT | GTCCTTTTGCTTCGTTCCCTTT |
| 147 | cnu_m345a | BG 184 | TGAGGGTGAGGATGGTGATG | GCACAGTACACCGACGCCTA |
| 148 | cnu_m348a | BG 186 | CCCCCAACAATTGATTACGC | CGAAAGTTAACCCGCTTGCTT |
| 149 | cnu_m349a | BG 187 | ACCCATGGCTGGGAAAAGTT | TCGGTAACACGGGCTACAAAA |
| 150 | cnu_m350a | BG 188 | TCGAGCCTACATCGACGTTTC | CGATCCAACCCGTCAACAG |
| 151 | cnu_m351a | BG 189 | TGATCAGCCAATCTTGTTGCTT | CCACTCTGAAAATTTTGTGATCAAT |
| 152 | cnu_m352a | BG 190 | TGAATTTCGCAAACGTCAAA | CGGGTATGACCTGGAGGCTA |
| 153 | cnu_m355a | BG 191 | CCCCAAAAACAGTGCCAAAG | GGCCCTAATCCCCATCACA |
| 154 | cnu_m357a | BG 192 | TCGCTCTCACACCCCTTCTC | GTGGCAAATTCGGACAGACC |
| 155 | cnu_m358a | BG 193 | AGTCTCATGATTTTTGTGTCACG | AGCAGACTGCAACCAAATGA |
| 156 | cnu_m359a | BG 194 | GCTTTGGCTTCAACAACTTCG | ACAGCTATGCGATGCAGCAA |
| 157 | cnu_m361a | BG 195 | TTCTGCACATGAGAGCACAAGA | TCGATAAAAGAAACTCAAATGACTGC |
| 158 | cnu_m363a | BG 196 | TGAGAAATGTAGTAAAGTTTTTGGAAT | ACCACCACTAAAGTGACATTTTCAA |
| 159 | cnu_m365a | BG 197 | TCTTTATTCAATTTGCTCACATTCC | TTCGTGGTTTCGCGGATTAT |
| 160 | cnu_m366a | BG 198 | CGAGGCGGGCTTAAACATT | TCGTTTATACCTTTAATGAATGAGA |
| 161 | cnu_m367a | BG 199 | AAAGCCCAATTTCAAACACCAA | AACGAAAACGAACCCAAACG |

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|---|---|---|---|---|
| 162 | cnu_m368a | BG 200 | CGGGTCCGAGTTACCTACCTT | TGGCACATGAAAACAATATAGTACCAA |
| 163 | cnu_m369a | BG 201 | GGATGGGCTGTAAGCTGGTG | TGGCAACATGCATTGGTTTT |
| 164 | cnu_m373a | BG 202 | GGGTGGAGACCGACGACTAA | TTCCTCTATCTGCGGCTTTATCA |
| 165 | cnu_m374a | BG 203 | AATCACACAGATATACACACCAGCA | CATGTGCCTAAGTTTACCCACAGAT |
| 166 | cnu_m375a | BG 204 | CGGGACCATGCCTGTAAATA | AAAGGGAACATTAAGGTTTATAAAATG |
| 167 | cnu_m376a | BG 205 | TCGGTTCCATAAAAATGTTGG | TTCAAATTTTGTGATCAATGGTT |
| 168 | cnu_m378a | BG 206 | ACATATTGAATGATCTACCTCGACA | TGCATCTTACTTGAATATCTTACGAC |
| 169 | cnu_m380a | BG 207 | TCATTTGAAAACCAATCCAAAA | GATCGTTCAAATGTCGTATGGA |
| 170 | cnu_m381a | BG 208 | TGCTTTTAACCAAACTCAAACG | TGCAGAGAGGCAAGTTTCAA |
| 171 | cnu_m382a | BG 209 | TGAACCAAATAGATAGTTTCTCGTG | TGCAGTGGAGAACCCTCTTC |
| 172 | cnu_m383a | BG 210 | CAAATTGTTGGGCATCAAAC | TCCTCACGGGGATCTTTATG |
| 173 | cnu_m385a | BG 211 | CCTCCATCAAGCTTCTCTGC | CTCTCTCCTCTCTGCCATGC |
| 174 | cnu_m386a | BG 212 | TGCTGATGGGATCCTTCTTC | CAAAAGAAATGGGCGTCACT |
| 175 | cnu_m390a | BG 213 | CTGGCCGGTTCTCATGTAAT | TCCAAACCGGACTAGATCGT |
| 176 | cnu_m391a | BG 214 | TGGACAAAATTAATTGCTACTCAC | GATATTTTTGGCCGGGTTC |
| 177 | cnu_m392a | BG 215 | CGAATTTAACTTCAGCATCTGG | TTGATCACTCCAAAACCAAGG |
| 178 | cnu_m393a | BG 216 | TAGTTAGCGTTTGCGGTTGC | CAAATGCAGCAGCGATTAAA |
| 179 | cnu_m394a | BG 217 | CGCACGGGAACTCATTTTAT | CAATTGTATGCAATATGTGTTTTGA |
| 180 | cnu_m395a | BG 218 | CAATTGTATGCAATATGTGTTTTGA | CGCACGGGAACTCATTTTAT |
| 181 | cnu_m401a | BG 220 | TGTGTTAAACTATCTCCGAACTAGAAA | TTTCTCTAGGATATTAGAAAAGGAAAA |
| 182 | cnu_m403a | BG 221 | CCAAAAACCTGTAATCGAGCTT | GAAGGGATCGACATGATGAGA |
| 183 | cnu_m404a | BG 222 | CCCTTGCATTCACTTCTCCT | CAAGACCAGCAGTCTGAGCA |
| 184 | cnu_m408a | BG 225 | GGGAGGAGAAAGAGGGAGAG | CTGTCTGCCACTCCACTTGA |
| 185 | cnu_m411a | BG 227 | TCACAACATGGTTTGAGTTCC | GAGGAAAAGTGCCAATTTCG |
| 186 | cnu_m413a | BG 228 | ATTGTGCCGTCGGAATTAAA | GATGATTAGAAAAGGTGTCTATTGC |
| 187 | cnu_m414a | BG 229 | TGATTCAAAATATGACACAAATCG | AAAATTCAACTTTCACGTAATTGTTAT |
| 188 | cnu_m415a | BG 230 | TGATGTAACCCCGGAGAAGA | TTTCTCCCCTGAAAGCACAC |
| 189 | cnu_m417a | BG 231 | ATTGCTTCTTGCTTGCGTTT | TCGGATACAGCCATACGTCA |
| 190 | cnu_m419a | BG 233 | TGGGAGCAATTTGGTGTACATA | TGCAGTTGCAATTGATGCTT |
| 191 | cnu_m420a | BG 234 | CGTTCACCTAAGAGCCCAGT | GAGGCACGTTCTGGAGGTAG |
| 192 | cnu_m421a | BG 235 | ATATCCAGTTCGCCAACACC | AGCAAACCCCATCAGACAAC |
| 193 | cnu_m423a | BG 236 | GAAAGAAACGCAACCAACAA | CGTATCATCCAACCCCATGT |
| 194 | cnu_m424a | BG 237 | CCTCCTCTCAATCCAAGTGC | ACGGCGTCTTGTGAACAAAT |
| 195 | cnu_m426a | BG 238 | AATTGCACCGAAAACTTTTGA | CGGATATCGGGTTGGTTTC |
| 196 | cnu_m427a | BG 239 | CGCGGTACTTAAACCTCTGC | CACCACCAACCGATTCTTCT |
| 197 | cnu_m428a | BG 240 | GAAAGCGGGATGGGATTAAC | AACACCAGAGCTCGAATCGT |
| 198 | cnu_m429a | BG 241 | CGGAGAGTCCGCTTCTATTG | CGAGGAGATCGGAGACTGAC |
| 199 | cnu_m430a | BG 242 | TTCGAAATTCAAATAACTAATCAGC | TTTGGTGAGGGTTTTGAACA |
| 200 | cnu_m431a | BG 243 | TGTCCCTGCTCGTGTTGATA | CACTCCATCGACCTCCTCAT |
| 201 | cnu_m432a | BG 244 | CAAACCTCGTCCTAAGCAGAA | ACCTGAAGATGACCCAGACG |
| 202 | cnu_m433a | BG 245 | ACCCGTGTCCAAAACTTCAG | CCAATTTGTCCTGCAACCTT |

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|---|---|---|---|---|
| 203 | cnu_m434a | BG 246 | AGCCAAACTGCTGAGCTTGT | CAGGGTAGGCGTTTTGTGAC |
| 204 | cnu_m436a | BG 247 | GGGTGATTGCATGATGTGTT | TAAAACCAAAGGGGGAGAGC |
| 205 | cnu_m437a | BG 248 | CCTTTATCTTCATTGTATCACTATTCA | GTGCTTGCCCTTTAGTTTGC |
| 206 | cnu_m438a | BG 249 | TGAACCCTCGAAACTGAAAGA | GGACGTTAACGAAGGCAAAA |
| 207 | cnu_m441a | BG 250 | TGGTTACCCCACTGACCCTA | AAATATGGGGCCTCTCCTGT |
| 208 | cnu_m444a | BG 251 | TCTAGGACCAACCGCATTTT | GTTGCACCTTCAAGCTCTCC |
| 209 | cnu_m445a | BG 252 | TTCAATTCTTTGAGGACCAACA | CCCCTGACTTGGGAATGTAG |
| 210 | cnu_m448a | BG 253 | TTCATAAAGCAGAGCAGGGGAA | TGGGCATATTGGTCTTTGATCC |
| 211 | cnu_m449a | BG 254 | GCAAGTGTGCTAAAACCGTGTG | GGGGTGATAACTGTGCCTCTTC |
| 212 | cnu_m450a | BG 255 | TAGCTGCGTTGCTTCCACAGTA | TGTCATCAAGTGGTTCTTGTCTGAA |
| 213 | cnu_m451a | BG 256 | AGTCAAAGGCCACAACATTTCC | ATATAAAAGGTCGAGCGGTTGC |
| 214 | cnu_m452a | BG 257 | ACTGAAACCAACATGCTTCAAC | ATGAGAAGATTAGCGACGAGGT |
| 215 | cnu_m453a | BG 258 | CGATTGTCCACAGGGATCTTG | TGAAATGGATGAGAGGGAGGAA |
| 216 | cnu_m454a | BG 259 | GAACTGAATGTAGCAGACAGGAGGA | CTGAGTATGAAACCGGCGAGAC |
| 217 | cnu_m455a | BG 260 | TCCACATCTACACAAAGCACAATG | TGCAACCACATGACTTGCAG |
| 218 | cnu_m456a | BG 261 | AAAAATGTTCAAAACCCTCACCAA | TCTCTGGCTTCGATTTATGTTGA |
| 219 | cnu_m460a | BG 262 | TGTTTTGGTGCGTTTTTGGA | ATCAAGGAGGGATGGGCATT |
| 220 | cnu_m462a | BG 263 | TGATTGATCGTGCCAGCAGT | GCGTGGTCATTTCCGAGTTC |
| 221 | cnu_m463a | BG 264 | CGCCTAAGATCAGTTCAGCA | GAAAGGCTAAGGCACACCTC |
| 222 | cnu_m464a | BG 265 | CCCTAATCCCCATCACACC | CCAAAAACAGTGCCAAAGGT |
| 223 | cnu_m466a | BG 266 | GGTCAGGTGCTACTCAGACTCC | TTGAAGAGGATCCACCAAAAG |
| 224 | cnu_m467a | BG 267 | AGAGCACCATTAACCCTAGCA | GCACCATTAACCAAGGGTACTT |
| 225 | cnu_m468a | BG 268 | CTGAAGCTTCCTCCGACAAC | TAACGAGATCGGCGAAGAAT |
| 226 | cnu_m469a | BG 269 | GCATGCTACGTTGGGAACTAA | ATACGAGCAAGGACGAGACG |
| 227 | cnu_m470a | BG 270 | CGAGGAAGAGAGTGGTTCGT | TCAAAGATGCAAAAGCCAAA |
| 228 | cnu_m473a | BG 271 | CCACTCTGAAAATTTTGTGATCAAT | TTCGGTTCGGTTCGTTTTT |
| 229 | cnu_m475a | BG 272 | GGCGTCTGATGTGAACTTTG | GACAGCAGATGCCTCAACAA |
| 230 | cnu_m476a | BG 273 | TGTCACAAGCCTATCCATTCA | CCCTTCATAGTGAAACAGTGGA |
| 231 | cnu_m478a | BG 274 | ACCTTGGCTCCTCCACCTAT | TGTCTCCTCCTCGTCTCACC |
| 232 | cnu_m481a | BG 275 | GAGAAATTTGGTGGATGTTTGA | GAACAAAGACAAAAGGGGTCA |
| 233 | cnu_m484a | BG 276 | AGGGAGTTGAAACGAAAGCA | CAATACAAAAACCGGGCAAG |
| 234 | cnu_m485a | BG 277 | GATGGTGGCCATGAAATGAT | TCACGAAATACTTTGATGTGGAA |
| 235 | cnu_m486a | BG 278 | CATGCTCCTAAACGTCCATGT | GGCCAAGCGTAAATCAGTAAA |
| 236 | cnu_m488a | BG 279 | CGGTTCGGTTCGGTTTTT | CCACTCTGAAAATTTTGTGATCAAT |
| 237 | cnu_m489a | BG 280 | CCACTACCAAGCCATTGTGA | GAGACCACGCAAGATCAGGT |

**APPENDIX-II: Mean performance of test genotypes for morphological and physiological traits under timely sown environment**

| Sr. No. | Genotype | DF | DM | PH | NPB | NSB | MSL | NSMS | SL | NSS | OC | TSW | SYP | A | Gs | E | Chl | Caro |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | RH 30 | 48 | 138 | 187.7 | 5.2 | 15.2 | 78.2 | 52.7 | 3.8 | 13.0 | 39.0 | 5.8 | 26.5 | 23.5 | 0.36 | 1.91 | 1.41 | 2.58 |
| 2 | RH 0119 | 55 | 144 | 200.0 | 5.3 | 13.7 | 76.5 | 44.7 | 5.3 | 14.9 | 38.8 | 5.6 | 24.9 | 15.5 | 0.43 | 2.45 | 1.38 | 2.45 |
| 3 | RH 406 | 56 | 145 | 202.2 | 4.2 | 10.2 | 79.0 | 49.7 | 4.5 | 15.7 | 38.9 | 6.0 | 26.9 | 14.8 | 0.60 | 2.91 | 1.35 | 2.54 |
| 4 | RH 725 | 51 | 141 | 199.7 | 5.2 | 13.0 | 78.0 | 55.2 | 4.8 | 14.2 | 38.3 | 5.8 | 30.8 | 24.8 | 0.80 | 3.84 | 1.46 | 2.64 |
| 5 | PBR 91 | 57 | 148 | 205.4 | 5.0 | 12.7 | 81.5 | 56.7 | 4.1 | 15.2 | 38.6 | 4.8 | 14.0 | 13.4 | 0.47 | 3.95 | 1.06 | 2.37 |
| 6 | RH 761 | 51 | 142 | 203.0 | 6.3 | 10.5 | 85.2 | 57.4 | 5.1 | 14.4 | 38.7 | 6.3 | 27.7 | 14.7 | 0.53 | 4.27 | 1.29 | 2.42 |
| 7 | RH 8113 | 56 | 146 | 209.5 | 8.8 | 12.7 | 73.7 | 55.7 | 3.9 | 12.4 | 40.0 | 5.1 | 17.0 | 17.0 | 0.48 | 3.93 | 1.10 | 2.30 |
| 8 | PBR 97 | 49 | 140 | 195.0 | 4.8 | 13.7 | 85.7 | 51.0 | 3.9 | 14.6 | 39.1 | 5.0 | 20.3 | 15.3 | 0.49 | 3.58 | 1.08 | 1.92 |
| 9 | RH 9304 | 48 | 138 | 197.0 | 5.7 | 13.7 | 78.0 | 52.7 | 3.6 | 14.2 | 38.9 | 5.8 | 27.0 | 18.8 | 0.48 | 3.30 | 1.34 | 2.33 |
| 10 | RH 9801 | 47 | 137 | 194.0 | 5.8 | 12.8 | 80.5 | 51.2 | 4.0 | 12.5 | 39.2 | 5.4 | 26.0 | 21.3 | 0.34 | 2.40 | 1.28 | 2.20 |
| 11 | RB 50 | 53 | 139 | 218.8 | 6.0 | 14.7 | 86.3 | 56.7 | 4.6 | 14.6 | 39.3 | 5.9 | 24.1 | 19.5 | 0.35 | 4.03 | 1.32 | 2.84 |
| 12 | RL 1359 | 50 | 142 | 194.8 | 5.3 | 11.0 | 78.0 | 47.2 | 4.4 | 13.9 | 39.4 | 4.1 | 18.2 | 15.3 | 0.31 | 4.34 | 0.96 | 2.12 |
| 13 | PM 21 | 52 | 142 | 219.2 | 7.2 | 16.8 | 71.7 | 53.8 | 4.2 | 12.3 | 39.6 | 5.1 | 23.5 | 14.8 | 0.41 | 4.35 | 1.14 | 2.12 |
| 14 | PM 22 | 49 | 140 | 214.5 | 5.2 | 10.5 | 61.0 | 45.3 | 4.3 | 15.2 | 38.6 | 4.9 | 22.0 | 14.1 | 0.35 | 4.32 | 1.18 | 2.13 |
| 15 | PM 24 | 52 | 139 | 219.7 | 5.2 | 13.0 | 69.3 | 52.2 | 4.0 | 13.1 | 38.8 | 4.2 | 20.3 | 14.4 | 0.41 | 4.23 | 1.09 | 2.25 |
| 16 | PM 25 | 44 | 132 | 186.2 | 4.7 | 14.7 | 74.8 | 52.7 | 4.6 | 15.3 | 38.7 | 4.9 | 22.0 | 16.8 | 0.51 | 4.74 | 1.34 | 2.23 |
| 17 | PM 26 | 45 | 135 | 201.7 | 6.3 | 15.2 | 65.0 | 51.0 | 4.2 | 15.1 | 38.7 | 5.5 | 23.3 | 23.6 | 0.77 | 4.18 | 1.35 | 2.25 |
| 18 | PM 28 | 45 | 135 | 194.8 | 5.3 | 13.3 | 72.7 | 49.3 | 4.0 | 15.5 | 39.2 | 4.3 | 23.8 | 19.0 | 0.61 | 4.09 | 1.07 | 2.04 |
| 19 | PDZM 31 | 51 | 140 | 208.7 | 5.5 | 16.0 | 75.0 | 54.5 | 3.6 | 14.5 | 39.4 | 3.1 | 21.7 | 17.2 | 0.73 | 4.04 | 1.04 | 2.03 |
| 20 | Pusa Vijay | 52 | 143 | 206.0 | 5.0 | 12.5 | 80.2 | 50.8 | 4.3 | 13.1 | 39.1 | 5.4 | 22.2 | 20.5 | 0.67 | 3.85 | 1.08 | 2.03 |
| 21 | Pusa Jai Kisan | 49 | 141 | 195.0 | 5.0 | 14.2 | 76.2 | 44.7 | 3.9 | 13.3 | 38.6 | 5.7 | 24.2 | 17.0 | 0.56 | 5.81 | 1.28 | 1.90 |
| 22 | Pusa Karishma | 53 | 144 | 208.0 | 7.2 | 16.0 | 82.2 | 61.8 | 3.4 | 14.5 | 38.6 | 4.0 | 23.8 | 17.6 | 0.57 | 5.79 | 1.24 | 2.02 |
| 23 | Pusa Bold | 53 | 143 | 204.0 | 4.3 | 11.0 | 75.2 | 48.7 | 4.2 | 14.0 | 38.7 | 5.0 | 25.9 | 15.8 | 0.55 | 4.99 | 1.36 | 2.08 |
| 24 | Pusa Jagannath | 48 | 141 | 202.3 | 4.5 | 11.0 | 68.8 | 53.2 | 4.4 | 14.3 | 38.7 | 4.6 | 25.9 | 17.8 | 0.55 | 4.41 | 1.29 | 2.81 |
| 25 | BPR 349-9 | 49 | 138 | 202.0 | 5.3 | 16.5 | 83.3 | 50.7 | 4.6 | 15.4 | 38.6 | 4.8 | 20.8 | 16.8 | 0.52 | 3.62 | 1.35 | 2.45 |
| 26 | BPR 540-6 | 45 | 135 | 193.9 | 5.7 | 14.7 | 85.3 | 56.2 | 5.2 | 13.9 | 38.7 | 5.2 | 19.7 | 16.4 | 0.47 | 3.87 | 1.09 | 1.94 |
| 27 | BPR 543-2 | 47 | 138 | 201.2 | 4.3 | 10.5 | 80.5 | 49.8 | 4.5 | 16.0 | 38.4 | 4.9 | 21.4 | 17.6 | 0.49 | 4.09 | 1.10 | 2.29 |
| 28 | BPR 549-9 | 50 | 141 | 208.3 | 5.2 | 12.5 | 82.3 | 51.0 | 4.7 | 15.0 | 39.5 | 5.5 | 21.0 | 13.5 | 0.51 | 5.24 | 1.11 | 2.32 |
| 29 | DRMR 150-35 | 49 | 140 | 205.9 | 4.2 | 12.9 | 80.8 | 45.2 | 4.1 | 13.1 | 38.8 | 5.6 | 18.2 | 14.2 | 0.53 | 4.91 | 1.23 | 1.97 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 30 | DRMR 1165-40 | 50 | 144 | 212.8 | 6.0 | 12.2 | 84.3 | 59.2 | 4.4 | 14.9 | 39.2 | 4.6 | 16.7 | 14.2 | 0.51 | 2.73 | 1.24 | 2.01 |
| 31 | NRCDR 2 | 46 | 138 | 214.5 | 5.5 | 14.4 | 84.2 | 50.8 | 4.9 | 15.5 | 38.8 | 5.2 | 22.4 | 17.9 | 0.42 | 3.35 | 1.19 | 2.45 |
| 32 | NRCDR 601 | 48 | 142 | 214.2 | 5.9 | 15.7 | 94.2 | 50.3 | 5.0 | 15.2 | 38.8 | 5.7 | 22.0 | 17.2 | 0.41 | 2.82 | 1.22 | 1.99 |
| 33 | RGN 48 | 49 | 141 | 242.2 | 5.2 | 15.0 | 85.2 | 63.7 | 4.5 | 14.3 | 38.8 | 4.9 | 23.6 | 18.6 | 0.42 | 3.31 | 1.18 | 1.86 |
| 34 | RGN 145 | 51 | 143 | 204.2 | 4.8 | 13.3 | 87.8 | 55.7 | 4.5 | 15.1 | 38.7 | 4.7 | 17.3 | 15.5 | 0.40 | 4.13 | 1.00 | 2.16 |
| 35 | RGN 229 | 55 | 145 | 235.0 | 5.3 | 13.3 | 83.8 | 59.8 | 4.2 | 14.8 | 38.8 | 4.9 | 20.3 | 16.0 | 0.58 | 3.74 | 1.10 | 2.31 |
| 36 | RGN 236 | 51 | 143 | 216.0 | 4.8 | 13.4 | 87.8 | 53.0 | 4.2 | 14.5 | 38.9 | 5.0 | 20.8 | 17.5 | 0.37 | 3.43 | 1.10 | 1.75 |
| 37 | RGN 298 | 52 | 142 | 218.0 | 5.5 | 14.8 | 93.7 | 61.2 | 4.8 | 14.3 | 38.7 | 4.7 | 21.1 | 19.0 | 0.46 | 4.86 | 1.02 | 1.85 |
| 38 | CS 56 | 53 | 140 | 222.5 | 5.7 | 16.3 | 90.5 | 59.2 | 4.2 | 15.5 | 38.6 | 4.6 | 21.9 | 16.3 | 0.51 | 2.83 | 1.08 | 1.99 |
| 39 | Radhika | 50 | 140 | 202.8 | 4.0 | 12.5 | 94.2 | 56.5 | 4.6 | 15.7 | 40.0 | 5.4 | 21.0 | 18.3 | 0.50 | 3.38 | 0.95 | 2.40 |
| 40 | Shivani | 51 | 141 | 210.7 | 5.5 | 15.3 | 79.5 | 50.5 | 4.4 | 13.4 | 38.8 | 4.1 | 19.0 | 17.6 | 0.39 | 3.33 | 0.94 | 2.39 |
| 41 | Aravali | 49 | 138 | 199.5 | 4.3 | 13.7 | 86.8 | 57.8 | 4.3 | 13.4 | 38.8 | 4.6 | 15.7 | 14.1 | 0.43 | 4.02 | 0.88 | 1.74 |
| 42 | Varuna | 52 | 146 | 219.0 | 8.0 | 17.2 | 78.3 | 57.5 | 4.1 | 11.7 | 39.6 | 5.1 | 25.4 | 20.1 | 0.41 | 3.45 | 1.24 | 2.46 |
| 43 | Pant Rai 18 | 53 | 144 | 200.0 | 4.2 | 9.0 | 74.0 | 47.8 | 4.1 | 13.4 | 38.8 | 4.2 | 16.5 | 13.0 | 0.39 | 3.22 | 1.10 | 1.98 |
| 44 | Pant Rai 19 | 42 | 133 | 172.2 | 4.5 | 12.2 | 81.7 | 51.7 | 4.4 | 14.5 | 38.9 | 4.7 | 14.7 | 14.1 | 0.46 | 2.57 | 1.09 | 1.70 |
| 45 | Pant Rai 20 | 50 | 137 | 200.0 | 5.3 | 15.0 | 76.3 | 55.5 | 4.3 | 14.2 | 39.6 | 4.2 | 17.4 | 16.0 | 0.29 | 2.94 | 1.07 | 1.93 |
| 46 | RH 781 | 53 | 144 | 214.0 | 5.5 | 12.2 | 81.7 | 55.3 | 4.1 | 12.9 | 38.4 | 5.0 | 22.7 | 21.9 | 0.29 | 2.66 | 1.26 | 2.25 |
| 47 | Giriraj | 50 | 141 | 201.3 | 4.5 | 8.5 | 84.5 | 55.7 | 4.4 | 14.7 | 38.7 | 5.0 | 26.2 | 20.8 | 0.47 | 2.92 | 1.25 | 2.36 |
| 48 | RH 819 | 50 | 138 | 231.5 | 5.3 | 12.8 | 86.0 | 60.7 | 4.4 | 15.0 | 38.9 | 4.5 | 17.8 | 16.0 | 0.35 | 2.09 | 0.98 | 2.06 |
| 49 | RVM 2 | 51 | 140 | 215.8 | 7.0 | 15.5 | 85.0 | 56.2 | 4.6 | 15.4 | 38.6 | 4.1 | 20.3 | 15.3 | 0.53 | 2.98 | 1.10 | 1.88 |
| 50 | CS 52 | 49 | 138 | 211.7 | 5.0 | 10.8 | 85.3 | 55.5 | 4.5 | 13.5 | 39.3 | 4.7 | 23.7 | 19.4 | 0.34 | 3.17 | 1.29 | 1.85 |
| 51 | IC 122287 | 49 | 139 | 196.3 | 5.0 | 11.2 | 79.7 | 49.0 | 4.1 | 12.9 | 39.4 | 4.9 | 18.0 | 16.1 | 0.39 | 3.18 | 1.03 | 2.04 |
| 52 | IC 333591 | 48 | 139 | 199.7 | 4.7 | 9.8 | 90.3 | 54.7 | 4.3 | 13.4 | 38.8 | 5.0 | 15.7 | 12.8 | 0.44 | 3.63 | 1.12 | 1.97 |
| 53 | IC 470135 | 48 | 139 | 204.3 | 5.8 | 14.0 | 84.2 | 52.2 | 4.8 | 16.2 | 39.2 | 5.1 | 17.5 | 15.5 | 0.49 | 3.80 | 1.04 | 1.94 |
| 54 | IC 491390 | 48 | 142 | 202.7 | 6.0 | 14.4 | 74.2 | 48.2 | 4.2 | 12.1 | 38.9 | 4.7 | 17.5 | 15.4 | 0.39 | 3.83 | 1.07 | 1.76 |
| 55 | RH 847 | 48 | 141 | 211.7 | 5.3 | 13.2 | 77.0 | 53.8 | 4.2 | 14.5 | 38.1 | 4.8 | 17.7 | 14.7 | 0.55 | 3.03 | 1.11 | 2.15 |
| 56 | RH 1400-2 | 46 | 138 | 196.2 | 5.5 | 16.7 | 82.8 | 57.3 | 4.0 | 13.3 | 38.9 | 4.5 | 20.0 | 15.0 | 0.41 | 2.88 | 0.99 | 1.96 |
| 57 | RC 2 | 51 | 143 | 214.5 | 6.5 | 16.0 | 96.7 | 63.5 | 4.1 | 14.8 | 38.9 | 3.2 | 17.3 | 15.8 | 0.50 | 2.19 | 1.09 | 1.81 |
| 58 | RC 5 | 54 | 143 | 207.8 | 7.0 | 16.2 | 71.8 | 53.0 | 3.6 | 12.6 | 39.1 | 3.6 | 15.7 | 17.0 | 0.59 | 3.73 | 0.93 | 2.05 |
| 59 | RC 12 | 52 | 138 | 224.8 | 6.0 | 13.8 | 83.2 | 61.2 | 4.4 | 13.1 | 38.7 | 3.9 | 15.2 | 13.9 | 0.37 | 3.17 | 0.92 | 1.68 |
| 60 | RC 48 | 50 | 141 | 214.8 | 5.3 | 14.8 | 95.0 | 60.2 | 4.0 | 13.8 | 38.8 | 3.9 | 17.9 | 14.8 | 0.36 | 4.17 | 1.07 | 2.05 |
| 61 | RC 81 | 48 | 137 | 218.0 | 5.7 | 13.0 | 100.5 | 59.7 | 5.3 | 15.7 | 38.1 | 4.4 | 18.4 | 15.7 | 0.35 | 3.78 | 1.02 | 2.00 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 62 | RC 91 | 45 | 138 | 199.0 | 5.5 | 15.7 | 80.0 | 52.2 | 11.0 | 14.4 | 38.3 | 4.2 | 16.5 | 14.2 | 0.26 | 3.07 | 0.97 | 1.90 |
| 63 | RC 104 | 48 | 138 | 202.7 | 6.4 | 12.5 | 88.7 | 51.7 | 4.2 | 15.0 | 38.7 | 4.3 | 18.5 | 15.3 | 0.35 | 2.52 | 1.08 | 2.15 |
| 64 | RC 106 | 48 | 139 | 201.5 | 4.8 | 13.8 | 89.7 | 55.2 | 4.1 | 14.7 | 38.8 | 3.8 | 18.9 | 16.3 | 0.29 | 3.12 | 1.02 | 1.97 |
| 65 | RC 108 | 48 | 137 | 192.8 | 5.8 | 15.2 | 83.5 | 56.2 | 3.8 | 13.2 | 38.6 | 4.0 | 15.9 | 14.2 | 0.27 | 2.90 | 0.93 | 2.00 |
| 66 | RC 118 | 48 | 140 | 213.5 | 5.2 | 13.5 | 92.5 | 57.7 | 4.3 | 13.8 | 39.5 | 3.9 | 18.9 | 13.3 | 0.28 | 3.26 | 1.09 | 2.05 |
| 67 | RC 134 | 51 | 139 | 219.3 | 5.9 | 16.7 | 84.2 | 63.8 | 4.6 | 13.7 | 38.7 | 4.1 | 18.0 | 16.2 | 0.32 | 2.36 | 1.12 | 1.89 |
| 68 | RC 162 | 48 | 138 | 210.3 | 4.7 | 12.7 | 83.3 | 46.3 | 4.1 | 14.1 | 39.5 | 4.3 | 19.1 | 14.8 | 0.26 | 2.42 | 0.99 | 1.91 |
| 69 | RC 280 | 50 | 141 | 205.8 | 4.7 | 15.3 | 90.7 | 57.7 | 4.2 | 15.0 | 39.3 | 3.5 | 18.8 | 14.8 | 0.34 | 2.93 | 0.98 | 1.96 |
| 70 | RC 330 | 50 | 140 | 210.3 | 4.5 | 10.7 | 93.2 | 57.7 | 4.2 | 15.4 | 39.0 | 4.1 | 18.1 | 16.5 | 0.45 | 2.79 | 1.24 | 2.13 |
| 71 | RC 448 | 46 | 135 | 197.0 | 5.7 | 14.5 | 91.3 | 57.5 | 4.0 | 14.1 | 39.3 | 3.9 | 17.4 | 14.8 | 0.29 | 2.94 | 1.11 | 1.91 |
| 72 | RC 449 | 47 | 137 | 205.7 | 6.5 | 17.7 | 86.0 | 58.0 | 4.3 | 13.6 | 38.6 | 4.5 | 16.6 | 13.4 | 0.37 | 1.53 | 1.21 | 1.63 |
| 73 | RC 587 | 51 | 140 | 208.9 | 6.5 | 19.0 | 89.8 | 52.2 | 4.0 | 14.4 | 38.8 | 3.6 | 22.2 | 15.2 | 0.35 | 2.94 | 1.13 | 1.74 |
| 74 | RC 713 | 51 | 139 | 223.2 | 6.5 | 16.3 | 82.7 | 52.5 | 4.7 | 15.9 | 38.8 | 4.0 | 17.3 | 12.7 | 0.30 | 2.36 | 1.06 | 1.87 |
| 75 | RC 734 | 50 | 142 | 209.7 | 5.5 | 13.3 | 83.3 | 56.5 | 4.4 | 14.6 | 38.5 | 3.9 | 20.2 | 15.8 | 0.44 | 2.30 | 1.27 | 2.13 |
| 76 | RC 806 | 56 | 146 | 193.9 | 5.7 | 10.0 | 68.0 | 47.4 | 4.5 | 13.7 | 38.7 | 4.3 | 16.6 | 14.2 | 0.28 | 2.91 | 1.19 | 2.03 |
| 77 | RC 840 | 52 | 142 | 202.8 | 6.3 | 13.7 | 83.0 | 56.5 | 4.0 | 12.3 | 38.8 | 4.4 | 17.7 | 11.6 | 0.39 | 2.72 | 1.21 | 1.88 |
| 78 | RC 904 | 55 | 142 | 209.7 | 5.7 | 8.8 | 70.5 | 50.3 | 4.2 | 12.8 | 38.8 | 4.6 | 17.9 | 14.9 | 0.44 | 4.36 | 0.96 | 2.12 |
| 79 | RLC 1 | 55 | 142 | 232.2 | 5.2 | 10.8 | 81.2 | 57.5 | 4.0 | 13.1 | 38.9 | 4.9 | 19.2 | 13.9 | 0.41 | 3.39 | 1.05 | 2.28 |
| 80 | RLC 2 | 57 | 145 | 228.5 | 5.3 | 13.2 | 86.5 | 58.3 | 3.8 | 13.8 | 39.3 | 4.1 | 17.8 | 12.3 | 0.38 | 2.84 | 1.06 | 2.28 |
| 81 | RLC 3 | 56 | 144 | 228.0 | 6.7 | 12.7 | 59.2 | 52.2 | 4.3 | 15.4 | 38.8 | 4.0 | 15.8 | 12.2 | 0.43 | 2.64 | 0.98 | 2.36 |
| 82 | RLM 619 | 57 | 146 | 201.7 | 5.5 | 11.0 | 68.8 | 48.5 | 4.2 | 12.4 | 38.9 | 4.4 | 18.9 | 14.0 | 0.47 | 3.90 | 1.15 | 2.50 |
| 83 | PB 357 | 53 | 142 | 214.5 | 5.7 | 10.7 | 76.8 | 52.5 | 3.5 | 14.8 | 38.8 | 5.3 | 22.4 | 16.5 | 0.44 | 2.84 | 1.08 | 2.27 |
| 84 | RH 1916 | 53 | 143 | 218.3 | 5.3 | 10.5 | 73.7 | 49.0 | 5.3 | 13.7 | 38.9 | 6.3 | 24.4 | 17.1 | 0.48 | 3.74 | 1.14 | 2.19 |
| 85 | RH 1917 | 48 | 142 | 214.7 | 5.0 | 12.8 | 82.4 | 53.3 | 4.6 | 13.6 | 38.9 | 6.3 | 23.8 | 14.4 | 0.53 | 5.11 | 1.24 | 2.11 |
| 86 | RH 1918 | 50 | 143 | 205.5 | 4.2 | 9.5 | 84.2 | 53.2 | 5.4 | 13.2 | 38.8 | 6.7 | 23.9 | 16.4 | 0.35 | 3.64 | 1.18 | 2.15 |
| 87 | RH 1919 | 50 | 142 | 214.0 | 5.7 | 9.7 | 78.3 | 54.7 | 5.0 | 12.8 | 39.3 | 7.2 | 17.4 | 15.2 | 0.47 | 3.80 | 1.08 | 1.97 |
| 88 | RH 1922 | 47 | 140 | 193.3 | 5.2 | 10.8 | 80.5 | 46.8 | 4.6 | 12.1 | 39.4 | 6.3 | 23.4 | 19.4 | 0.45 | 4.15 | 1.24 | 2.01 |
| 89 | RH 1923 | 49 | 144 | 213.2 | 5.3 | 12.3 | 82.5 | 48.7 | 5.0 | 12.5 | 38.5 | 6.4 | 17.0 | 14.9 | 0.41 | 2.78 | 1.13 | 1.90 |
| 90 | RH 1924 | 48 | 141 | 205.8 | 4.3 | 7.8 | 88.3 | 57.2 | 5.5 | 12.3 | 39.5 | 7.0 | 21.2 | 17.6 | 0.50 | 4.38 | 1.05 | 1.80 |
| 91 | RH 1927 | 49 | 142 | 205.7 | 5.3 | 11.5 | 81.5 | 59.7 | 5.0 | 14.0 | 38.7 | 5.2 | 22.7 | 19.5 | 0.53 | 3.56 | 1.11 | 1.79 |
| 92 | RH 1928 | 48 | 141 | 202.2 | 6.5 | 17.0 | 81.2 | 54.2 | 4.7 | 13.1 | 38.6 | 5.5 | 23.3 | 17.4 | 0.47 | 3.00 | 1.18 | 2.17 |
| 93 | RH 1929 | 48 | 142 | 202.2 | 4.5 | 9.2 | 84.0 | 51.9 | 5.4 | 12.7 | 38.7 | 6.5 | 25.1 | 20.4 | 0.45 | 3.75 | 1.31 | 1.80 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 94 | RH 1930 | 48 | 142 | 207.8 | 4.8 | 11.2 | 80.3 | 52.7 | 5.5 | 12.1 | 38.9 | 6.6 | 29.4 | 22.5 | 0.56 | 3.37 | 1.33 | 2.27 |
| 95 | RH 1931 | 46 | 140 | 204.7 | 4.0 | 10.0 | 84.7 | 46.7 | 5.1 | 12.5 | 38.9 | 7.2 | 25.3 | 18.5 | 0.49 | 4.04 | 1.37 | 2.63 |
| 96 | RH 1932 | 49 | 141 | 204.2 | 5.7 | 12.7 | 90.0 | 54.5 | 4.9 | 12.1 | 39.2 | 6.7 | 27.9 | 20.2 | 0.49 | 2.65 | 1.36 | 2.45 |
| 97 | RH 1934 | 51 | 143 | 207.0 | 5.8 | 14.8 | 78.7 | 51.7 | 4.6 | 12.7 | 39.2 | 5.8 | 26.7 | 17.7 | 0.50 | 2.21 | 1.12 | 2.05 |
| 98 | RH 1935 | 47 | 139 | 204.5 | 5.3 | 12.2 | 85.7 | 59.7 | 4.9 | 14.0 | 38.7 | 6.7 | 28.1 | 20.2 | 0.61 | 3.08 | 1.33 | 2.15 |
| 99 | RH 1936 | 49 | 141 | 204.3 | 4.5 | 12.5 | 78.3 | 50.3 | 5.5 | 12.4 | 38.9 | 5.7 | 24.3 | 18.7 | 0.51 | 3.52 | 1.41 | 2.26 |
| 100 | RH1937 | 45 | 136 | 193.0 | 5.3 | 13.7 | 77.5 | 48.0 | 5.7 | 14.3 | 39.4 | 5.7 | 27.2 | 19.5 | 0.58 | 3.64 | 1.32 | 2.47 |
| 101 | RH 1938 | 44 | 135 | 206.8 | 5.2 | 11.5 | 83.5 | 48.9 | 5.7 | 13.2 | 38.8 | 6.0 | 26.5 | 19.2 | 0.44 | 2.92 | 1.20 | 2.26 |
| 102 | RH 1939 | 47 | 137 | 200.9 | 4.0 | 11.0 | 85.0 | 52.0 | 5.4 | 14.2 | 38.8 | 6.1 | 24.5 | 18.8 | 0.50 | 3.27 | 1.14 | 2.23 |
| 103 | RH 1940 | 47 | 138 | 201.3 | 4.7 | 9.5 | 83.2 | 54.2 | 5.8 | 13.1 | 38.9 | 6.1 | 27.8 | 21.4 | 0.53 | 2.45 | 1.23 | 2.15 |
| 104 | RH 2001 | 44 | 136 | 189.0 | 4.7 | 13.0 | 86.8 | 50.2 | 5.7 | 12.5 | 38.7 | 6.3 | 26.0 | 20.4 | 0.53 | 2.70 | 1.05 | 1.88 |
| 105 | RH 2002 | 47 | 140 | 207.0 | 4.2 | 10.0 | 89.2 | 52.8 | 5.5 | 12.5 | 38.9 | 6.3 | 28.3 | 20.4 | 0.54 | 3.79 | 1.31 | 2.56 |
| 106 | RH 2003 | 53 | 145 | 205.5 | 4.8 | 10.3 | 83.4 | 57.4 | 4.5 | 12.8 | 39.3 | 6.0 | 27.2 | 18.8 | 0.49 | 3.60 | 1.34 | 2.56 |
| 107 | RH 2004 | 55 | 148 | 211.5 | 5.7 | 13.7 | 82.2 | 44.3 | 5.7 | 14.6 | 38.7 | 7.1 | 29.3 | 17.2 | 0.51 | 2.40 | 1.26 | 2.51 |
| 108 | RH 2005 | 55 | 147 | 220.5 | 5.2 | 17.8 | 75.8 | 41.8 | 5.8 | 13.2 | 38.7 | 6.4 | 24.9 | 16.5 | 0.43 | 3.56 | 1.04 | 2.27 |
| 109 | RH 2006 | 51 | 144 | 212.0 | 5.2 | 13.0 | 90.8 | 62.2 | 4.7 | 13.1 | 38.7 | 5.8 | 26.2 | 20.3 | 0.46 | 3.58 | 1.35 | 2.48 |
| 110 | RH 2007 | 52 | 144 | 205.0 | 4.8 | 9.5 | 80.8 | 56.7 | 5.0 | 13.3 | 38.3 | 6.6 | 25.3 | 16.9 | 0.43 | 2.33 | 1.38 | 2.23 |
| 111 | RH 2008 | 48 | 142 | 205.7 | 5.5 | 14.7 | 83.5 | 54.5 | 5.1 | 13.3 | 39.4 | 6.7 | 24.9 | 16.3 | 0.38 | 3.85 | 1.19 | 1.97 |
| 112 | RH 2012 | 47 | 141 | 197.0 | 4.8 | 12.7 | 81.8 | 49.7 | 5.6 | 12.3 | 38.8 | 6.5 | 21.2 | 14.7 | 0.41 | 3.37 | 1.05 | 2.08 |
| 113 | RH 2013 | 51 | 143 | 198.0 | 4.7 | 10.0 | 84.2 | 47.2 | 5.5 | 13.5 | 38.8 | 6.6 | 24.8 | 17.8 | 0.52 | 3.42 | 1.07 | 2.32 |
| 114 | RH 2014 | 47 | 139 | 191.2 | 4.8 | 9.2 | 78.0 | 43.8 | 5.5 | 13.7 | 38.9 | 6.3 | 26.2 | 18.4 | 0.55 | 2.35 | 1.28 | 2.22 |
| 115 | RH 2015 | 43 | 134 | 204.0 | 4.5 | 10.7 | 89.0 | 52.8 | 6.0 | 14.9 | 39.4 | 6.0 | 25.8 | 19.3 | 0.50 | 3.61 | 1.19 | 2.38 |
| 116 | RH 2016 | 49 | 140 | 208.9 | 4.5 | 9.7 | 91.2 | 55.5 | 5.1 | 14.0 | 39.4 | 6.1 | 23.7 | 18.7 | 0.46 | 1.15 | 1.33 | 2.38 |
| 117 | RH 2017 | 50 | 141 | 207.8 | 5.2 | 10.5 | 83.8 | 49.2 | 5.7 | 14.0 | 38.8 | 5.9 | 25.1 | 18.0 | 0.48 | 1.43 | 1.31 | 2.38 |
| 118 | RH 2018 | 48 | 141 | 211.2 | 5.0 | 9.8 | 93.7 | 57.8 | 5.1 | 13.3 | 39.1 | 5.8 | 24.4 | 16.3 | 0.45 | 2.49 | 1.29 | 2.32 |
| 119 | RH 2019 | 51 | 142 | 199.2 | 5.0 | 14.5 | 84.3 | 49.8 | 5.5 | 13.7 | 38.8 | 5.9 | 24.2 | 17.9 | 0.43 | 2.86 | 1.13 | 1.99 |
| 120 | RH 2020 | 51 | 144 | 203.0 | 5.5 | 13.4 | 77.0 | 45.3 | 6.2 | 14.2 | 38.8 | 6.8 | 23.9 | 17.9 | 0.47 | 3.02 | 1.26 | 2.08 |
| 121 | RH 2021 | 51 | 142 | 208.2 | 5.5 | 13.8 | 85.2 | 53.5 | 5.1 | 14.0 | 38.6 | 6.3 | 24.8 | 19.7 | 0.43 | 2.58 | 1.13 | 2.28 |
| 122 | RH 2022 | 56 | 147 | 188.8 | 5.0 | 9.8 | 86.2 | 66.8 | 5.0 | 12.2 | 39.0 | 6.8 | 25.2 | 17.2 | 0.52 | 3.40 | 1.16 | 2.14 |
| 123 | RH 2023 | 50 | 139 | 201.0 | 5.5 | 10.2 | 81.3 | 48.2 | 5.9 | 12.4 | 39.4 | 6.8 | 23.0 | 14.6 | 0.50 | 3.24 | 1.05 | 2.03 |
| 124 | RH 2024 | 49 | 139 | 197.2 | 4.2 | 8.3 | 94.7 | 54.0 | 6.0 | 13.1 | 39.1 | 6.8 | 23.9 | 17.0 | 0.36 | 3.02 | 1.08 | 2.14 |
| 125 | RH 2025 | 52 | 144 | 207.3 | 5.2 | 12.7 | 79.8 | 56.5 | 5.1 | 14.0 | 38.7 | 7.0 | 29.5 | 21.6 | 0.47 | 2.60 | 1.35 | 1.61 |

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| 126 | RH 2026 | 51 | 142 | 208.8 | 4.8 | 13.9 | 91.2 | 60.0 | 5.1 | 14.2 | 38.7 | 6.1 | 21.9 | 14.5 | 0.36 | 2.51 | 1.13 | 1.88 |
| 127 | RH 2027 | 53 | 144 | 197.9 | 5.0 | 14.3 | 82.0 | 52.5 | 4.8 | 13.8 | 39.3 | 5.2 | 24.0 | 15.3 | 0.42 | 3.52 | 1.11 | 2.18 |
| 128 | RH 2028 | 55 | 146 | 214.5 | 6.0 | 11.0 | 78.0 | 52.5 | 4.4 | 14.4 | 39.5 | 5.7 | 25.2 | 17.3 | 0.44 | 2.86 | 1.10 | 1.93 |
| 129 | RH 2029 | 54 | 145 | 202.2 | 4.5 | 22.0 | 81.7 | 60.2 | 4.5 | 13.8 | 39.1 | 6.1 | 27.9 | 19.7 | 0.51 | 3.09 | 1.31 | 2.56 |
| 130 | RH 2030 | 50 | 140 | 197.8 | 4.0 | 8.8 | 83.8 | 49.5 | 5.4 | 15.4 | 39.5 | 7.0 | 26.4 | 18.3 | 0.46 | 2.64 | 1.21 | 2.42 |
| 131 | RH 2031 | 51 | 142 | 200.7 | 5.5 | 12.7 | 85.3 | 56.7 | 4.7 | 13.5 | 38.9 | 6.7 | 25.1 | 15.7 | 0.49 | 2.72 | 1.30 | 2.04 |
| 132 | RH 2032 | 49 | 142 | 204.7 | 4.5 | 12.5 | 86.5 | 46.7 | 4.8 | 13.5 | 38.8 | 6.6 | 27.8 | 19.0 | 0.50 | 3.15 | 1.17 | 2.54 |
| 133 | RH 2033 | 53 | 145 | 205.7 | 4.9 | 11.2 | 73.8 | 40.3 | 5.1 | 14.8 | 39.4 | 6.7 | 26.1 | 16.8 | 0.53 | 2.47 | 1.37 | 2.43 |
| 134 | RH 2034 | 51 | 143 | 196.0 | 4.8 | 8.3 | 70.5 | 43.4 | 5.1 | 15.1 | 38.9 | 5.7 | 22.3 | 16.1 | 0.43 | 1.99 | 1.21 | 1.99 |
| 135 | RH 2035 | 55 | 146 | 205.5 | 4.3 | 10.8 | 76.3 | 42.2 | 5.4 | 14.4 | 39.4 | 6.7 | 25.1 | 16.4 | 0.46 | 3.45 | 1.25 | 2.18 |
| 136 | RH 2036 | 48 | 138 | 201.8 | 4.8 | 14.5 | 78.8 | 46.2 | 5.9 | 14.7 | 38.8 | 6.3 | 29.3 | 20.9 | 0.48 | 2.70 | 1.29 | 2.63 |
| 137 | RH 2038 | 53 | 144 | 207.8 | 4.0 | 12.0 | 81.8 | 45.5 | 5.6 | 13.1 | 38.6 | 7.6 | 29.3 | 19.8 | 0.42 | 2.57 | 1.16 | 2.24 |
| 138 | RH 2039 | 48 | 140 | 207.3 | 5.5 | 11.7 | 76.5 | 40.7 | 5.2 | 12.2 | 38.8 | 6.6 | 27.1 | 18.7 | 0.35 | 2.29 | 1.11 | 2.41 |
| 139 | RH 2040 | 48 | 142 | 211.5 | 5.2 | 13.2 | 88.2 | 47.5 | 5.1 | 14.0 | 38.9 | 6.4 | 27.1 | 19.9 | 0.53 | 3.49 | 1.19 | 2.04 |
| 140 | RH 2041 | 50 | 142 | 216.8 | 5.5 | 10.9 | 77.2 | 48.3 | 5.3 | 12.9 | 38.9 | 6.5 | 27.8 | 19.0 | 0.47 | 3.45 | 1.34 | 2.03 |
| 141 | RH 2042 | 51 | 143 | 219.8 | 4.8 | 11.2 | 79.2 | 55.8 | 5.4 | 13.2 | 38.9 | 7.1 | 28.3 | 16.6 | 0.50 | 2.79 | 1.23 | 2.00 |
| 142 | RH 2043 | 52 | 142 | 211.2 | 4.8 | 9.3 | 87.0 | 47.2 | 4.9 | 12.4 | 39.1 | 7.6 | 25.1 | 17.0 | 0.41 | 4.01 | 1.23 | 2.09 |
| 143 | RH 2044 | 50 | 141 | 195.0 | 5.7 | 13.4 | 85.8 | 48.2 | 5.1 | 13.6 | 38.7 | 7.7 | 28.1 | 19.1 | 0.58 | 3.81 | 1.29 | 1.99 |
| 144 | RH 2045 | 52 | 143 | 208.7 | 5.5 | 9.8 | 84.8 | 49.0 | 5.0 | 12.5 | 38.7 | 6.6 | 24.9 | 15.2 | 0.46 | 3.29 | 1.16 | 1.74 |
| 145 | RH 2046 | 54 | 144 | 222.2 | 5.7 | 11.4 | 80.2 | 51.8 | 5.6 | 13.3 | 39.5 | 7.8 | 27.1 | 16.5 | 0.43 | 3.19 | 1.25 | 2.19 |
| 146 | RH 2047 | 48 | 141 | 210.3 | 5.7 | 11.3 | 89.3 | 55.8 | 5.1 | 12.5 | 38.2 | 7.4 | 25.8 | 17.4 | 0.52 | 3.05 | 1.23 | 2.01 |
| 147 | RH 2048 | 47 | 138 | 192.2 | 5.0 | 10.3 | 88.5 | 50.8 | 5.5 | 14.2 | 38.6 | 6.6 | 25.4 | 15.8 | 0.42 | 2.76 | 1.20 | 2.10 |
| 148 | RH 2049 | 46 | 136 | 190.2 | 5.2 | 12.4 | 82.3 | 44.3 | 6.6 | 14.1 | 38.8 | 7.2 | 30.9 | 22.1 | 0.56 | 1.56 | 1.36 | 2.04 |
| 149 | RH 2050 | 46 | 137 | 201.5 | 5.4 | 13.7 | 79.0 | 58.2 | 6.4 | 14.7 | 39.2 | 6.5 | 29.9 | 23.4 | 0.52 | 1.68 | 1.34 | 2.20 |
| 150 | RH 2051 | 47 | 140 | 204.5 | 4.8 | 13.0 | 79.8 | 51.0 | 5.7 | 12.9 | 39.4 | 7.3 | 27.7 | 19.5 | 0.48 | 4.12 | 1.25 | 2.06 |
| **Mean** | | **50** | **141** | **206.4** | **5.3** | **12.8** | **83.2** | **53.0** | **4.8** | **13.8** | **38.9** | **5.5** | **22.7** | **17.1** | **0.45** | **3.28** | **1.17** | **2.14** |
| **Range** | | **42-57** | **132-148** | **172.2-242.2** | **4.0-8.8** | **7.8-22.7** | **59.2-100.5** | **40.3-66.8** | **3.4-11.0** | **11.7-16.2** | **38.1-40.0** | **3.1-7.8** | **14.0-30.9** | **11.6-24.8** | **0.26-0.80** | **1.15-5.81** | **0.88-1.46** | **1.61-2.84** |
| **C.D. #** | | **4.56** | **5.30** | **10.94** | **0.77** | **1.75** | **9.68** | **6.81** | **0.78** | **0.70** | **0.49** | **1.09** | **4.86** | **3.03** | **0.09** | **1.91** | **0.12** | **0.21** |

# - C.D. at 5% between two varieties not in same block; DF-Days to 50% flowering; DM-Days to maturity; PH-Plant height (cm); NPB-Number of primary branches/plant; NSB-Number of secondary branches/plant; MSL-Main shoot length (cm), NSMS-Number of siliquae on main shoot; SL-Siliqua length (cm); NSS-Number of seeds/siliqua; OC-Oil content (%); TSW-1000-seed weight (g); SYP-Seed yield/plant (g); A–Photosynthetic rate (µmol $CO_2/m^2/s$); Gs – Stomatal conductance (mmol/$m^2$/s); E – Transpiration rate (mmol $H_2O/m^2/s$); Chl – Total chlorophyll content (mg/g); Caro – Carotenoid content (mg/g).

**APPENDIX-III: Mean performance of test genotypes for morphological and physiological traits under late sown environment**

| Sr. No. | Genotype | DF | DM | PH | NPB | NSB | MSL | NSMS | SL | NSS | OC | TSW | SYP | A | Gs | E | Chl | Caro |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | RH 30 | 64 | 128 | 173.5 | 5.0 | 12.2 | 68.0 | 46.4 | 3.6 | 10.5 | 38.8 | 4.7 | 20.2 | 14.5 | 0.20 | 3.22 | 1.18 | 2.60 |
| 2 | RH 0119 | 72 | 131 | 182.7 | 4.3 | 10.0 | 68.3 | 41.0 | 5.1 | 12.0 | 38.7 | 4.4 | 18.6 | 9.1 | 0.21 | 3.67 | 0.94 | 2.49 |
| 3 | RH 406 | 74 | 133 | 181.2 | 3.8 | 6.3 | 68.2 | 42.8 | 4.4 | 12.8 | 38.8 | 4.6 | 23.4 | 8.7 | 0.27 | 5.67 | 0.96 | 2.57 |
| 4 | RH 725 | 72 | 131 | 183.0 | 4.7 | 9.5 | 66.8 | 48.2 | 4.6 | 12.7 | 37.7 | 5.0 | 27.0 | 16.1 | 0.33 | 6.12 | 1.18 | 2.74 |
| 5 | PBR 91 | 74 | 134 | 197.7 | 3.7 | 9.5 | 78.7 | 50.9 | 3.8 | 10.5 | 38.4 | 4.2 | 11.5 | 9.1 | 0.19 | 6.02 | 0.84 | 2.40 |
| 6 | RH 761 | 73 | 131 | 193.8 | 4.0 | 6.2 | 65.7 | 46.5 | 4.6 | 11.9 | 38.6 | 4.3 | 23.0 | 8.8 | 0.26 | 5.30 | 1.00 | 2.53 |
| 7 | RH 8113 | 76 | 132 | 200.8 | 4.7 | 12.8 | 69.2 | 50.7 | 3.7 | 10.7 | 38.6 | 4.1 | 14.2 | 10.4 | 0.18 | 7.09 | 0.85 | 2.43 |
| 8 | PBR 97 | 73 | 132 | 191.5 | 4.8 | 10.0 | 71.0 | 41.8 | 3.6 | 12.2 | 38.4 | 3.6 | 13.6 | 9.4 | 0.21 | 6.94 | 0.81 | 1.97 |
| 9 | RH 9304 | 68 | 126 | 181.0 | 4.2 | 9.3 | 71.5 | 47.3 | 3.4 | 12.1 | 38.6 | 4.3 | 21.7 | 12.8 | 0.14 | 6.65 | 1.09 | 2.54 |
| 10 | RH 9801 | 69 | 126 | 182.7 | 4.8 | 11.8 | 75.7 | 43.3 | 3.7 | 11.4 | 38.8 | 3.7 | 20.7 | 10.8 | 0.14 | 5.09 | 0.90 | 2.31 |
| 11 | RB 50 | 74 | 126 | 198.7 | 3.8 | 8.7 | 75.8 | 45.8 | 4.2 | 10.8 | 37.9 | 4.5 | 19.9 | 12.6 | 0.18 | 6.80 | 0.91 | 2.85 |
| 12 | RL 1359 | 74 | 130 | 184.2 | 3.8 | 10.3 | 76.0 | 45.3 | 4.2 | 12.9 | 38.5 | 3.1 | 12.3 | 8.7 | 0.19 | 7.07 | 0.78 | 2.37 |
| 13 | PM 21 | 77 | 131 | 203.8 | 5.2 | 9.5 | 75.5 | 48.2 | 4.0 | 9.8 | 38.7 | 4.3 | 19.3 | 8.7 | 0.25 | 8.47 | 0.78 | 2.19 |
| 14 | PM 22 | 70 | 127 | 196.2 | 4.5 | 13.2 | 74.7 | 40.0 | 4.1 | 12.9 | 38.1 | 5.0 | 18.7 | 7.2 | 0.22 | 7.47 | 0.85 | 2.28 |
| 15 | PM 24 | 74 | 130 | 202.8 | 5.0 | 11.4 | 64.0 | 49.4 | 3.9 | 11.0 | 37.9 | 3.7 | 16.5 | 8.5 | 0.21 | 6.67 | 0.78 | 2.38 |
| 16 | PM 25 | 62 | 119 | 171.7 | 4.5 | 8.9 | 82.5 | 44.0 | 4.6 | 12.5 | 38.4 | 4.2 | 18.3 | 11.4 | 0.23 | 8.44 | 1.05 | 2.32 |
| 17 | PM 26 | 63 | 120 | 184.0 | 5.5 | 13.7 | 76.5 | 52.8 | 4.2 | 14.0 | 38.7 | 4.7 | 21.2 | 21.2 | 0.59 | 5.45 | 1.27 | 2.97 |
| 18 | PM 28 | 63 | 121 | 178.5 | 4.5 | 13.2 | 77.8 | 44.7 | 4.0 | 12.6 | 38.4 | 3.6 | 18.7 | 12.0 | 0.28 | 8.03 | 0.87 | 2.26 |
| 19 | PDZM 31 | 68 | 127 | 198.0 | 4.7 | 10.3 | 66.3 | 47.2 | 3.3 | 13.3 | 38.6 | 2.9 | 15.7 | 11.0 | 0.18 | 7.47 | 0.85 | 2.26 |
| 20 | Pusa Vijay | 67 | 123 | 193.4 | 4.2 | 9.5 | 77.5 | 41.2 | 3.7 | 9.9 | 38.5 | 4.5 | 13.3 | 11.4 | 0.24 | 8.00 | 0.80 | 2.37 |
| 21 | Pusa Jai Kisan | 68 | 127 | 186.9 | 4.7 | 10.7 | 76.2 | 43.9 | 3.8 | 10.8 | 38.3 | 4.3 | 16.0 | 11.1 | 0.27 | 9.04 | 1.02 | 2.25 |
| 22 | Pusa Karishma | 79 | 131 | 201.5 | 6.0 | 9.9 | 71.0 | 49.8 | 3.2 | 12.0 | 38.5 | 2.8 | 16.7 | 10.4 | 0.28 | 10.15 | 0.99 | 2.15 |
| 23 | Pusa Bold | 69 | 127 | 187.2 | 4.0 | 5.2 | 73.0 | 41.3 | 4.3 | 11.2 | 38.0 | 3.9 | 18.6 | 9.9 | 0.28 | 9.69 | 1.07 | 2.20 |
| 24 | Pusa Jagannath | 69 | 129 | 193.2 | 3.8 | 11.3 | 72.8 | 38.8 | 4.0 | 11.7 | 37.9 | 4.2 | 20.2 | 11.3 | 0.26 | 10.51 | 0.99 | 2.86 |
| 25 | BPR 349-9 | 68 | 123 | 187.5 | 4.4 | 14.7 | 82.5 | 47.3 | 4.1 | 13.6 | 38.5 | 4.1 | 19.0 | 14.9 | 0.40 | 4.76 | 1.28 | 3.02 |
| 26 | BPR 540-6 | 64 | 123 | 184.9 | 4.5 | 13.0 | 81.5 | 46.7 | 4.5 | 12.1 | 37.9 | 4.1 | 16.4 | 10.3 | 0.22 | 9.04 | 0.91 | 2.13 |
| 27 | BPR 543-2 | 68 | 127 | 158.0 | 4.2 | 11.0 | 81.5 | 49.2 | 4.4 | 11.8 | 38.4 | 4.2 | 18.1 | 9.1 | 0.22 | 9.35 | 0.89 | 2.43 |
| 28 | BPR 549-9 | 66 | 125 | 194.3 | 4.7 | 7.3 | 77.7 | 47.4 | 4.3 | 14.4 | 38.7 | 5.3 | 16.1 | 7.3 | 0.30 | 9.91 | 0.88 | 2.49 |
| 29 | DRMR 150-35 | 66 | 122 | 187.2 | 3.5 | 7.5 | 67.3 | 41.3 | 3.9 | 10.7 | 38.5 | 4.7 | 13.6 | 7.4 | 0.30 | 8.90 | 0.99 | 2.26 |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 30 | DRMR 1165-40 | 67 | 127 | 192.3 | 4.8 | 8.7 | 74.2 | 41.0 | 4.2 | 13.0 | 38.6 | 4.0 | 11.9 | 9.1 | 0.13 | 9.41 | 0.80 | 2.33 |
| 31 | NRCDR 2 | 65 | 125 | 188.2 | 5.0 | 12.4 | 71.3 | 45.0 | 4.9 | 12.5 | 38.1 | 4.9 | 18.4 | 12.1 | 0.16 | 7.59 | 0.89 | 2.65 |
| 32 | NRCDR 601 | 67 | 128 | 183.8 | 5.2 | 10.2 | 70.7 | 50.3 | 4.3 | 12.3 | 38.7 | 4.5 | 17.1 | 10.0 | 0.15 | 7.82 | 0.89 | 2.27 |
| 33 | RGN 48 | 73 | 132 | 208.0 | 4.7 | 11.4 | 76.7 | 57.9 | 4.0 | 11.7 | 38.7 | 3.9 | 17.0 | 11.2 | 0.26 | 7.24 | 0.92 | 1.97 |
| 34 | RGN 145 | 69 | 126 | 193.0 | 4.7 | 10.7 | 78.5 | 51.0 | 4.1 | 11.7 | 38.0 | 4.2 | 10.5 | 6.9 | 0.19 | 8.89 | 0.78 | 2.28 |
| 35 | RGN 229 | 72 | 127 | 231.5 | 4.5 | 10.7 | 79.8 | 54.2 | 4.0 | 10.8 | 38.8 | 4.0 | 15.3 | 9.5 | 0.23 | 7.13 | 0.84 | 2.37 |
| 36 | RGN 236 | 71 | 127 | 205.3 | 4.3 | 10.3 | 80.0 | 49.5 | 4.1 | 12.3 | 38.7 | 4.1 | 12.6 | 8.9 | 0.20 | 7.75 | 0.81 | 2.13 |
| 37 | RGN 298 | 72 | 129 | 214.2 | 5.3 | 11.0 | 83.7 | 54.9 | 4.3 | 10.7 | 38.2 | 4.1 | 15.5 | 9.5 | 0.25 | 8.45 | 0.83 | 2.15 |
| 38 | CS 56 | 71 | 124 | 200.7 | 4.8 | 11.8 | 84.0 | 55.2 | 4.2 | 12.6 | 38.1 | 4.4 | 17.0 | 9.5 | 0.24 | 7.01 | 0.83 | 2.25 |
| 39 | Radhika | 68 | 126 | 198.7 | 4.2 | 7.8 | 76.0 | 37.5 | 4.3 | 12.6 | 38.7 | 4.3 | 12.1 | 9.4 | 0.26 | 9.03 | 0.75 | 2.51 |
| 40 | Shivani | 69 | 126 | 201.7 | 5.2 | 8.7 | 60.7 | 41.3 | 3.8 | 11.7 | 38.6 | 3.6 | 15.1 | 7.1 | 0.21 | 9.76 | 0.77 | 2.57 |
| 41 | Aravali | 66 | 125 | 175.7 | 4.7 | 9.5 | 72.0 | 50.0 | 4.2 | 11.3 | 37.9 | 3.4 | 10.8 | 6.1 | 0.18 | 10.80 | 0.75 | 1.82 |
| 42 | Varuna | 68 | 132 | 191.8 | 4.2 | 8.8 | 71.8 | 46.3 | 3.8 | 9.5 | 38.5 | 4.0 | 14.9 | 7.6 | 0.19 | 6.93 | 0.81 | 2.55 |
| 43 | Pant Rai 18 | 69 | 127 | 181.2 | 4.3 | 8.5 | 70.3 | 44.0 | 4.0 | 12.2 | 38.7 | 3.6 | 10.8 | 4.7 | 0.25 | 7.62 | 0.84 | 2.10 |
| 44 | Pant Rai 19 | 62 | 119 | 162.7 | 4.3 | 9.7 | 77.5 | 45.0 | 4.4 | 12.1 | 38.7 | 3.5 | 9.6 | 4.9 | 0.22 | 10.36 | 0.73 | 1.85 |
| 45 | Pant Rai 20 | 70 | 124 | 190.3 | 5.3 | 8.0 | 70.2 | 40.7 | 4.3 | 12.0 | 39.3 | 4.0 | 13.4 | 7.0 | 0.10 | 5.28 | 0.89 | 2.06 |
| 46 | RH 781 | 71 | 128 | 198.0 | 4.7 | 7.0 | 76.2 | 47.2 | 3.9 | 10.7 | 38.8 | 4.7 | 18.1 | 10.6 | 0.16 | 7.81 | 1.03 | 2.29 |
| 47 | Giriraj | 68 | 128 | 183.0 | 4.0 | 5.0 | 69.0 | 41.0 | 4.3 | 11.5 | 37.7 | 4.3 | 17.2 | 8.0 | 0.21 | 8.90 | 0.82 | 2.43 |
| 48 | RH 819 | 74 | 130 | 201.3 | 4.8 | 9.2 | 68.3 | 46.3 | 4.3 | 13.1 | 38.2 | 3.3 | 13.2 | 6.8 | 0.13 | 7.12 | 0.81 | 2.35 |
| 49 | RVM 2 | 74 | 128 | 204.3 | 5.0 | 12.0 | 68.0 | 51.0 | 4.1 | 12.4 | 38.6 | 3.3 | 13.2 | 8.6 | 0.25 | 9.15 | 0.85 | 1.99 |
| 50 | CS 52 | 72 | 128 | 199.5 | 4.5 | 7.7 | 76.0 | 52.7 | 3.5 | 12.3 | 38.4 | 3.7 | 15.0 | 7.2 | 0.17 | 10.21 | 0.98 | 2.10 |
| 51 | IC 122287 | 70 | 127 | 189.8 | 5.0 | 8.5 | 64.8 | 45.0 | 3.4 | 12.6 | 38.9 | 4.1 | 13.7 | 5.8 | 0.28 | 9.11 | 0.80 | 2.41 |
| 52 | IC 333591 | 70 | 128 | 195.0 | 3.9 | 8.3 | 73.7 | 47.0 | 4.0 | 10.6 | 38.1 | 4.1 | 11.2 | 5.5 | 0.24 | 11.18 | 0.85 | 2.22 |
| 53 | IC 470135 | 71 | 128 | 192.7 | 4.7 | 10.3 | 71.5 | 49.5 | 4.4 | 14.3 | 38.0 | 3.7 | 12.5 | 6.9 | 0.23 | 9.29 | 0.88 | 2.12 |
| 54 | IC 491390 | 70 | 130 | 187.8 | 4.7 | 8.7 | 70.0 | 37.0 | 3.7 | 11.8 | 37.9 | 5.1 | 12.0 | 7.2 | 0.24 | 8.18 | 0.82 | 1.93 |
| 55 | RH 847 | 72 | 131 | 204.3 | 4.7 | 10.5 | 70.3 | 45.0 | 4.0 | 11.9 | 38.1 | 4.7 | 12.7 | 6.7 | 0.30 | 9.96 | 0.95 | 2.41 |
| 56 | RH 1400-2 | 64 | 125 | 190.3 | 4.7 | 14.5 | 79.5 | 50.2 | 3.9 | 11.2 | 38.6 | 4.3 | 17.8 | 7.6 | 0.23 | 7.26 | 0.74 | 2.30 |
| 57 | RC 2 | 67 | 129 | 195.5 | 4.7 | 9.3 | 64.3 | 41.5 | 3.6 | 11.7 | 38.8 | 2.9 | 11.5 | 6.8 | 0.15 | 6.72 | 0.95 | 1.90 |
| 58 | RC 5 | 74 | 130 | 196.2 | 5.8 | 11.2 | 61.8 | 43.5 | 3.3 | 11.2 | 38.7 | 2.6 | 11.1 | 7.3 | 0.30 | 7.64 | 0.64 | 2.12 |
| 59 | RC 12 | 67 | 128 | 192.5 | 3.7 | 9.0 | 75.8 | 47.7 | 4.0 | 12.0 | 38.7 | 3.8 | 10.6 | 7.5 | 0.16 | 7.47 | 0.57 | 1.83 |
| 60 | RC 48 | 68 | 127 | 190.3 | 4.2 | 8.8 | 72.0 | 43.7 | 3.5 | 11.3 | 38.7 | 3.0 | 11.9 | 6.7 | 0.23 | 8.41 | 0.86 | 2.22 |
| 61 | RC 81 | 68 | 126 | 200.2 | 5.0 | 9.7 | 84.8 | 53.2 | 5.1 | 12.1 | 37.7 | 4.1 | 13.3 | 4.6 | 0.16 | 6.45 | 0.80 | 2.20 |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 62 | RC 91 | 66 | 125 | 177.8 | 4.7 | 12.0 | 70.3 | 44.5 | 3.7 | 11.8 | 37.9 | 3.2 | 12.8 | 5.8 | 0.11 | 6.35 | 0.80 | 1.99 |
| 63 | RC 104 | 68 | 124 | 184.5 | 4.7 | 11.5 | 70.3 | 46.7 | 3.9 | 12.9 | 38.0 | 3.2 | 12.5 | 6.9 | 0.17 | 9.15 | 0.87 | 2.34 |
| 64 | RC 106 | 72 | 126 | 198.2 | 4.8 | 12.9 | 71.5 | 46.7 | 4.0 | 11.9 | 37.9 | 3.3 | 12.3 | 8.1 | 0.15 | 6.96 | 0.85 | 2.06 |
| 65 | RC 108 | 68 | 125 | 178.4 | 4.8 | 12.2 | 69.0 | 48.8 | 3.4 | 11.8 | 38.4 | 3.3 | 12.3 | 8.1 | 0.13 | 6.07 | 0.80 | 1.62 |
| 66 | RC 118 | 65 | 124 | 190.7 | 4.7 | 11.5 | 76.2 | 50.0 | 3.6 | 11.2 | 38.8 | 3.6 | 13.4 | 8.3 | 0.15 | 5.68 | 0.76 | 2.16 |
| 67 | RC 134 | 71 | 126 | 204.2 | 5.5 | 8.0 | 78.5 | 45.8 | 3.4 | 10.0 | 38.6 | 3.0 | 11.1 | 8.3 | 0.11 | 3.57 | 0.84 | 2.02 |
| 68 | RC 162 | 70 | 126 | 178.3 | 4.5 | 11.7 | 71.0 | 45.7 | 3.9 | 13.4 | 38.7 | 3.0 | 12.1 | 5.2 | 0.12 | 5.18 | 0.73 | 2.16 |
| 69 | RC 280 | 68 | 126 | 197.5 | 4.7 | 12.7 | 77.2 | 52.3 | 4.0 | 12.1 | 38.8 | 3.2 | 12.4 | 7.9 | 0.18 | 5.89 | 0.82 | 2.25 |
| 70 | RC 330 | 65 | 123 | 185.3 | 4.2 | 9.8 | 65.2 | 44.8 | 3.8 | 13.0 | 38.9 | 3.5 | 11.8 | 7.3 | 0.20 | 7.16 | 0.85 | 2.33 |
| 71 | RC 448 | 64 | 122 | 166.7 | 5.2 | 12.8 | 62.0 | 42.3 | 3.6 | 12.3 | 38.5 | 2.9 | 12.4 | 5.0 | 0.12 | 7.21 | 0.92 | 2.16 |
| 72 | RC 449 | 69 | 126 | 197.8 | 5.9 | 12.3 | 72.0 | 49.7 | 3.1 | 11.9 | 38.5 | 3.2 | 13.2 | 6.8 | 0.13 | 6.57 | 0.85 | 1.90 |
| 73 | RC 587 | 67 | 125 | 190.5 | 4.8 | 13.2 | 70.8 | 45.2 | 3.6 | 11.9 | 38.8 | 3.3 | 18.8 | 5.4 | 0.20 | 7.52 | 0.84 | 1.81 |
| 74 | RC 713 | 69 | 125 | 193.0 | 5.8 | 12.5 | 64.0 | 41.0 | 4.2 | 12.4 | 38.9 | 3.5 | 13.2 | 3.6 | 0.11 | 4.88 | 0.75 | 1.96 |
| 75 | RC 734 | 66 | 124 | 198.0 | 4.0 | 11.5 | 71.2 | 52.2 | 4.0 | 12.2 | 38.3 | 3.6 | 14.7 | 6.3 | 0.15 | 5.68 | 1.07 | 2.18 |
| 76 | RC 806 | 71 | 129 | 170.2 | 3.3 | 7.5 | 65.5 | 45.8 | 4.5 | 12.6 | 38.6 | 3.2 | 12.6 | 6.6 | 0.15 | 8.85 | 0.97 | 2.17 |
| 77 | RC 840 | 71 | 130 | 169.8 | 4.3 | 10.7 | 63.5 | 50.9 | 3.8 | 11.8 | 37.8 | 4.0 | 13.8 | 6.1 | 0.14 | 7.01 | 0.82 | 2.07 |
| 78 | RC 904 | 73 | 128 | 203.3 | 5.3 | 8.2 | 56.2 | 48.4 | 4.4 | 12.3 | 38.8 | 4.5 | 13.6 | 8.6 | 0.26 | 7.83 | 0.88 | 2.35 |
| 79 | RLC 1 | 72 | 130 | 199.3 | 3.8 | 6.7 | 63.3 | 48.9 | 3.8 | 11.1 | 38.2 | 3.8 | 13.7 | 5.9 | 0.17 | 6.68 | 0.79 | 2.35 |
| 80 | RLC 2 | 72 | 130 | 192.0 | 4.5 | 8.2 | 56.0 | 47.7 | 3.7 | 12.8 | 39.1 | 3.1 | 13.6 | 7.6 | 0.13 | 6.91 | 0.87 | 2.40 |
| 81 | RLC 3 | 69 | 127 | 179.5 | 5.2 | 10.6 | 46.8 | 37.8 | 4.1 | 13.2 | 38.5 | 3.0 | 11.5 | 8.8 | 0.22 | 5.47 | 0.72 | 2.41 |
| 82 | RLM 619 | 71 | 129 | 186.0 | 4.5 | 9.7 | 61.2 | 41.2 | 3.5 | 11.2 | 38.6 | 4.2 | 11.8 | 7.4 | 0.27 | 9.09 | 0.77 | 1.51 |
| 83 | PB 357 | 70 | 129 | 187.0 | 4.2 | 9.2 | 62.3 | 42.2 | 3.4 | 12.4 | 38.2 | 4.2 | 14.2 | 9.6 | 0.21 | 7.32 | 0.82 | 2.43 |
| 84 | RH 1916 | 72 | 130 | 195.0 | 4.8 | 7.3 | 68.4 | 44.2 | 4.7 | 12.2 | 38.5 | 4.9 | 17.4 | 8.3 | 0.29 | 7.47 | 0.94 | 2.21 |
| 85 | RH 1917 | 69 | 129 | 191.8 | 4.4 | 10.8 | 77.5 | 47.8 | 4.6 | 11.1 | 38.7 | 4.6 | 19.8 | 8.2 | 0.29 | 10.28 | 0.99 | 2.35 |
| 86 | RH 1918 | 71 | 130 | 186.7 | 3.3 | 6.4 | 74.5 | 43.0 | 5.3 | 11.6 | 38.6 | 5.6 | 18.0 | 8.6 | 0.18 | 9.85 | 0.86 | 2.35 |
| 87 | RH 1919 | 72 | 131 | 200.0 | 4.7 | 8.3 | 70.0 | 48.7 | 4.9 | 10.9 | 38.6 | 4.7 | 12.3 | 7.1 | 0.17 | 10.47 | 0.83 | 2.33 |
| 88 | RH 1922 | 66 | 127 | 177.3 | 3.7 | 8.7 | 71.2 | 45.7 | 4.2 | 11.0 | 38.5 | 4.7 | 15.6 | 11.9 | 0.27 | 7.77 | 0.96 | 2.39 |
| 89 | RH 1923 | 69 | 125 | 195.7 | 5.0 | 8.2 | 74.5 | 47.7 | 5.0 | 11.7 | 38.2 | 5.4 | 13.0 | 7.5 | 0.18 | 7.91 | 0.84 | 2.27 |
| 90 | RH 1924 | 70 | 129 | 194.0 | 4.5 | 7.5 | 81.0 | 48.3 | 5.3 | 11.7 | 37.9 | 5.7 | 16.4 | 10.3 | 0.16 | 9.05 | 0.86 | 1.87 |
| 91 | RH 1927 | 70 | 126 | 180.7 | 3.8 | 6.5 | 67.5 | 43.2 | 4.7 | 12.2 | 37.9 | 5.1 | 16.3 | 10.1 | 0.21 | 8.40 | 0.78 | 2.22 |
| 92 | RH 1928 | 70 | 128 | 186.5 | 4.0 | 10.8 | 75.8 | 49.8 | 4.4 | 10.8 | 38.8 | 4.7 | 17.9 | 6.5 | 0.22 | 7.58 | 0.84 | 2.32 |
| 93 | RH 1929 | 70 | 129 | 190.7 | 3.7 | 6.7 | 72.7 | 35.2 | 4.8 | 11.6 | 38.8 | 5.5 | 21.6 | 8.1 | 0.23 | 9.06 | 0.96 | 2.20 |

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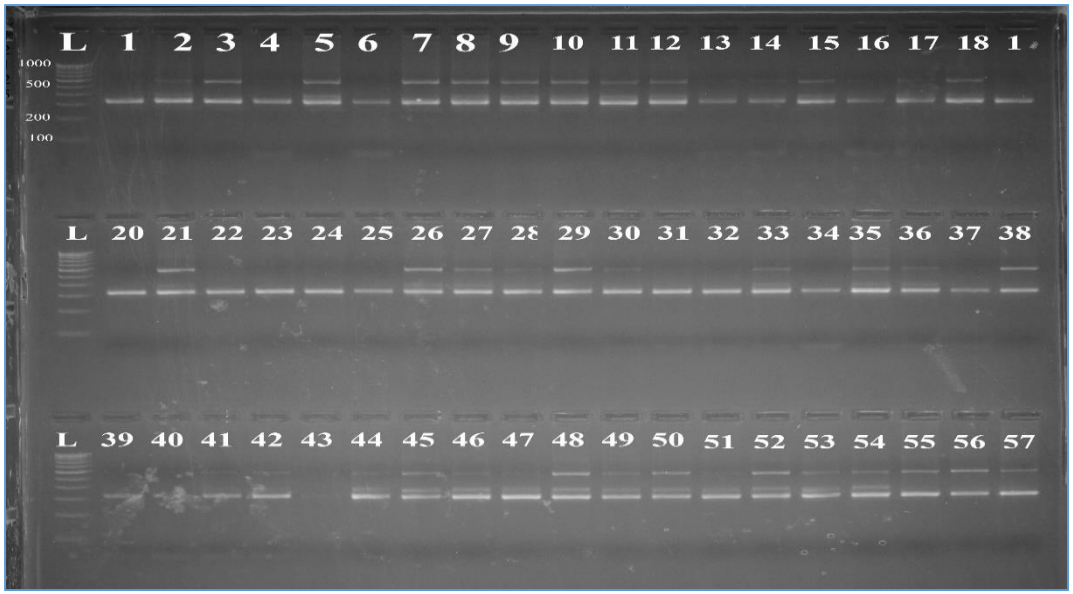
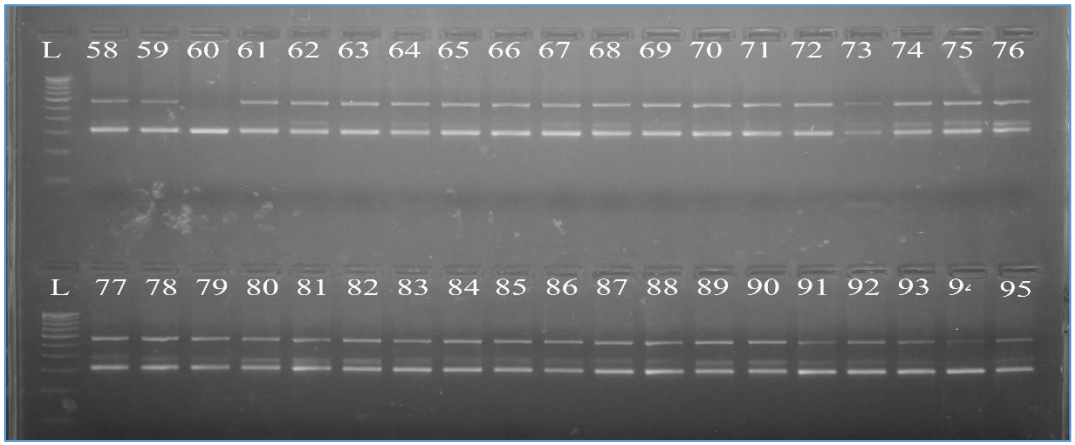
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 94 | RH 1930 | 70 | 131 | 190.5 | 4.5 | 6.4 | 79.0 | 44.7 | 4.7 | 10.9 | 38.4 | 5.6 | 23.6 | 10.8 | 0.27 | 8.10 | 1.06 | 2.48 |
| 95 | RH 1931 | 68 | 128 | 180.2 | 4.0 | 5.8 | 78.8 | 45.0 | 4.7 | 11.1 | 38.5 | 5.6 | 17.9 | 8.9 | 0.25 | 8.92 | 0.90 | 2.87 |
| 96 | RH 1932 | 69 | 128 | 196.7 | 4.3 | 7.7 | 70.5 | 41.2 | 4.4 | 11.0 | 37.9 | 5.1 | 22.2 | 9.3 | 0.23 | 7.17 | 0.98 | 2.71 |
| 97 | RH 1934 | 70 | 129 | 201.2 | 4.7 | 10.2 | 66.3 | 43.3 | 4.4 | 12.1 | 38.9 | 5.0 | 22.4 | 11.0 | 0.27 | 8.69 | 0.98 | 2.15 |
| 98 | RH 1935 | 70 | 124 | 196.3 | 4.4 | 11.2 | 77.5 | 52.7 | 4.5 | 12.4 | 38.2 | 6.5 | 25.0 | 10.5 | 0.25 | 6.34 | 1.10 | 2.27 |
| 99 | RH 1936 | 70 | 125 | 197.2 | 4.0 | 9.3 | 73.7 | 45.5 | 5.1 | 11.6 | 38.7 | 4.7 | 19.8 | 8.5 | 0.25 | 8.41 | 0.97 | 2.27 |
| 100 | RH1937 | 70 | 125 | 185.7 | 4.2 | 10.7 | 69.0 | 46.3 | 5.7 | 11.9 | 38.2 | 5.2 | 22.4 | 10.1 | 0.30 | 8.19 | 0.90 | 2.54 |
| 101 | RH 1938 | 69 | 126 | 177.7 | 4.0 | 5.8 | 72.5 | 43.3 | 4.7 | 12.2 | 38.6 | 5.3 | 22.2 | 8.1 | 0.26 | 7.46 | 0.95 | 2.36 |
| 102 | RH 1939 | 70 | 127 | 190.5 | 4.2 | 9.0 | 73.3 | 45.2 | 5.2 | 13.8 | 38.2 | 5.3 | 19.8 | 7.5 | 0.31 | 9.28 | 0.90 | 2.35 |
| 103 | RH 1940 | 69 | 125 | 189.0 | 4.3 | 8.8 | 74.2 | 36.9 | 5.1 | 11.9 | 38.7 | 5.3 | 22.7 | 9.4 | 0.27 | 7.47 | 0.99 | 2.30 |
| 104 | RH 2001 | 66 | 124 | 187.0 | 3.5 | 10.5 | 77.5 | 44.0 | 4.8 | 10.8 | 38.2 | 5.5 | 22.4 | 10.6 | 0.22 | 6.66 | 0.87 | 2.29 |
| 105 | RH 2002 | 67 | 126 | 194.5 | 3.3 | 5.7 | 78.2 | 47.0 | 5.2 | 10.4 | 38.8 | 5.1 | 22.0 | 9.1 | 0.25 | 8.00 | 1.01 | 2.58 |
| 106 | RH 2003 | 75 | 133 | 194.2 | 3.8 | 8.7 | 68.9 | 41.0 | 4.7 | 10.5 | 38.8 | 4.7 | 22.9 | 8.4 | 0.27 | 8.88 | 1.19 | 2.68 |
| 107 | RH 2004 | 75 | 133 | 203.2 | 4.7 | 10.8 | 74.5 | 35.3 | 5.5 | 12.3 | 38.5 | 5.9 | 24.8 | 8.7 | 0.22 | 7.81 | 1.05 | 2.69 |
| 108 | RH 2005 | 75 | 133 | 190.7 | 4.3 | 13.0 | 68.7 | 36.5 | 5.4 | 11.4 | 37.7 | 5.9 | 21.9 | 6.0 | 0.21 | 9.58 | 0.84 | 2.39 |
| 109 | RH 2006 | 70 | 129 | 199.3 | 4.0 | 7.5 | 84.0 | 56.7 | 4.4 | 11.5 | 38.2 | 4.9 | 19.1 | 9.0 | 0.21 | 8.04 | 1.09 | 2.54 |
| 110 | RH 2007 | 74 | 132 | 192.0 | 4.7 | 7.2 | 72.7 | 55.0 | 4.5 | 12.0 | 38.2 | 4.9 | 21.9 | 10.9 | 0.18 | 9.03 | 0.89 | 2.33 |
| 111 | RH 2008 | 70 | 129 | 198.5 | 3.5 | 7.7 | 77.7 | 50.5 | 4.4 | 11.4 | 37.9 | 4.8 | 20.2 | 8.1 | 0.25 | 7.44 | 0.93 | 2.23 |
| 112 | RH 2012 | 70 | 130 | 187.5 | 4.0 | 8.2 | 74.8 | 40.8 | 5.1 | 11.5 | 38.6 | 6.3 | 17.5 | 7.7 | 0.26 | 8.83 | 0.84 | 2.36 |
| 113 | RH 2013 | 70 | 129 | 182.0 | 4.0 | 5.8 | 72.8 | 42.7 | 5.4 | 12.3 | 38.8 | 5.9 | 20.5 | 7.8 | 0.31 | 9.42 | 0.89 | 2.69 |
| 114 | RH 2014 | 70 | 127 | 188.0 | 3.8 | 7.7 | 67.5 | 36.3 | 5.4 | 12.4 | 38.8 | 5.5 | 23.0 | 9.3 | 0.32 | 9.75 | 0.93 | 2.32 |
| 115 | RH 2015 | 69 | 126 | 188.7 | 4.5 | 8.2 | 80.2 | 47.8 | 5.9 | 12.8 | 38.9 | 5.5 | 22.8 | 7.4 | 0.30 | 8.86 | 0.95 | 2.59 |
| 116 | RH 2016 | 74 | 128 | 204.8 | 4.2 | 6.7 | 72.8 | 45.8 | 4.5 | 10.8 | 38.8 | 5.8 | 19.5 | 8.1 | 0.25 | 6.68 | 1.03 | 2.61 |
| 117 | RH 2017 | 74 | 128 | 201.7 | 4.7 | 11.7 | 72.7 | 42.7 | 4.7 | 11.2 | 38.9 | 5.6 | 18.1 | 7.3 | 0.24 | 8.75 | 0.99 | 2.56 |
| 118 | RH 2018 | 72 | 129 | 195.0 | 4.0 | 8.2 | 75.0 | 50.0 | 5.0 | 12.0 | 38.1 | 5.1 | 19.2 | 8.6 | 0.26 | 9.11 | 1.07 | 2.52 |
| 119 | RH 2019 | 70 | 129 | 196.8 | 4.8 | 10.0 | 78.0 | 40.0 | 5.4 | 11.5 | 37.8 | 5.6 | 20.5 | 6.7 | 0.23 | 6.83 | 0.93 | 2.32 |
| 120 | RH 2020 | 70 | 129 | 199.3 | 5.0 | 11.2 | 75.2 | 40.2 | 5.9 | 11.6 | 38.3 | 6.3 | 22.5 | 15.1 | 0.37 | 4.00 | 1.21 | 2.96 |
| 121 | RH 2021 | 75 | 131 | 191.4 | 4.7 | 6.5 | 66.2 | 46.0 | 4.3 | 12.7 | 37.9 | 4.6 | 19.7 | 9.2 | 0.25 | 8.15 | 0.96 | 2.50 |
| 122 | RH 2022 | 72 | 130 | 166.2 | 4.7 | 6.3 | 80.2 | 53.0 | 4.9 | 11.1 | 38.5 | 5.5 | 19.9 | 8.1 | 0.32 | 9.87 | 0.94 | 2.37 |
| 123 | RH 2023 | 72 | 127 | 185.0 | 3.8 | 6.3 | 68.2 | 42.0 | 5.4 | 10.4 | 38.7 | 5.4 | 19.2 | 4.9 | 0.27 | 8.34 | 0.90 | 2.20 |
| 124 | RH 2024 | 70 | 123 | 182.8 | 4.0 | 6.7 | 72.2 | 48.2 | 5.3 | 10.4 | 38.9 | 4.9 | 19.2 | 9.7 | 0.17 | 7.75 | 0.88 | 2.22 |
| 125 | RH 2025 | 70 | 125 | 177.7 | 4.7 | 12.0 | 74.0 | 50.5 | 4.5 | 12.6 | 38.2 | 4.8 | 21.4 | 11.0 | 0.26 | 7.21 | 0.94 | 1.97 |

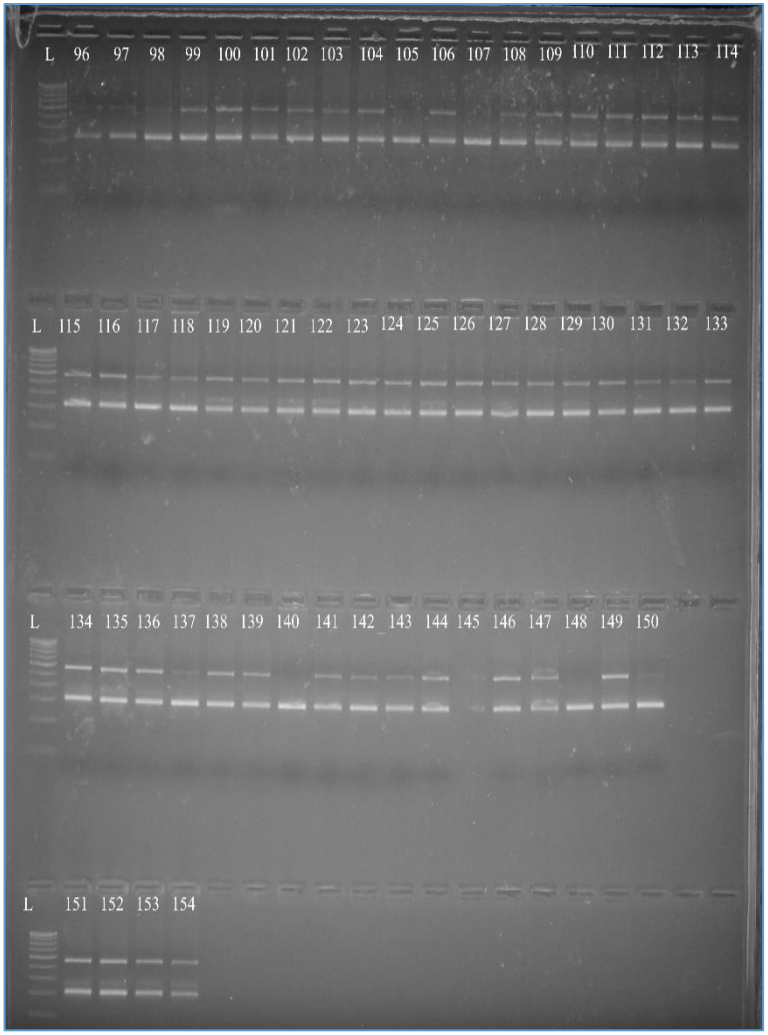
| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 126 | RH 2026 | 70 | 129 | 187.2 | 4.3 | 8.2 | 76.8 | 50.5 | 4.5 | 12.4 | 38.3 | 5.2 | 17.1 | 7.2 | 0.12 | 8.18 | 0.84 | 1.92 |
| 127 | RH 2027 | 72 | 130 | 176.2 | 4.5 | 10.0 | 69.2 | 48.2 | 4.1 | 12.2 | 38.6 | 5.0 | 18.6 | 8.6 | 0.23 | 7.06 | 0.83 | 2.52 |
| 128 | RH 2028 | 73 | 127 | 197.5 | 4.2 | 9.0 | 62.7 | 43.7 | 4.2 | 12.3 | 38.8 | 4.7 | 17.3 | 6.7 | 0.24 | 9.79 | 0.83 | 2.31 |
| 129 | RH 2029 | 71 | 128 | 184.0 | 4.0 | 15.0 | 76.2 | 46.7 | 4.3 | 12.7 | 38.6 | 5.2 | 21.8 | 9.0 | 0.27 | 7.54 | 0.93 | 2.63 |
| 130 | RH 2030 | 70 | 126 | 190.8 | 4.2 | 7.0 | 68.0 | 39.5 | 4.6 | 12.5 | 38.8 | 5.5 | 22.2 | 7.4 | 0.26 | 8.61 | 0.93 | 2.70 |
| 131 | RH 2031 | 70 | 124 | 188.7 | 4.3 | 12.3 | 79.4 | 52.0 | 4.4 | 12.7 | 38.8 | 5.0 | 20.8 | 5.9 | 0.23 | 8.91 | 1.06 | 2.39 |
| 132 | RH 2032 | 68 | 127 | 188.7 | 3.5 | 8.0 | 79.5 | 45.3 | 4.4 | 13.4 | 38.6 | 4.3 | 20.8 | 4.4 | 0.25 | 6.70 | 0.87 | 2.85 |
| 133 | RH 2033 | 69 | 129 | 197.5 | 4.0 | 7.7 | 62.2 | 37.7 | 4.9 | 12.3 | 38.7 | 5.0 | 22.1 | 6.0 | 0.22 | 6.78 | 1.06 | 2.69 |
| 134 | RH 2034 | 70 | 129 | 194.2 | 4.0 | 7.3 | 69.3 | 39.9 | 4.7 | 13.1 | 38.8 | 4.9 | 20.0 | 13.1 | 0.25 | 4.31 | 1.16 | 2.71 |
| 135 | RH 2035 | 71 | 129 | 199.2 | 3.8 | 7.8 | 67.0 | 37.8 | 4.7 | 13.0 | 38.2 | 5.4 | 20.4 | 6.6 | 0.20 | 7.87 | 0.97 | 2.43 |
| 136 | RH 2036 | 70 | 126 | 196.2 | 4.0 | 10.0 | 75.2 | 42.2 | 4.7 | 12.0 | 37.9 | 5.4 | 19.3 | 6.2 | 0.25 | 8.10 | 1.07 | 2.72 |
| 137 | RH 2038 | 70 | 127 | 200.0 | 3.8 | 7.0 | 62.7 | 33.0 | 4.6 | 12.2 | 38.2 | 5.3 | 20.0 | 5.6 | 0.20 | 8.98 | 0.94 | 2.39 |
| 138 | RH 2039 | 70 | 128 | 198.3 | 4.0 | 7.0 | 69.7 | 38.0 | 4.5 | 11.8 | 38.2 | 6.1 | 18.6 | 4.6 | 0.17 | 8.24 | 0.98 | 2.72 |
| 139 | RH 2040 | 70 | 130 | 208.8 | 3.7 | 8.7 | 81.0 | 45.3 | 4.6 | 12.5 | 38.5 | 5.1 | 22.3 | 7.0 | 0.25 | 6.91 | 0.91 | 2.29 |
| 140 | RH 2041 | 72 | 130 | 208.7 | 4.3 | 9.7 | 69.7 | 40.2 | 5.2 | 12.4 | 38.6 | 6.2 | 26.0 | 14.2 | 0.36 | 4.61 | 1.25 | 2.92 |
| 141 | RH 2042 | 70 | 129 | 205.3 | 3.8 | 6.7 | 74.5 | 40.5 | 5.2 | 12.7 | 38.8 | 5.8 | 21.7 | 11.3 | 0.30 | 8.32 | 0.89 | 2.27 |
| 142 | RH 2043 | 71 | 128 | 208.4 | 4.5 | 8.4 | 75.3 | 45.2 | 4.4 | 12.4 | 38.7 | 5.7 | 18.2 | 8.1 | 0.24 | 8.20 | 0.90 | 2.25 |
| 143 | RH 2044 | 70 | 128 | 188.8 | 5.2 | 11.2 | 77.8 | 42.5 | 4.6 | 12.5 | 38.6 | 5.3 | 20.7 | 5.6 | 0.29 | 7.96 | 1.02 | 2.40 |
| 144 | RH 2045 | 71 | 129 | 187.5 | 5.0 | 9.8 | 73.8 | 45.3 | 5.2 | 11.4 | 38.6 | 6.7 | 19.5 | 7.5 | 0.20 | 7.35 | 0.99 | 2.04 |
| 145 | RH 2046 | 72 | 129 | 199.5 | 5.5 | 8.8 | 72.3 | 47.0 | 5.3 | 11.9 | 38.0 | 5.7 | 21.9 | 8.5 | 0.23 | 7.76 | 1.07 | 2.28 |
| 146 | RH 2047 | 70 | 129 | 200.3 | 4.5 | 8.2 | 65.2 | 34.3 | 5.0 | 10.5 | 37.9 | 5.5 | 20.6 | 8.9 | 0.25 | 9.07 | 1.04 | 2.31 |
| 147 | RH 2048 | 69 | 126 | 188.7 | 4.7 | 8.3 | 74.7 | 45.2 | 5.0 | 13.1 | 37.8 | 5.1 | 21.0 | 8.1 | 0.23 | 7.82 | 1.03 | 2.45 |
| 148 | RH 2049 | 68 | 124 | 187.5 | 4.5 | 11.2 | 73.5 | 35.7 | 5.9 | 12.9 | 38.6 | 5.9 | 28.4 | 17.0 | 0.41 | 3.39 | 1.28 | 2.74 |
| 149 | RH 2050 | 69 | 126 | 186.2 | 5.0 | 10.9 | 74.0 | 51.8 | 5.8 | 13.7 | 38.7 | 5.7 | 28.3 | 19.3 | 0.40 | 3.64 | 1.26 | 3.05 |
| 150 | RH 2051 | 71 | 130 | 196.8 | 4.5 | 10.4 | 73.0 | 45.0 | 5.1 | 12.3 | 39.1 | 7.1 | 23.2 | 10.8 | 0.29 | 8.76 | 1.08 | 2.57 |
| **Mean** | | **70** | **127** | **191.3** | **4.4** | **9.5** | **72.3** | **45.4** | **4.4** | **11.9** | **38.4** | **4.5** | **17.5** | **8.7** | **0.23** | **7.70** | **0.92** | **2.34** |
| **Range** | | **62-79** | **119-134** | **158.0-231.5** | **3.3-6.0** | **5.0-15.0** | **46.8-84.8** | **33.0-57.9** | **3.1-5.9** | **9.5-14.4** | **37.7-39.3** | **2.6-7.1** | **9.6-28.4** | **3.6-21.2** | **0.10-0.59** | **3.22-11.18** | **0.57-1.28** | **1.51-3.05** |
| **C.D. #** | | **2.85** | **3.62** | **12.69** | **0.56** | **1.58** | **9.15** | **6.52** | **0.62** | **1.01** | **0.46** | **1.11** | **5.57** | **3.12** | **0.10** | **1.88** | **0.11** | **0.24** |

# - C.D. at 5% between two varieties not in same block; DF-Days to 50% flowering; DM-Days to maturity; PH-Plant height (cm); NPB-Number of primary branches/plant; NSB-Number of secondary branches/plant; MSL-Main shoot length (cm), NSMS-Number of siliquae on main shoot; SL-Siliqua length (cm); NSS-Number of seeds/siliqua; OC-Oil content (%); TSW-1000-seed weight (g); SYP-Seed yield/plant (g); A–Photosynthetic rate (µmol $CO_2/m^2/s$); Gs – Stomatal conductance (mmol/$m^2$/s); E – Transpiration rate (mmol $H_2O/m^2/s$); Chl – Total chlorophyll content (mg/g); Caro – Carotenoid content (mg/g).

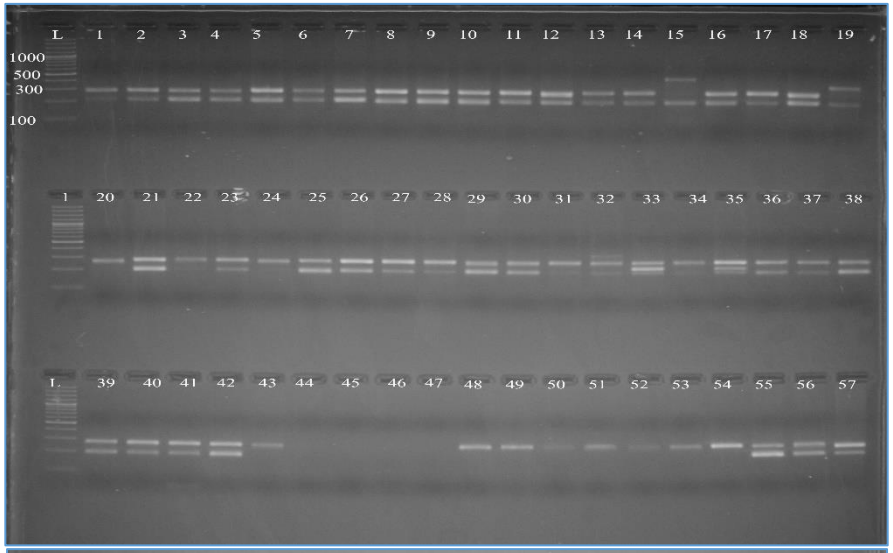
## Primer BG 102 Gel Pictures

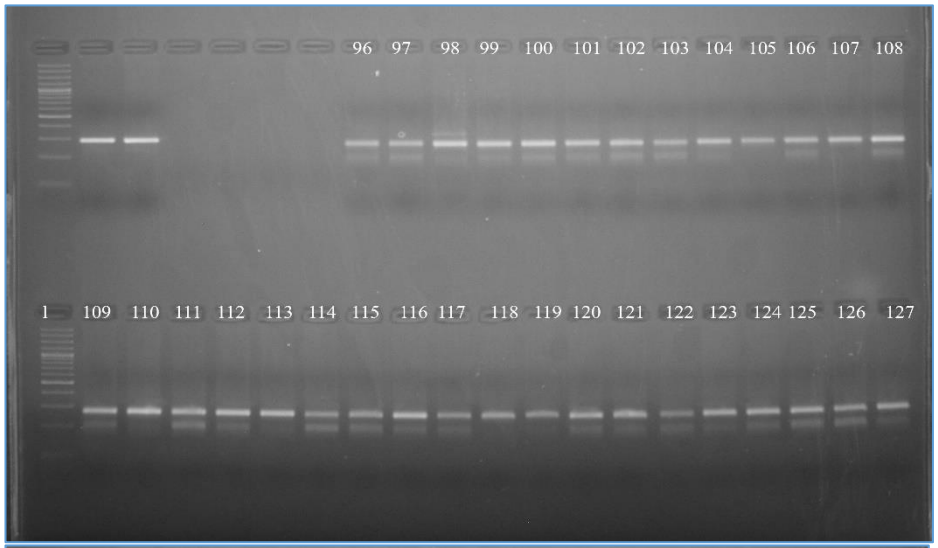


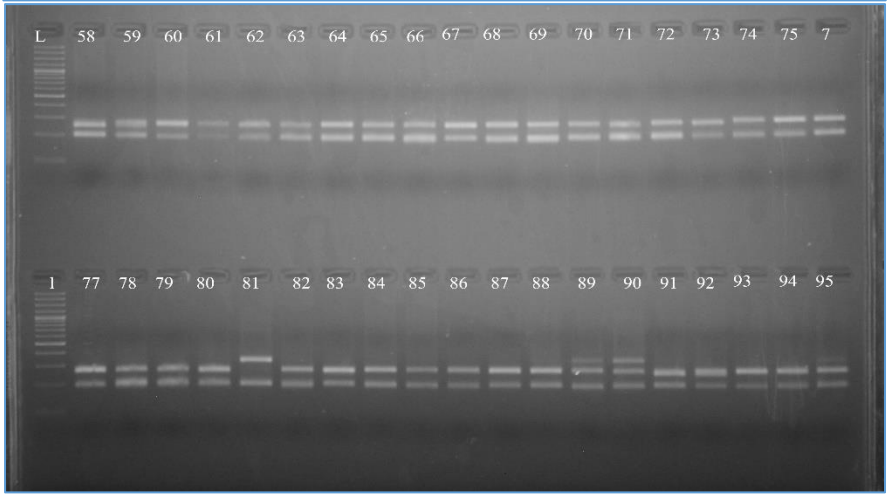



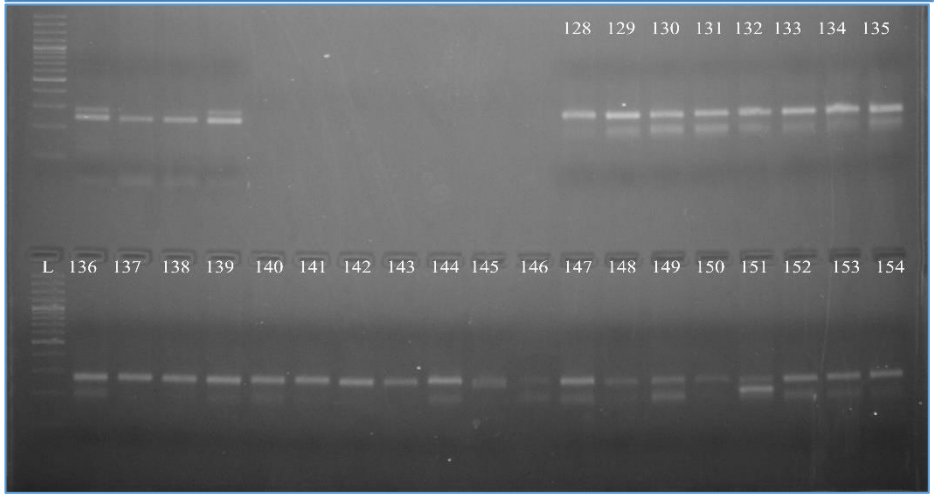



# Primer BG 111 Gel Pictures



# ABSTRACT

| | | |
|---|---|---|
| **Title** | : | **"Population structure and genetic diversity studies for terminal heat stress tolerance in Indian mustard [*Brassica juncea* (L.) Czern & Coss.]"** |
| **Name of Degree Holder** | : | RAJU RAM CHOUDHARY |
| **Admission Number** | : | 2019A48D |
| **Title of degree** | : | Doctor of Philosophy |
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| **Year of award of degree** | : | 2022 |
| **Major Subject** | : | Genetics and Plant Breeding |
| **Minor Subject** | : | Molecular Biology, Biotechnology & Bioinformatics |
| **Total No. of pages in the thesis** | : | 102 + vii + XVIII |
| **Number of Words in the abstract** | : | 386 |

**Keywords:** Trait association, Linkage disequilibrium, Association mapping, Heat susceptibility index, Mixed linear model

Indian mustard is one of the most important oilseed crops and contributes more than 30% to the Indian vegetable oil pool. Heat stress is one of the major yield-limiting factors under changing climate conditions. Its impact is most severe at the reproductive stage, resulting in low seed yield. Therefore, assessment of genetic diversity, population structure, and molecular marker-traits associated with terminal heat stress tolerance in Indian mustard germplasm is necessary to accelerate the breeding effort. In the present study, a set of advanced breeding lines and genetic stocks representing 154 genotypes of Indian mustard were phenotyped for various morpho-physiological traits under control and terminal heat stress conditions. Besides, these genotypes were genotyped using 237 SSR markers. The results of the present study revealed significant effects of year, sowing date, and genotypes on various morpho-physiological traits under investigation. For the majority of the traits studied, high heritability and genetic advance were estimated. Trait association results revealed that seed yield/plant was significantly and positively correlated with plant height, number of primary branches/plant, number of secondary branches/plant, main shoot length, number of siliquae on the main shoot, siliqua length, number of seeds/siliqua, 1000-seed weight, photosynthetic rate, stomatal conductance, total chlorophyll content, and carotenoid content. DNA was isolated from all 154 genotypes by the standard method and subjected to SSR marker analysis. Out of total of 237 SSR markers, 111 were polymorphic. PIC values for all polymorphic SSR markers ranged from 0.013 to 0.627, with an average PIC value of 0.31. Unweighted Neighbor Joining-based dendrogram and population structure analysis divided the 154 genotypes into three clusters and two sub-populations, respectively. A total of 29 SSRs under timely sown and 33 SSRs under late sown environment were found to be associated with morphological and physiological traits by the MLM (Q + K) method. Chromosome B06 harbored the maximum number of SSRs (12), followed by chromosomes A08 (11) and A07 (five SSRs). Under terminal heat conditions, a total of 20 SSRs were specifically detected that were not associated under normal sown conditions. This meant that these particular genomic regions and QTLs were linked under extreme heat conditions. Overall, the heat-tolerant genotypes identified in this study and the SSR markers associated with terminal heat stress tolerance attributes will be helpful for the development of a heat-tolerant cultivar of Indian mustard through marker-assisted selection.

**MAJOR ADVISOR** **SIGNATURE OF STUDENT**

**HEAD OF THE DEPARTMET**

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| Degree | University/Board | Year of passing | Percentage | Subjects |
|---|---|---|---|---|
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| Intermediate | B.S.E.R. Ajmer | 2013 | 74.40% | Biology, Chemistry, Physics, Hindi and English |
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## j) Achivements

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*Qualified GATE (Life Sciences) in 2021.
*Admitted in PhD through award of ICAR-JRF/SRF with AIR 9.

## k) List of Publications


Choudhary, R. R., Avtar, R. S. R. and Kumar, D. (2020). Heterosis studies based upon *Mori* CMS system in *Brassica juncea* L. *Journal of Oilseed Brassica*, 11(2), 116-120.

Choudhary, R. R., Avtar, R., Sheoran, R. K., Samita and Kumar, D. (2020). Combining Ability Studies Based on Mori CMS System in Indian Mustard [*Brassica juncea* (L.) Czern. & Coss.]. *Current Journal of Applied Science and Technology*, 39(20), 58-66.

Choudhary, R. R., Avtar, R., Singh M., Samita and Amit (2021). Genetic Variability Studies for Yield and its Components in Indian Mustard (*Brassica juncea* L.) *Frontiers in Crop Improvement*, 9 (7): 2983-2986.

Choudhary, R. R., Avtar, R., Kumar, P., Singh, M., & Bishnoi, M. (2022). Molecular and morphological diversity of the mori CMS lines and restores in Indian mustard (*Brassica juncea* L.). *Journal of Oilseed Brassica*, *13*(2), 94-99.

Kumar, P., Nimbal, S., Sangwan, R. S., Budhlakoti, N., Singh, V., Mishra, D. C. and Choudhary, R. R. (2021). Identification of novel marker–trait associations for lint yield contributing traits in upland cotton (*Gossypium hirsutum* L.) using SSRs. *Frontiers in plant science*, 855.

Singh, M., Avtar, R., Lakra, N., Hooda, E., Singh, V. K., Bishnoi, M., Kumari, N., Punia, R., Kumar N. and Choudhary, R. R. (2021). Genetic and Proteomic Basis of Sclerotinia Stem Rot Resistance in Indian Mustard [*Brassica juncea* (L.) Czern & Coss.]. *Genes*, 12(11), 1784.


**Raju Ram Choudhary**

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I, **Raju Ram Choudhary**, Admission No. **2019A48D**, under takes that I give copyright of my thesis entitled, **"Population structure and genetic diversity studies for terminal heat stress tolerance in Indian mustard "[*Brassica juncea* (L.) Czern & Coss.]"** to the CCS Haryana Agricultural University, Hisar.

I also under take the patent if any, arising out of the research work conducted during the programme shall be filed by only with due permission of the competent authority of CCS Haryana Agricultural University, Hisar.

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