MOLECULAR CHARACTERIZATION FOR TARGETING FOLIAR BLAST DISEASE IN PEARL MILLET [*Pennisetum glaucum* (L.) R. Br.]

By

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CERTIFICATE – I

This is to certify that this thesis entitled, **“Molecular characterization for targeting foliar blast disease in pearl millet [*Pennisetum glaucum* (L.) R. Br.**]**”** submitted for the degree of **Doctor of Philosophy** in the subject of ‘**Molecular Biology & Biotechnology’** of the Chaudhary Charan Singh Haryana Agricultural University, Hisar, is a bonafide research work carried out by **Ms. Aarti Kamboj** 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 **“Molecular characterization for targeting foliar blast disease in pearl millet [*Pennisetum glaucum* (L.) R. Br.”** submitted by **Ms. Aarti Kamboj**, Admission No. **2019BS32D,** to Chaudhary Charan Singh Haryana Agricultural University, Hisar, in partial fulfilment of the requirements for the degree of **Doctor of Philosophy** in the subject of **Molecular Biology & Biotechnology,** has been approved by the Student’s Advisory Committee after an oral examination on the same.

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

ABBREVIATIONS

µl	Micro litre
µM	Micro Mole
µmol min^{-1}g^{-1}	Micromole per minute per gram
µmol t-CA h^{-1}g^{-1}	Micromole trans-cinnamic acid per hour per gram
AFLP	Amplified Fragment Length Polymorphisms
AMOVA	Analysis of Molecular Variance
ANOVA	Analysis of Variance
BABA	β-aminobutyric acid
Bp	Base pair
℃	Degree Celsius
CBDP	CAAT-box Derived Polymorphism
cm	Centimetre
CTAB	Cetyl Trimethyl Ammonium Bromide
CV	Coefficient of variation
DF	Days to 50% flowering
DFPP	Dry fodder yield per plant
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
ED	Ear Diameter
EDTA	Ethylene diamine tetra acetic acid
EL	Ear Length
et. al.	*et alia* (and others)
EtBr	Ethidium Bromide
ETPP	Effective Number of tillers per plant
Fig.	Figure
g	Gram
GCV	Genotypic Coefficient of Variation
GYPP	Grain Yield Per Plant
ha	Hectare
HRGP	Hydroxyproline Rich Glycoprotein
ILP	Intron Length Polymorphism
kg/ha	Kilogram/ hectare
LOX	Lipoxygenase
MAS	Marker Assisted Selection

MeJ	Methyl Jasmonate
mg/ml	Milligram per millilitre
ml	Millilitre
mM	Millimolar
ng	Nanogram
nm	Nanometre
NTSYS-pc	Numerical Taxonomy SYStem for personal computer
PAL	Phenylalanine Ammonia Lyase
PCA	Principal Component Analysis
PCoA	Principal Coordinate Analysis
PCR	Polymerase Chain reaction
PCV	Phenotypic coefficient of variation
PH	Plant Height
PIC	Polymorphism Information Content
POX	Peroxidase
PPO	Polyphenol Oxidase
PVP	Polyvinylpyrrolidone
QTL	Quantitative Trait Loci
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
RNA	Ribonucleic acid
rpm	Revolution per minute
SA	Salicylic Acid
SCoT	Start Codon Targeted
SSR	Simple Sequence Repeat
STS	Sequence Tagged Sites
TE	Tris-EDTA
TW	1000-Grain weight
UPGMA	Unweighted Pair Group Method for Arithmetic Mean
viz.	Namely
w/v	Weight/ volume

CHAPTER – I

INTRODUCTION

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] belonging to the family Gramineae is an important cereal and forage crop in the arid and semi-arid regions of India (Kumar & Manga, 2011). It is a preferred experimental millet grass in various genetic investigations on account of its diploid genome (2n=2x=14) of ~1.79 Gb with around 38,579 genes (Varshney *et al.*, 2017), short life cycle (60-90 days) and protogynous flowering, helpful in controlling undesirable cross-pollination (Chelpuri *et al.*, 2019). It accounts for more than one half of the total worldwide production of all the millets and is globally cultivated on an area of 3 million ha with a production of 32 million tonnes **(**FAOSTAT, 2021). India produces 26.6% of the total pearl millet produced in the world (Kumar *et al.*, 2022). It accounts for around two-third of the millets produced in India and is grown in the states of Maharashtra, Rajasthan, Uttar Pradesh, Gujrat, Andhra Pradesh, Karnataka, and Haryana which makes it the fourth most extensively cultivated crop after rice, wheat, and maize (ICAR, 2021), cultivated on an area of 7.4 million ha with a production of 9.13 million tonnes and productivity of 1237 kg/ha (Sharma *et al.*, 2021).

It is classified as a C_4 cereal, thus absorbs more CO_2 and have high water use efficiency, is well adapted to low soil fertility, low pH, salinity, and high temperature and can fit readily into any cropping system (Taunk *et al.*, 2018). It is most drought tolerant among all the domesticated cereals and is more nutritious than rice, wheat, sorghum, and maize because of the presence of high levels of proteins (12%), carbohydrates (65%), fats (6%), minerals (2-3%), essential amino acids, essential micronutrients such as Iron (Fe) & Zinc (Zn) and antioxidants (Serba *et al.*, 2017). Pearl millet in India is mostly grown in *kharif* season (June-September). It is also being progressively cultivated in the summer season (February- May) in the parts of Rajasthan, Gujrat, and Uttar Pradesh and in the post rainy season (November-February) at a small scale in Gujrat and Maharashtra (Yadav & Rai, 2013). Pearl millet productivity has been hindered as a result of several constraints and is not consistent since last more than two decades (Chelpuri *et al.*, 2019). Development of F_1 hybrids having relatively high productivity as compared to the open pollinated crop is suggested as one of the convincing substitutes for maintaining the pearl millet grain yield. However, the hybrids are highly vulnerable to biotic and abiotic stresses because of their narrow genetic base.

Downy mildew of pearl millet is a major biotic constraint for crop productivity causing the grain yield and fodder yield instability (Sharma *et al.*, 2015). Blast, also known as the leaf spot disease caused by *Pyricularia grisea* (teleomorph: *Magnaporthe grisea*) has come out as a serious menace in the major pearl millet growing regions in India having an adverse effect on the grain production and forage (Pawar *et al.*, 2016). *M. grisea* is a filamentous, heterothallic fungi which is pathogenic to around 50 plant species in 30 genera of Gramineae family which includes economically important crops like wheat, barley, rice, and millets (Lavanya *et al.*, 2017). This disease was first reported in Kanpur, Uttar Pradesh (Mehta *et al.*, 1952) and persisted as a minor disease for a very long time. However, in the past 8-10 years, it has turned out to be a serious threat to pearl millet fodder and grain production. The pathogen has a proclivity to cause disease in all the stages of crop growth beginning from the seedling up to grain formation, thus causing serious crop losses.

Although, some disease management alternatives are available involving fungicides like ‘Tricyclazole’ (Joshi *et al.*, 2015), but in view of their adverse impact on the environment, the most suitable approach for managing the disease is by host-plant resistance as it is cost-effective and eco-friendly for the resource poor farmers. The blast disease severity in the previous years has indicated the necessity for a tactical breeding program which incorporates disease resistance with the improvement in the yield to develop improved cultivars which are acceptable to the farmers. Host-plant resistance brought about by the marker-assisted selection (MAS) has various advantages in terms of transferring the desirable genes. It is a prompt breeding process in which the selections are based on the tests for the appropriate markers rather than testing or screening of the phenotypes in the field. Massive progress in the arena of plant genomics and its agreement with conventional breeding has been beheld in the form of several blast resistant agronomically superior identified lines (Goud *et al.*, 2016). Furthermore, these lines can be used in the breeding programs for the development of blast resistant hybrid parents or as one of the parents for developing blast resistant hybrids in order to diversify the genetic base of blast resistance in the future pearl millet hybrids. The studies on genetic diversity among the parental lines are also essential in order to choose the appropriate parents as the selections made purely based on phenotypic performance does not lead to the expected results in the breeding program. Extensive phenotypic variability is manifested by the cultivated pearl millet for traits such as ear length, flowering time, stover and grain characteristics, tolerance to pests, drought, and diseases as well as the nutritional value (Bhattacharjee *et al.*, 2007; Anuradha *et al.*, 2017). Also, it is a well-established fact that crosses between the genetically diverse parents shows more hybrid vigour as compared to the crosses between the closely related parents (Groszmann *et al.*, 2013). Thus, classification of germplasm within the heterotic groups with the help of genetic diversity studies is primarily important in hybrid breeding programs. Therefore, any crop improvement program involves the genetic variability estimation and identification of the superior genotypes as the first step for further improvement.

So, keeping in view the above points, present study was planned with the following objectives:

1. **To evaluate pearl millet germplasm lines and hybrids for morpho-phenological traits and blast disease**
2. **To study DNA polymorphism and initiate the crossing program between selected blast resistant and elite lines**
3. **To screen F_1 hybrids for morphological and biochemical traits and blast disease**

CHAPTER – II

REVIEW OF LITERATURE

The fourth-most significant food crop in the world, after rice, wheat, and sorghum, is pearl millet (*Pennisetum glaucum* (L.) R. Br.). It is produced for both grain and fodder purposes, and its cultivation spans widely from well-irrigated places to the world's most dry regions in Asia and Africa. Limited hybridization areas and the prevalence of several biotic and abiotic stress are the main factors limiting the productivity of this crop (Kapila *et al.*, 2008). Currently, the development of hybrids rather than open-pollinated varieties has been chosen for pearl millet genetic improvement; therefore, understanding genetic diversity is essential. Although, there are several reports available on genetic variability, heritability, correlation and path coefficient between the grain yield and its attributing traits, still there are limited studies available which pertains to stable germplasm lines that also shows resistance against blast disease as well.

A brief review of the available literature is given in this section under the following sub headings on the above reported aspects of pearl millet

1. **Genetic variability in morpho-phenological characters of pearl millet**
2. **Screening studies**
3. **Applications of molecular markers for diversity analysis studies**
4. **Enzyme activity analysis in pearl millet**

2.1 Genetic variability in morpho-phenological characters of pearl millet

In 2013, Kumari *et al.* evaluated the genetic diversity and associations among 30 pearl millet genotypes for 11 characteristics. For all 11 characters, the analysis of variance revealed significant variability. Character correlation revealed a significant positive correlation between biological yield/plant (g), ear head length (cm) and the number of active tillers/plant for grain yield/plant. Biological yield per plant and the quantity of efficient tillers per plant, both had high values of genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV). Moreover, it possessed a high heritability rate and a high genetic advance percentage. They recommended that when making a direct selection using these genotypes, proper emphasis should be placed on these traits. It was predicted that selection for these characters would also be successful based on the estimates of GCV, PCV, heritability, and genetic advance for seed yield/plant.

According to Kumar *et al.* (2014) estimation of the PCV & GCV among 26 pearl millet genotypes, grain yield, productive tillers per plant, dry fodder yield and 1000-seed weight were all highest. While grain yield character exhibited 75% heredity, the estimated heritability ranged from 26% (plant population trait) to 99% (1000-seed weight trait). Dry fodder yield, grain yield, effective tillers per plant, 1000-seed weight, plant height and ear length, all had estimates of heredity that ranged from moderate to high as a percentage of the mean.

According to Bind *et al.* (2015), the 36 genotypes of fodder pearl millet [*Pennisetum glaucum* (L.) R. Br.] showed numerous variations for the parameters investigated. Green fodder yield/plant had the largest estimated difference between PCV and GCV, whereas dry matter yield per plant, grain yield per plant and panicle length had the lowest differences. They found significant heritability among the traits, such as grain yield/plant, dry matter yield/plant, and panicle length, along with strong genetic progress as a percentage of the mean, and concluded that these traits could be controlled by additive gene effects.

Dhedhi *et al.* (2016) investigated 29 fodder hybrids of pearl millet for genetic diversity, genetic advance, heritability, and correlation of variables for green fodder production and its characteristics. With an exception in lodging score and harvest index, the analysis of variance (ANOVA) showed extremely significant variations among hybrids for the investigated characters. The potential for genetic improvement using selection and other breeding techniques was revealed in the green fodder output/plant, plant height and dry fodder yield/plant, all of which displayed a wide range of phenotypic variability. Plant height, dry fodder production/plant, harvest index, and grain yield/plant all showed strong heritability and high to moderate genetic progress represented as a percentage of mean.

In a set of 376 pearl millet genotypes, Ramya *et al.* (2018) investigated the genetic variability for yield and its other contributing factors. Plant height (cm), number of tillers per plant, days to 50% flowering, leaf blade length (cm), leaf blade width (cm), spike diameter (cm), spike length (cm), grain yield/plant and 1000-seed weight (g) were measured. For specific attributes, a wide range of variance, high PCV, and GCV were recorded. For the number of productive tillers per plant and the width of the leaf blade, higher values of heritability with high substantial genetic progress as a percent of the mean were observed. Therefore, selecting individuals with these traits would improve crops.

Abubakar *et al.* (2019) evaluated thirty-five pearl millet germplasm lines in a randomized complete block design to examine the morphological and yield variables. Plant height, number of internodes/plant, number of tillers/plant, panicle length, stem diameter, day until anthesis, panicle diameter, 1000-seed weight, panicle weight, and weight of seed/panicle were the traits studied. Every characteristic had a PCV larger than the corresponding GCV, with a moderate to high heritability.

Kumar *et al.* (2020) investigated 48 different pearl millet genotypes in two replications for heritability, genetic variability, and genetic progress as a percent of the mean, in addition to yield and its contributing traits. It was found that the GCV was lower than the PCV, which signified that all the traits under study expressed themselves with a little influence from the environment. The GCV and PCV of most of the traits were in the moderate to high range. The findings indicate that additive gene action was main determinant of the traits, and that direct selection would be useful for these.

Using thirty inbred lines of pearl millet at the ICAR-AICRP, Project Coordinating Unit, Mandor-Jodhpur, Rani *et al.* (2022) examined the genetic diversity for dry fodder yield and its corresponding features. All the characters showed a significant variance, indicating a lot of variation. Green fodder yield/plant, dry fodder yield/plant, grain yield/plant, number of productive tillers/plant, and area of leaf, all showed a range of phenotypic and genotypic variability, signifying that direct selection may be useful for all these traits. Green fodder yield/plant, followed by grain yield/plant, number of productive tillers/plant and dry fodder yield/plant was reported to have high heritability and moderate genetic progress as percent mean.

2.2 Screening studies

Thakur *et al.* (2009) screened 211 pearl millet lines against the blast disease and developed the field and greenhouse screening techniques and identified 25 blast resistance lines i.e., having score less than 3 on 1-9 scale. It was also found that all the blast resistance lines were also resistant to downy mildew when grown under the field conditions.

Gupta *et al.* (2012) screened two resistant restorer lines i.e., ICMR 07555 & ICMR 06222 and two susceptible maintainer lines i.e., ICMB 89111 and ICMB 95444, selected on the basis of blast screening scores, to derive the information about the inheritance pattern of the blast disease. They found that the resistance to blast disease is controlled by a single dominant gene in pearl millet.

Sharma *et al.* (2013) observed 238 accessions of pearl millet mini-core for studying the pathogenic variations among blast disease pathogen and identified many resistant sources under the greenhouse screening technique conditions. They identified five different pathotypes of *M. grisea* isolates i.e., Pg45, Pg53, Pg56, Pg118 & Pg119.

Prakash *et al.* (2016) standardized the phenotyping protocols for blast disease in the pearl millet and further screened the resistant entries i.e., PPMI 660, PPMI 1089, and PPMI

1087 from the 15 evaluated inbred lines of pearl millet. These procedures were found to be very useful for large scale screening of lines against the pathogen.

Singh *et al.* (2018) generated many populations namely, F_1s, F_2s and the backcrosses by crossing 2 susceptible genotypes *viz.*, ICMB 95444 and ICMB 95111 with 6 genotypes that were resistant to blast disease *viz.*, ICMB 06222, ICMB 11003, ICMR 97222, ICMR 06444, IP 21187-PI and ICMR 93333 to study blast disease inheritance. They performed the screening of various generations in glasshouse against two isolates of *M. grisea* i.e., Pg45 and Pg53. The study revealed that the resistance is governed by a single dominant gene.

Sharma *et al.* (2020) surveyed upon 305 pearl millet accessions to identify the diverse sources of rust and blast resistance under greenhouse conditions. One local isolate of *Pennisetum substriata* and five *M. grisea* isolates were used in the screening of these accessions. 17 accessions were found to be resistant to all the five isolates having a score of less than 3 on a 1-9 scale.

Sharma *et al.* (2021) collected 80 *M. grisea* isolates from the major pearl millet growing states in India and studied pathogenic variation on pearl millet host differentials i.e., ICMB 95444, ICMB 93333, ICMB 01333, ICMB 97222, ICMB 02444, 863B, ICMR 06444, ICMR 06222, IP-21187 and ICMR 11003 under greenhouse conditions. Based on the reaction of the isolates on host differentials, 80 isolates were clustered in 14 pathogenic groups.

Dagar *et al.* (2022) screened six pearl millet genotypes against blast disease in field conditions for lesion length, number of lesions, latent period, and disease severity, and found that lowest mean lesion number and lowest mean lesion length were found in resistant (18-0035) genotype. They found that the genotype 18-0114 scored highest for terminal illness while 18-0035 scored lowest.

2.3 Applications of molecular markers for diversity analysis studies

Molecular markers are recognised as brief DNA fragments that are positioned at a certain location on the genome and are passed down from one generation to the next according to the rules of inheritance. The use of molecular marker technologies can significantly speed up crop development initiatives when they are combined with high-quality phenotyping. They have several benefits, including the ability to map and tag genes or Quantitative Trait Loci (QTLs) for disease resistance and agronomic traits, as well as in assessing genetic diversity at the molecular level in a germplasm collection to determine the best parents for crosses (such as hybrid breeding). The use of isozyme markers to hasten the introgression of monogenic characteristics from foreign germplasm into a cultivar background helped to popularise the use of molecular markers in plant breeding applications in the early 1980s (Tanksley and Rick, 1980; Tanksley, 1983). Beckmann and Soller (1986) described the first application of restriction fragment length polymorphism (RFLP) markers in crop development. In pearl millet, molecular markers in the form of RFLPs were discovered as early as 1992 (Gale and Witcombe, 1992). Due of their suitability for use in high throughput and user-friendly tests, PCR-based molecular markers are favoured over RFLP markers. Sequence tagged site (STS) markers based on primers acquired by end-sequencing of RFLP probes were created to convert RFLPs to PCR-based markers, but these typically failed to discover polymorphism that can be consistently scored on gels.

A significant advancement in molecular marker technology occurred with the development of microsatellites or simple sequence repeats (SSRs), a type of variable number of tandem repeats that can be of two to many (often up to six) repeated nucleotides. These markers are co-dominant, typically detect larger levels of polymorphism, and are regarded as the best class of molecular markers for marker-assisted breeding projects (Gupta and Varshney, 2000). A few isozyme loci and a few RFLP loci made up the majority of the first molecular marker-based genetic linkage map of pearl millet, which was described by Liu *et al.* (1994). This map has been the starting point for future studies using pearl millet markers (Busso *et al.*, 2000; Liu *et al.*, 1996; Devos *et al.*, 2000). In later years, the linkage map has been expanded (Qi *et al.*, 2004), and its intricate connections to the genomes of foxtail millet, rice, and sorghum have been identified (Devos *et al.*, 2000; Bowers *et al.*, 2003). Specific benefits provided by molecular markers can be used to evaluate crop genetic diversity.

2.3.1 SSR markers for diversity analysis studies in other crops

Using bulk DNA samples and 45 SSR sites located across 10 maize chromosomes, a total of 286 alleles were found among the landraces. The landraces were divided into five categories using a clustering technique based on genetic similarity coefficients. Most of the landraces obtained from the same area grouped together. 180 individuals representing 12 landraces represented a total of 357 alleles. The estimated mean allele count, effective allele count, observed heterozygosity, and anticipated heterozygosity were 7.93, 4.03, 0.70, and 0.39, respectively. Both inside and between landraces, there was a clear genetic divergence from the Hardy-Weinberg assumption, and there was significantly more genetic variation within the landraces than among them. The 11 collected results revealed that the Wuling mountain region's maize land-race core collection might be built from 180 different genotypes (Qi-Lun *et al.,* 2008).

Salem *et al.* (2008) used 48 SSRs and 9 morphological features to assess the genetic diversity of the seven wheat (*Triticum aestivum* L.) varieties at the DNA level. Using wheat microsatellite markers, they identified 15 loci distributed across 15 chromosomes and were able to identify 48 alleles, with an average of 3.2 alleles per locus (WMS). With an average of

0.548, PIC values varied from 0.27 for the Xgwm95 to 0.81 for the Xgwm437. In comparison to SSR markers (0.53 with a range of 0.42-0.63), the average genetic diversity based on the morphological features was higher (23.49, with a range of 8.51-38.46). According to their findings, breeders can arrange crosses for advantageous features by using the classification based on the morphological characters and genotype. Microsatellite markers make it possible to fingerprint samples quickly and thoroughly from a variety of accessions, as demonstrated by this study.

Using five SSR markers, Prabakaran *et al.* (2010) assessed the genetic divergence between 12 rice landraces. The number of alleles present per locus varied from 2 to 3, with an average of 2.2 per locus, and a total of 11 alleles were found in 12 land races. Among the used primers, RM 481 detected more alleles, with an average PIC value of 0.43. Dendrogram grouped the twelve rice accession samples into six clusters based on SSR marker analyses.

Reid *et al.* (2011) classified 119 elite maize inbred lines into heterotic groups by utilising pedigree information. Inbred lines formed eight groupings, six of which were known main heterotic groups. The inbreds were grouped into 10 groups after an SSR analysis of 105 loci. The SSR clustering groups differed slightly from the pedigree-based grouping, and pedigree-based groups of genetically related germplasm were not always validated by molecular markers.

The significant number of alleles found in the 90 inbreds genotyped with 80 SSR markers by Suteu *et al.* (2013) best characterised the Romanian maize germplasm as a reservoir of genetic variability. 920 alleles were present in total, with 11.5 per locus on average. For 73 out of the 80 markers utilised, a PIC value less than 0.5 was found, with an average of 0.73.

Bashir *et al.* (2015) used 30 SSRs to analyse the genetic diversity in 214 pearl millet accessions from various Sudanese areas and 11 accessions from West Africa. Additionally, 15 agro-morphological parameters were examined in the accessions. There were found to be 400 different alleles. In terms of gene diversity, observed heterozygosity, and PIC, the mean values for 30 SSRs were 0.82, 0.27, and 0.77, respectively. The genetic matrix and the agro-morphological matrix had a low association (r=0.20).

Al-Ashkar *et al.* (2020) exhibited the phenotypic and genetic variation of 18 wheat genotypes under salt stress using a complete set of morpho-physiological and biochemical data, SSR marker technology, and multivariate analysis. The findings demonstrated that heritability (>60%) and genetic gain (>20%) were both high for all parameters in terms of the relative change of genetic variation. Utilizing clustering techniques, the changes in the genotypes' relative turgidity and shoot dry matter during salt stress were confirmed. The genotypes were divided into three categories for cluster analysis: tolerant, moderate, and sensitive, each comprising five, six, and seven genotypes. 17 of the 23 SSR markers that displayed polymorphism were connected to all the characteristics observed. Therefore, the markers were very helpful in identifying tolerant and sensitive genotypes based on the reported molecular marker-phenotypic trait association.

With the help of two gene-based markers [Start Codon Targeted (SCoT) polymorphism and CAAT-box Derived Polymorphism (CBDP)] and SSRs, Pour-Aboughadareh *et al.* (2022) examined 100 individuals from four *Triticum* and *Aegilops* species for genetic diversity and population structure. The polymorphism segments from the SCoT, CBDP, and SSR markers were 76, 116, and 48, respectively. Due to the CBDP marker's higher PIC, resolving power (Rp), and marker index (MI), it was more effective than the SCoT and SSR markers. The distribution of genetic variation was more evenly distributed within species than between them, according to AMOVA. The two species with the highest values for all genetic variation parameters were *Ae. cylindrica* and *Ae. tauschii*. The SSR marker was more effective in grouping the tested accessions, as seen by the results of principal coordinate analysis (PCoA) and population structure also confirmed the obtained clustering patterns.

2.3.2 SSR markers for diversity analysis studies in pearl millet

The isozyme investigations carried out by the ORSTOM team (Tostain *et al.*, 1987; Tostain and Marchais, 1989; Tostain, 1994) marked the beginning of marker-assisted diversity assessment in pearl millet. Then came a few investigations using Random amplified polymorphic DNA (RAPD), Amplified fragment length polymorphism (AFLP), and RFLP (Chowdari *et al.*, 1998a, 1998b; Busso *et al.*, 2000; Bhattacharjee *et al.*, 2002). Because they are highly informative, stably inherited, co-dominant, have good genome coverage, and have the potential for automation, microsatellite or SSR markers have evolved as the markers of choice.

Budak *et al.* (2003) established and made use of a set of microsatellite markers to analyse the genetic diversity of 53 lines of pearl millet. 11 of the 18 novel microsatellites discovered using the (CT)15 oligonucleotide probe to examine the genomic library were used to quantify the genetic diversity. It was determined that the unweighted pair group method for arithmetic mean (UPGMA) method of cluster analysis was suitable to distinguish between two major and eight smaller clusters.

Mariac *et al.* (2006) developed a new set of microsatellite markers using publicly available EST found in GenBank. In Niger, 46 wild and 421 cultivated pearl millet accessions were examined for genetic diversity using a total of 25 microsatellite markers, eight of which were created by the researchers. Comparing cultivated pearl millet accessions to wild accessions, there were significantly fewer alleles present, as shown by lower genetic diversity and allelic richness. A study of introgressions between wild and domesticated accessions revealed negligible but statistically significant evidence of introgressions.

Kapila *et al.* (2008) used 34 primer pairs to analyse the genetic diversity at Simple Sequence Repeat loci in 70 maintainers and 2 pollinators of sub-Saharan and Indian descent. The 72 lines clustered into five groups, which was consistent with their ancestry and distinguishing characteristics. Stich *et al.* (2010) found that the 145 landrace-derived pearl millet inbreds had a total gene diversity of 0.74 and a significantly larger number of alleles per locus (16.4) for 20 SSRs.

Thirty-eight polymorphic SSRs and a high throughput test were used by Nepolean *et al.* (2012) to genotype 213 lines (98 B-lines and 115 R-lines). Genetically, B-lines were less varied than Restorer lines. B-lines and R-lines were clearly separated into two clusters by a neighbour-joining tree based on a basic matching dissimilarity distance matrix. B-lines were further divided into four sub-clusters and R-lines into five sub-clusters. 30 R-lines and 14 B-lines each have distinct alleles that can be utilised to separate them from one another.

Singh *et al.* (2013) reported the genetic diversity of 20 commercially available cultivars of open-pollinated and hybrid pearl millet. 64 alleles were amplified using 21 polymorphic SSR primer pairs, which were used to explore the diversity. All the cultivars were distinguished by UPGMA cluster analysis. In a subgroup of Group I, all the cultivars created at IARI, New Delhi, were present. Similar to this, the bulk of hybrids created at HAU, Hisar, were classified in a different subgroup of group 1. Genetic divergence was greatest in a special forage variety called BAIF Bajra 1. In addition, a group of five polymorphic primers were discovered to distinguish between all the cultivars. The findings showed that there was a moderate level of genetic variability among the cultivars of pearl millet.

Bhardwaj *et al.* (2018) studied genetic diversity among 190 pearl millet accessions using SSR markers. They used 40 SSR markers for screening out of which 34 were found to be polymorphic. PIC values ranged from 0.002 to 0.749 with an average of 0.445. The dendrogram tree revealed that the germplasm lines differentiated R from B lines into two separate clusters with a few exceptions of B lines grouping with R lines and vice versa.

Kumar *et al.* (2020) studied diversity in 17 Indian pearl millet inbreds using SSR markers. They found 342 total polymorphic alleles with a mean of 4.62 alleles per locus from 74 SSR primers. The PIC values ranged from 0.10 to 0.89. Low levels of heterozygosity were detected in the genotypes. The average genetic dissimilarity between the pearl millet inbred lines was found to be 0.66. the results showed the presence of sufficient genetic variability among the genotypes and also led to the identification of a suit of SSR markers that could effectively be used for genetic diversity analysis in pearl millet.

Choudhary *et al.* (2021) screened 96 pearl millet germplasm lines tested for drought utilising a variety of physiological and morphological characteristics, as well as SSR markers. The goal of the study was to document several morphological and physiological characteristics associated with drought sensitivity and tolerance. Additionally, 35 microsatellite markers were utilised to examine the variation among the genotypes of pearl millet. Genotypes were grouped according to their morpho-physiological traits. Twenty-two of the 35 SSR markers were successfully amplified across all germplasm lines, and fifteen of the markers were monomorphic while seven were polymorphic. A total of 22 pearl millet genotypes were discovered to be categorised separately from the other genotypes based on the morpho-physiological data. These genotypes had demonstrated their ability to withstand drought, however the remaining genotypes had been discovered to be susceptible to it.

Priya *et al.* (2022) evaluated 150 pearl millet genotypes using 50 SSR markers out of which 28 were found to be polymorphic. UPGMA was used to calculate the genetic distances. With an average of 5.37 alleles per locus, 148 alleles in total were found. The polymorphic information content (PIC) values had an average of 0.83 and varied from 0.74 (PSMP 2078) to 0.93 (PSMP 2214). The UPGMA analysis showed that the genotypes ICMR 07999 and ICMB 98222 were most similar, whereas 670R and 2B had the lowest similarity index. The estimation of genetic diversity and the distinction of the genetic relationships between genotypes made possible by the analysis of SSR data allowed for the identification of diverse genotypes that can be used in a hybridization programme.

2.4 Enzyme activity analysis in pearl millet

Nagarathna *et al.* (1992) tested lipoxygenase (LOX) activity in the seeds of different pearl millet genotypes having differential susceptibility for downy mildew. The LOX assay in the seeds showed a good correlation between the enzyme activity and their reactions against downy mildew in the field. They recorded maximum activity in the seeds of highly resistant genotypes and minimum activity in highly susceptible genotypes. Seeds derived from the plants which recovered from the downy mildew disease were found to have more LOX activity as compared to the parent seeds. Also, they found decreased LOX activity in susceptible genotypes. Thus, they concluded that the LOX activity can be used for the screening of different genotypes against downy mildew disease as a biochemical marker in pearl millet.

After inoculating pearl millet with Pathotype 1 of *Sclerospora graminicola*, Nagarathna *et al.* (1993) investigated Phenylalanine Ammonia Lyase (PAL) activity in various genotypes with differing degrees of sensitivity to downy mildew. After 24 hours of fungal inoculation, the enzyme activity considerably increased in resistant genotypes whereas it reduced in susceptible genotypes. The degree of host resistance to the infection was highly correlated with the rise or fall in enzyme activity. The researchers concluded that PAL is crucial in imparting pearl millet resistance against the downy mildew disease.

Basavaraj *et al.* (2018) evaluated the capacity of some abiotic elicitors, including salicylic acid (SA), methyl jasmonate (MeJ) and β-aminobutyric acid (BABA) to promote growth and generate resistance against *Magnaporthe grisea* caused blast disease of Pearl millet. Seeds treated with 50mM BABA showed germination percentage of 90.67% followed by seeds treated with 0.50mM SA which showed germination percentage of 83.61% compared to controls. When compared to untreated seedlings, the BABA demonstrated the highest level of disease protection against blast disease, with a protection rate of 61.3%, followed by SA, with a protection rate of 52.4%. Additionally, it was discovered that in the pathogen challenged resistant and inoculated seedlings, the activity of defense-related enzymes such as PAL, peroxidase (POX), and β-1,3-glucanase considerably increased.

Basavaraj *et al.* (2019) evaluated the efficacy of biotic agents like *Trichoderma virens*, *Pseudomonas fluorescens* and neem leaf extract to induce resistance in pearl millet against blast disease caused by *M. grisea. P. fluorescens* treated seeds showed maximum seed germination and seed vigour followed by seeds treated with *T. virens* and neem leaf extract. They also found that the activity of defence related enzymes such as LOX, POX, PAL and β-1,3-glucanase were considerably increased in *P. fluorescens* treated seedlings which indicated that these enzymes greatly aid in inducing blast disease resistance in pearl millet.

Lavanya *et al.* (2022) deciphered the immunity eliciting properties of 3,5-dichloroanthranilic acid, lipopolysaccharide, cell wall glucan and glycine betaine through protein and enzymatic studies based on the elicitor treatment activated defense mechanism. They checked the gene expression and activities of defense enzymes *viz.*, PAL, LOX, POX, polyphenol oxidase (PPO), β-1,3-glucanase and hydroxyproline rich glycoproteins (HRGPs). The results revealed high level of gene expression and enzyme activities in elicitor treated *P. glaucum* to be in positive correlation with increased downy mildew resistance. They concluded that the magnitude and rate of defense gene expression is important for the effective manifestation of defense against the pathogen.

CHAPTER – III

MATERIALS AND METHODS

The present study was carried out in the Department of Molecular Biology and Biotechnology, College of Biotechnology, CCS Haryana Agricultural University, Hisar in collaboration with the Department of Genetics and Plant Breeding, and Department of Plant Pathology, College of Agriculture, CCS Haryana Agricultural University, Hisar. The techniques and materials used during the course of investigation are described in this chapter.

3.1 Experimental site and conditions

The field experiments for the present investigation were conducted in the research area of Bajra Section, Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar. It is situated at 2.1492°N latitude and 75.7217°E longitude with 215.52 m altitude above the mean sea level and the soil characteristic is sandy loam.

3.2 Experiment 1: Evaluation of pearl millet genotypes for morpho-phenological traits and blast disease

3.2.1 Experimental material

In this experiment, 60 pearl millet genotypes (Table 3.1) were evaluated during *kharif* season 2021.

3.2.2 Experimental design and layout plan:

- Plant material : 60 pearl millet genotypes
- Experimental design : Randomized Block Design
- Replications : Two
- Number of rows : Two
- Row length : 4 m
- Row to row distance : 0.45 m
- Plant to plant distance : 10-12 cm
- Season : *kharif* 2021
- Location : Research Area, Bajra Section, Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar

Table 3.1: List of pearl millet genotypes evaluated in *kharif* 2021

S.No.	Genotypes	S.No.	Genotypes
1	R-line 2	**31**	R-line 34
2	R-line 3	**32**	R-line 39
3	R-line 4	**33**	R-line 40
4	R-line 5	**34**	R-line 42
5	R-line 6	**35**	R-line 62
6	R-line 11	**36**	R-line 63
7	R-line 16	**37**	R-line 64
8	R-line 17	**38**	R-line 66
9	R-line 18	**39**	R-line 67
10	R-line 21	**40**	R-line 68
11	R-line 24	**41**	ICMB 843-22
12	R-line 25	**42**	ICMB 89111
13	R-line 31	**43**	ICMB 95444
14	R-line 33	**44**	ICMB 04888
15	R-line 36	**45**	ICMB 97111
16	R-line 37	**46**	ICMB 02333
17	R-line 44	**47**	ICMB 04999
18	R-line 71	**48**	H77/833-2-202
19	R-line 75	**49**	HTP 94/54
20	R-line 187	**50**	HBL-11
21	R-line 8	**51**	AC 04/13
22	R-line 10	**52**	G73-107
23	R-line 12	**53**	H77/29-2
24	R-line 13	**54**	H13/001
25	R-line 14	**55**	H90/4-5
26	R-line 15	**56**	HMS 70B
27	R-line 19	**57**	HMS 23B
28	R-line 22	**58**	HMS 58B
29	R-line 30	**59**	HMS 33B
30	R-line 32	**60**	EBL-12-237

3.2.3 Observations recorded

The following morpho-phenological traits and yield attributes were recorded on five random representative plants from each genotype in each replication.

3.2.3.1 Plant height (cm)

Plant height was recorded in centimetres from the tip of the main panicle to ground level in five representative plants.

3.2.3.2 Ear length (cm)

Ear length was recorded in centimetres from tip to the base of ear of the main tiller of the plant in five representative plants.

3.2.3.3 Ear diameter (cm)

The diameter of the ear was measured at the point slightly below from the centre of the ear in centimetres using Vernier calliper.

3.2.3.4 Leaf blade width (cm)

Leaf blade width of flag leaf was measured in centimetres on five randomly selected plants using scale.

3.2.3.5 Effective number of tillers/plant

Effective number of tillers per plant of five randomly selected competitive plants from each entry were recorded only from the ear head bearing the tillers.

3.2.3.6 Days to 50% flowering

It was calculated by noting the number of days from the date of sowing up to the date when the main panicle in 50% of the plants in a plot exhibited stigma emergence.

3.2.3.7 Days to maturity

Days to maturity were calculated by observing the number of days from the date of sowing until the grain reached maturity in five randomly selected plants.

3.2.3.8 1000 grain weight (g)

Well-developed 1000 grains were collected from the panicles harvested from the productive tillers of the five randomly selected plants and their weight was noted.

3.2.3.9 Grain yield/plant (g)

The weight of grains from each plant obtained by the threshing of panicles from productive tillers was recorded in grams as grain yield per plant.

3.2.3.10 Dry fodder yield/plant (g)

After the plants from each row were harvested, they were sun dried for a week and the weight of the dry fodder was recorded in grams.

3.2.4 Screening and scoring for the blast disease

A total of 60 pearl millet genotypes were screened under the natural field conditions using the methods standardized at ICRISAT (Thakur *et al.*, 2011). For this, the artificial spore suspension of *P. grisea* was obtained from Department of Plant Pathology, College of Agriculture, CCS Haryana Agricultural University, Hisar. The seedlings were spray-inoculated at pre-tillering stage with aqueous spore suspension to run-off. High humidity was provided by operating irrigation twice a day; 30-60 minutes each; in the morning and in afternoon. The blast disease severity was recorded at hard dough stage on five representative plants in the plot for all the genotypes on 1-9 progressive scale developed by International Rice Research Institute (IRRI) for rice blast (Table 3.2).

Table 3.2: Rating scale (1-9) for foliar blast severity

Rating scale	Symptoms and lesions	Disease Reaction
1	No lesions to small brown specks of pinhead size	Highly Resistant
2	Large brown specks	Resistant
3	Small, roundish to slightly elongated, grey necrotic spots, about 1-2 mm in diameter having a brown margin	
4	Typical blast lesions, elliptical, 1-2 cm long, confined to the area in between the main veins, covering <2% of the area of leaf	Moderately Resistant
5	Typical blast lesions covering <10% of the area of leaf	
6	Typical blast lesions covering 10-25% of the area of leaf	Susceptible
7	Typical blast lesions covering 26-50% of the area of leaf	
8	Typical blast lesions covering 51-75% of the area of leaf along with many dead leaves	Highly Susceptible
9	>75% of the leaf area covered with lesions and most leaves dead	

3.3 Experiment 2: Studying the DNA polymorphism and initiating the crossing program between the selected blast resistant and elite lines

3.3.1 DNA polymorphism study

In this experiment, young leaf samples from 60 pearl millet genotypes were collected and DNA polymorphism was studied.

3.3.1.1 Reagents and chemicals

All the chemicals used in DNA extraction, polymerase chain reaction (PCR), and gel electrophoresis were obtained from Genei and SRL. All other chemicals were of analytical grade and obtained from Sigma Chemicals Co., USA and G-Biosciences.

3.3.1.2 Glassware and plasticware

Glassware and plasticware used in the investigation were obtained from Borosil India Ltd. and Tarsons Products Pvt. Ltd. respectively.

3.3.1.3 Molecular markers

A total of 140 SSR primer pairs were selected to study the molecular diversity among the genotypes. They were synthesized from Integrated DNA Technologies, Inc. The sequence information of the forward and reverse primers used in genotyping the pearl millet SSR loci is given in Table 3.3.

Table 3.3 List of SSR markers used to study polymorphism in 60 pearl millet genotypes

S. No.	Primer	Forward Sequence (5’-3’)	Reverse Sequence (5’-3’)
1	Xcump001	GCACGAGGCTTATCTGTGTTTC	CAACTCTTGCCTTTCTTGGCCT
2	Xcump002	GCACGAGGCAAAATATAAAGGTG	ACGTAGACTTGCACCACCAGA
3	Xcump003	CATGCGACGTGGTCTATCTG	GAGAGAGAACCAGCAGCACC
4	Xcump004	CACGAGGCTCACTAGGGTTT	ACCCGGGTCTGGTTAGACTT
5	Xcump005	GCACGAGGGCCAGATTCTAGAA	CACGGTGATGACACGACATGGT
6	Xcump006	GAAATCGGCAGAGGGCAT	CAATGAGTATGTGCACGCTGCA
7	Xcump007	GAGGGATTCCAGGCGGTTC	GCGAGGAGCACATTCGATGAA
8	Xcump008	GTTGACTACCACTATTATGCTCC	GACCAAGAACTTCATACAATTCAG
9	Xcump009	ATCTGATCGTGAGGCCTCAAC	GCCGACCAAGAACTTCATACAAT
10	Xcump010	GCTGAACTATTCTGTAAACTTAAC	TATCGAAACGGTACTAAAATCATG
11	Xcump011	TGATGGGAACCGAGAGCATGA	TAGCACAGCAATAACATGGCATC
12	Xcump012	TGTGATCTGTGGTCTCAGGC	CGTGAAAGCTCTCCAGGACT
13	Xcump013	ACCGACAGCAACAAATCCTCC	GCTCTTGTGTGTAGTTGTGCTT

14	Xcump014	CTGACCTCTCCTCTCCTTCG	GAGCAGATCCTTGGCCTTCTTG
15	Xcump015	GAAGCATAGGAGAGGAGGG	CTTGCTGCTCGGACTTCTCT
16	Xcump016	CATTTCTCTCGCCAGTGCTC	ATCTCCAGAACCGAGCGCA
17	Xcump017	ATAGCTGGGTGTTGTCTGGC	CCCTGGCGCTTAATTGTAAA
18	Xcump018	TGCTTTCTTCCCAACCAGTGG	TGCTGAGTGGGGTGCTGCT
19	Xcump019	GGCCTAACTCTCTGTTCTTCTTC	GAGAAGCTAACATTTGGGGCCTA
20	CTM-1	TCTGGGGATTGGCTGGAATTACA	AAGTTGGGTAACGCCAGGGTTTTC
21	CTM-2	GGTGATTAAAATCGAGGGTT	AGCAACTTGAGCAGCGG
22	CTM-3	GTCCATCGTCGCCGACGAA	GGATTTGCTAGTTGTGGGCT
23	CTM-8	GCTGCATCGGAGATAGGGAA	CTCAGCAAGCACGCTGCTCT
24	CTM-9	GCCTCCTCTTGATACCATATT	TAGCCTTGGCTGCTATATTC
25	CTM-10	GAGGCAAAAGTGGAAGACAG	TTGATTCCCGGTTCTATCGA
26	CTM-11	GACCGATCTTCTTTGCTGTTG	TCTATCGTACGTTAACCTCA
27	CTM-12	GTTGCAAGCAGGAGTAGATCGA	CGCTCTGTAGGTTGAACTCCTT
28	CTM-21	ATGCCTCCCACCCCACGTCG	CGTCGCACTAGCCACAGTCA
29	CTM-25	GCGAAGTAGAACACCGCGCT	GCACTTCCTCCTCGCCGTCA
30	ICMP 3019	GCGCACCACCTGTGTCTAT	CATGCAGAGAAAAATCAAGCA
31	ICMP 3056	ACGGAGCTACGGTTGGAATA	CACAAGGGACCCCACGATA
32	ICMP3017	CACCAAACAGCATCAAGCAG	AGGTAGCCGAGGAAGGTGAG
33	ICMP3018	ACGAGGACAAGCTCTTGGAA	ACGGCGCATACTCGATCATA
34	ICMP3088	TCAGGTGGAGATCGATGTTG	TTACGGGAGGATGAGGATG
35	ICMP 10	ATCCCCTACAGCATCAGCAC	CGGCGGAGAGATCTTATTCA
36	PGIRD 46	GAACAATTGCTTCTGTAATATTGCTT	GCCGACCAAGAACTTCATACA
37	PGIRD 49	AGCTCCTCGACGGAGAAGT	GACGGTGTCGACGAAGATG
38	PGIRD 50	CTCTCGGTTTGACGGTTTGT	GGGGAAAACAAAGTTGCTCA
39	PGIRD 54	GCCTGGGATGTGTTTCTTCT	GCCTTTCATTTCCACCATGA
40	PSMP 20	CATTACACGTTTCTTCAAACGC	TCTTCGGCCTAATAGCTCTAAC
41	PSMP 2001	CATGAAGCCAATTAGGTCTC	ACCATCTGACTTGTTCTTATCC
42	PSMP 2013	GTAACCCACTAACCCTTACC	GTCGCACAGAAAAAGAATAG
43	PSMP 2030	ACCAGAGCTTGGAAATCAGCAC	CATAATGCTTCAAATCTGCCACAC
44	PSMP 2059	GGGGAGATGAGAAAACACAATCAC	TCGAGAGAGGAACCTGATCCTAA
45	PSMP 2076	GGAATAGTATATTGGCAAAATGTG	ATACTACACCTGTAAGCATTGTC
46	PSMP 2080	CAGAATCCCCACATCTGCAT	TGCAACTGAGCGAAGATCAA

47	PSMP 2081	CTGTGCTGTCATTGTTACCA	TCAGATCACCTATTACTTTCCCT
48	PSMP 2084	AATCTAGTGATCTAGTGTGCTTCC	GGTTAGTTTGTTTGAGGCAAATGC
49	PSMP 2086	CGCTTGTTTTCCTTTCTTGCTGTT	CCTTCTCAGATCCTGTGCTTTCTT
50	PSMP 2087	GGAACAGACTCCATACCTGAAA	TACCTGCCTGTGCTGTTAGT
51	PSMP 2089	TTCGCCGCTGCTACATACTT	TGTGGATGTTGCTGGTCATT
52	PSMP 2090	AGCAGCCCAGTAATACCTCAGCTC	AGCCCTAGCGCACAACACAAACTC
53	PSMP 22	TCTGTTTGTTTGGGTCAGGTCCTTC	CGAATACGTATGGAGAACTGCGCATC
54	PSMP 2201	CCCGACGTTATGCGTTAAGTT	TCCATCCATCCATTAATCCACA
55	PSMP 2224	GGCGAAATTGGAATTCAGATTG	CGTAATCGTAGCGTCTCGTCTAA
56	PSMP 2227	ACACCAAACACCAACCATAAA	TCGTCAGCAATCACTAATGACC
57	PSMP 2229	CCACTACCTTCGTCTTCCTCCATTC	GTCCGTTCCGTTAGTTGTTGCC
58	PSMP 2232	TGTTGTTGGGAGAGGGTATGAG	CTCTCGCCATTCTTCAAGTTCA
59	PSMP 2237	TGGCCTTGGCCTTTCCACGCTT	CAATCAGTCCGTATCCACACCCCA
60	PSMP 2246	CGGATGCTAAATTAACCGAAGC	CCAGCTTGCTTCTGTTCGGTTC
61	PSMP 2248	TCTGTTTGTTTGGGTCAGGTCCTTC	CGAATACGTATGGAGAACTGCGCATC
62	PSMP 2249	CAGTCTCTAACAAACAAACACGGC	GACAGCAACCAAGTCCAAACTCCA
63	PSMP 2263	AAAGTGAATACGATACAGGAGCTGAG	CATTTCAGCCGTTAAGTGAGACAA
64	PSMP 2267	GGAAGGCGTAGGGATCAATCTCAC	ATCCACCCGACGAAGGAAACGA
65	PSMP 2270	AACCAGAGAAGTACATGGCCCG	CGACGAACAAATTAAGGCTCTC
66	PSMP 2271	CCTTATATTGGACCGACTGCTGAC	CTCCCCCATACACGAGCGAGAA
67	PSMP 2273	AACCCCACCAGTAAGTTGTGCTGC	GATGACGACCAAGACTTCTCTCC
68	PSMP 2274	CACCTAGACTCTACACAATGCAAC	AATATCAAGTGATCCACCTCCCAA
69	PSMP 2275	CCAGTGCCTGCATTCTTGGC	GCATCGAATACTTCATCTCA
70	PSMP 2008	GATCATGTTGTCATGAATCACC	ACACTACACCTACATACGCTCC
71	PSMP 2027	AGCAATCCGATAACAAGGAC	AGCTTTGGAAAAGGTGATCC
72	PSMP 20	CATTACACGTTTCTTCAAACGC	TCTTCGGCCTAATAGCTCTAAC
73	PSMP 2233	TGTTTTCTCCTCTTAGGCTTCGTTC	ACCTTCTCCGCCACTAAACAACT
74	Xctm 03	GTCCATCGTCGCCGACGAA	GGATTTGCTAGTTGTGGGCT
75	CTM-26	GCAAGTGATCCATGACATTACGA	ACTTGCTAGCTGCTGCTCTTG
76	CTM-27	GTTGCAAGCAGGAGTAGATCGA	CGCTCTGTAGGTTGAACTCCTT
77	CTM-55	CGTCTTCTACCACGTCCT	CATAATCCCACTCAACAATCC
78	CTM-56	GCGTTGTTTCGGTGACCAC	GCGTATCTTTAAATTGCCTTTGTT
79	CTM-57	TGGTGGCAATGCAAGCTACAG	AGCGAGACGATCGACAGGG

80	CTM-58	TACGTGCTACAAGAATGG	GCTGGCTAGGACACAA
81	CTM-59	TCCTCGACATCCTCCA	GACACCTCGTAGCACTCC
82	CTM-60	AAGCCCCGATCACATCAA	AGCCGAGCCTCATCCC
83	Xicmp3032	AGGTAGCCGAGGAAGGTGAG	CAACAGCATCAAGCAGGAGA
84	Xipes 0146	GTGTATGGTATGCGTGTCC	GCATTGTGATCGAATAAACTACTG
85	XIPES 0216	AAAGGCAGCAGATCCCTACA	TCTTTCGTTGCATCTGTTCG
86	Xipes 0066	CAACATGTCAAGGAAGTAAAATTGA	GCCTCTTGATACCCAAGATCA
87	Xpsmp 3029	CAACATGTCAAGGAAGTAAAATTGA	GGTTAGTTTGTTTGAGGCAAATGC
88	Xpsmp2006	GACTTATAGTCATGGGAAAGCTC	GCTTTAATAACTTTGTGCGTATT
89	Xpsmp2019	TGTGCCACAGCTTGTTCCTC	CAAGCAGCCAGTTCCTCATC
90	Xpsmp2040	CATTACACGTTTCTTCAAACGC	TCTTCGGCCTAATAGCTCTAAC
91	Xpsmp2069	CCCATCTGAAATCTGGCTGAGAA	CCGTGTTCGTACAAGGTTTTGC
92	Xpsmp2074	AGGACTGTAGGAGTGTGGACAACACAA	CCAGACCTACCAGTGAATGAGA
93	Xpsmp2086	CGCTTGTTTTCCTTTCTTGCTGTT	CCTTCTCAGATCCTGTGCTTTCTT
94	Xpsmp2214	CGCACAGTACGTGTGAGTGAAG	GATTGAGCAGCAAAAACCAGC
95	CTM-26	GCAAGTGATCCATGACATTACGA	ACTTGCTAGCTGCTGCTCTTG
96	CTM-27	GTTGCAAGCAGGAGTAGATCGA	CGCTCTGTAGGTTGAACTCCTT
97	CTM-55	CGTCTTCTACCACGTCCT	CATAATCCCACTCAACAATCC
98	CTM-56	GCGTTGTTTCGGTGACCAC	GCGTATCTTTAAATTGCCTTTGTT
99	CTM-57	TGGTGGCAATGCAAGCTACAG	AGCGAGACGATCGACAGGG
100	CTM-58	TACGTGCTACAAGAATGG	GCTGGCTAGGACACAA
101	CTM-59	TCCTCGACATCCTCCA	GACACCTCGTAGCACTCC
102	CTM-60	AAGCCCCGATCACATCAA	AGCCGAGCCTCATCCC
103	ICMP 3019	GCGCACCACCTGTGTCTAT	CATGCAGAGAAAAATCAAGCA
104	ICMP 3056	ACGGAGCTACGGTTGGAATA	CACAAGGGACCCCACGATA
105	ICMP3017	CACCAAACAGCATCAAGCAG	AGGTAGCCGAGGAAGGTGAG
106	ICMP3018	ACGAGGACAAGCTCTTGGAA	ACGGCGCATACTCGATCATA
107	ICMP3088	TCAGGTGGAGATCGATGTTG	TTACGGGAGGATGAGGATG
108	ICMP 10	ATCCCCTACAGCATCAGCAC	CGGCGGAGAGATCTTATTCA
109	PGIRD 46	GAACAATTGCTTCTGTAATATTGCTT	GCCGACCAAGAACTTCATACA
110	PGIRD 49	AGCTCCTCGACGGAGAAGT	GACGGTGTCGACGAAGATG
111	PGIRD 50	CTCTCGGTTTGACGGTTTGT	GGGGAAAACAAAGTTGCTCA
112	PGIRD 54	GCCTGGGATGTGTTTCTTCT	GCCTTTCATTTCCACCATGA

113	PSMP 20	CATTACACGTTTCTTCAAACGC	TCTTCGGCCTAATAGCTCTAAC
114	PSMP 2001	CATGAAGCCAATTAGGTCTC	ACCATCTGACTTGTTCTTATCC
115	PSMP 2013	GTAACCCACTAACCCTTACC	GTCGCACAGAAAAAGAATAG
116	PSMP 2030	ACCAGAGCTTGGAAATCAGCAC	CATAATGCTTCAAATCTGCCACAC
117	PSMP 2059	GGGGAGATGAGAAAACACAATCAC	TCGAGAGAGGAACCTGATCCTAA
118	PSMP 2076	GGAATAGTATATTGGCAAAATGTG	ATACTACACCTGTAAGCATTGTC
119	PSMP 2080	CAGAATCCCCACATCTGCAT	TGCAACTGAGCGAAGATCAA
120	PSMP 2081	CTGTGCTGTCATTGTTACCA	TCAGATCACCTATTACTTTCCCT
121	PSMP 2084	AATCTAGTGATCTAGTGTGCTTCC	GGTTAGTTTGTTTGAGGCAAATGC
122	PSMP 2086	CGCTTGTTTTCCTTTCTTGCTGTT	CCTTCTCAGATCCTGTGCTTTCTT
123	PSMP 2087	GGAACAGACTCCATACCTGAAA	TACCTGCCTGTGCTGTTAGT
124	PSMP 2089	TTCGCCGCTGCTACATACTT	TGTGGATGTTGCTGGTCATT
125	PSMP 2090	AGCAGCCCAGTAATACCTCAGCTC	AGCCCTAGCGCACAACACAAACTC
126	PSMP 22	TCTGTTTGTTTGGGTCAGGTCCTTC	CGAATACGTATGGAGAACTGCGCATC
127	PSMP 2201	CCCGACGTTATGCGTTAAGTT	TCCATCCATCCATTAATCCACA
128	PSMP 2224	GGCGAAATTGGAATTCAGATTG	CGTAATCGTAGCGTCTCGTCTAA
129	PSMP 2227	ACACCAAACACCAACCATAAA	TCGTCAGCAATCACTAATGACC
130	PSMP 2229	CCACTACCTTCGTCTTCCTCCATTC	GTCCGTTCCGTTAGTTGTTGCC
131	PSMP 2232	TGTTGTTGGGAGAGGGTATGAG	CTCTCGCCATTCTTCAAGTTCA
132	PSMP 2237	TGGCCTTGGCCTTTCCACGCTT	CAATCAGTCCGTATCCACACCCCA
133	PSMP 2246	CGGATGCTAAATTAACCGAAGC	CCAGCTTGCTTCTGTTCGGTTC
134	PSMP 2248	TCTGTTTGTTTGGGTCAGGTCCTTC	CGAATACGTATGGAGAACTGCGCATC
135	PSMP 2249	CAGTCTCTAACAAACAAACACGGC	GACAGCAACCAAGTCCAAACTCCA
136	PSMP 2274	CACCTAGACTCTACACAATGCAAC	AATATCAAGTGATCCACCTCCCAA
137	PSMP 2275	CCAGTGCCTGCATTCTTGGC	GCATCGAATACTTCATCTCA
138	PSMP 2008	GATCATGTTGTCATGAATCACC	ACACTACACCTACATACGCTCC
139	PSMP 2027	AGCAATCCGATAACAAGGAC	AGCTTTGGAAAAGGTGATCC
140	PSMP 2233	TGTTTTCTCCTCTTAGGCTTCGTTC	ACCTTCTCCGCCACTAAACAACT

3.3.1.4 Genomic DNA isolation

Genomic DNA was isolated from the young leaf samples of sixty pearl millet genotypes using CTAB extraction method (Saghai-Maroof *et al.*, 1984). Quantity and quality of isolated DNA was checked by the nanodrop and gel electrophoresis.

3.3.1.5 Reagents used in DNA isolation

Table 3.4: Composition of CTAB buffer

Reagents	Amount required for 100 ml
Tris Hcl (1M, pH 8.0)	20 ml
EDTA (disodium, 0.5 M, pH 8.0)	4 ml
NaCl (5M)	28 ml
CTAB	2 g
β-Mercaptoethanol	200 µl
Sterile distilled water	46 ml

Table 3.5: Composition of TE buffer

Reagents	Amount required for 100 ml
Tris (1M, pH 8.0)	1 ml
EDTA (disodium, 0.5M; pH 8.0)	200 µl

3.3.1.6 DNA isolation Protocol

(i) Young leaves were crushed in liquid nitrogen into a fine powder with the help of sterilized mortar and pestle and then immediately transferred to 1 ml pre-heated CTAB extraction buffer in the microcentrifuge tubes.

(ii) The microcentrifuge tubes were then incubated at 65℃ for 1 hour and the contents in tube were gently inverted and mixed intermittently after every 15 minutes.

(iii) The tubes were then allowed to cool at room temperature after which 1 ml of chloroform: isoamyl alcohol (24:1) was added and samples were mixed thoroughly by gently inverting the tubes many times.

(iv) The entire mixture was subjected to centrifugation for 10 minutes at 10000 rpm at 25℃.

(v) Aqueous phase from the tubes was then collected and transferred to a fresh centrifuge tube and equal amount of chilled isopropanol was added for the DNA to precipitate and tubes were placed at 4℃ for 1 hour. Then the tubes were centrifuged for 10 minutes at 10000 rpm to pellet out the DNA.

(vi) The DNA pellet obtained was washed with 70% ethanol.

(vii) The pellet was dried at room temperature and then suspended in adequate volume of TE buffer. DNA was then stored at 4℃ for further use.

(viii) The DNA samples were then treated with 2 µl of 10 mg/ml RNase A and kept for incubation in hot water bath at 37℃ for 1 hour to remove RNA contamination.

(ix) The steps from (iv) to (vii) were repeated to re-precipitate the DNA.

(x) The DNA was then checked for its quality and quantity.

3.3.1.7 Qualitative and quantitative estimation of DNA

3.3.1.7.1 DNA estimation using nanodrop

Nanodrop (Thermo Scientific) was used for DNA concentration estimation. Blank measurement was done using TE buffer and 1 µl DNA dissolved in TE buffer was loaded on the lower pedestal of nanodrop and lever arm was closed. The software automatically analysed and showed the DNA concentrations along with the purity ratios.

3.3.1.7.2 Quality of DNA

Quality of the DNA was checked with agarose gel electrophoresis using 0.8% (w/v) agarose gel. Intact, smear less and bright bands of DNA observed on the gel were considered to be of good quality.

3.3.1.8 Polymerase chain reaction Amplification

PCR amplification was carried out using Benchtop^{TM} Lab Systems (programmable thermal cycler). The reaction was carried out in a 20 µl of reaction mixture having components given in Table 3.6.

Table 3.6: Composition of PCR reaction mixture

Components	**Volume used**
PCR Buffer (10X)	2.0 µl
dNTPs (10mM)	0.5 µl
Forward Primer (2.5 µM)	1.0 µl
Reverse Primer (2.5 µM)	1.0 µl
Taq DNA Polymerase (5U/ µl)	0.5 µl
Nuclease Free Water	14 µl
DNA Template (50 ng)	1 µl
Total Volume	20 µl

Table 3.7 Reaction conditions used for PCR amplification

Step	Temperature with time	No. of Cycles
Initial Denaturation	94°C for 3 min	
Denaturation	94°C for 20 sec	35 cycles
Annealing	46-61°C for 30 sec	
Extension	72°C for 1:30 min	
Final Extension	72°C for 10 min	

Amplified products were stored at 4°C till further use.

3.3.1.9 Agarose gel electrophoresis

PCR amplified products were resolved on 2.0% (w/v) agarose gel and were visualized under UV light by staining with ethidium bromide (EtBr). Table 3.8 shows the composition of EtBr.

Table 3.8: Composition of Ethidium Bromide (EtBr)

Reagents	Amount to be added for 100 ml
Ethidium Bromide (EtBr) (10mg/ml)	1g

Procedure

(i) Agarose gel (2.0%) was prepared in 1X TAE buffer. Table 3.9 shows the composition of TAE buffer. 2g agarose was melted in 1X TAE buffer and EtBr was added at a concentration of 0.1 µg/ml and mixed well.

(ii) Gel was poured into the gel casting tray containing an appropriate comb. Gel was allowed to solidify after which the comb was removed and gel tray was placed in the electrophoresis chamber containing 1X TAE buffer.

(iii) PCR products were prepared by adding 1X loading dye and then loaded in the gel using a micropipette.

(iv) Electrophoresis was carried out giving constant voltage supply (Hoefer Inc., USA) i.e., 5 volts/cm of the gel until the dye migrated to the other end of the gel.

(v) PCR amplified products were visualized using UV light and photographed using ‘BioRad Gel Documentation System’.

Table 3.9: Composition of 50X TAE buffer (pH 8.0)

Reagents	**Amount to be added for 1 litre**
Tris	242 g
Glacial Acetic acid	57.1 ml
0.5 M EDTA (pH 8.0)	100 ml

3.3.1.10 Allele scoring

SSR amplified DNA fragments were scored visually for the presence (1) and absence (0) of the bands for each pearl millet genotype. The size of the amplified products was determined in nucleotide base pairs based on their migration relative to the 100 bp DNA ladder that was used as a marker.

3.3.1.11 Data analysis

Based on the presence and absence of bands, the similarity matrix analysis was done using ‘SIMQUAL’ sub-program of the software ‘Numerical Taxonomy and multivariate Analysis System (NTSYS-pc) (Rohlf, 1993). Cluster analysis was done for estimating the relationship among all the pearl millet genotypes. Dendrogram was constructed with the help of distance matrix by the ‘Unweighted Pair-Group’ method using ‘UPGMA’ sub-program of NTSYS-pc. Principle Component Analysis was done using the ‘EIGEN’ sub-program of NTSYS-pc.

3.3.2 Crossing between blast resistance and elite lines

3.3.2.1 Experimental material

In this experiment, all the 60 pearl millet genotypes (Table 3.1) were re-sown at Deendayal Upadhyay Centre of Excellence for Organic Farming, CCS Haryana Agricultural University, Hisar during *summer* season, 2022 and 39 crosses were initiated using the parents from *kharif*, 2021 and some additional lines were also taken as the crossing material (Table 3.10) to develop blast resistance hybrids.

Table 3.10: Parents used to initiate blast resistant hybrids

S.No.	**♀ parent**	×	**♂ parent**
1	HMS 47A	×	HPT-2-12-32
2	HMS 47A	×	HB 15/085
3	HMS 47A	×	R-line 2
4	HMS 47A	×	R-line 10
5	HMS 47A	×	R-line 17
6	HMS 47A	×	R-line 49

7	HMS 47A	×	H78/711
8	HMS 47A	×	SGP-10-107
9	HMS 47A	×	R-line 2
10	HMS 74A	×	R-line 4
11	HMS 74A	×	78/711
12	ICMA 04999	×	ISK-51
13	ICMA 04999	×	SGP-10-107
14	ICMA 04999	×	EMRL 15/109
15	ICMA 04999	×	R-line 17
16	ICMA 04999	×	78/711
17	ICMA 94555	×	EMRL 15/109
18	ICMA 94555	×	ISK-51
19	HMS 69A	×	SGP-10-107
20	HMS 69A	×	R-line 17
21	HMS 04888A	×	R-line 17
22	HMS 04888A	×	SGP-10-107
23	HMS 04888A	×	EMRL-15-109
24	HMS 48A	×	R-line 17
25	HMS 48A	×	SGP-10-107
26	HMS 48A	×	78/711
27	HMS 48A	×	EMRL-15-109
28	HMS 02333	×	R-line 2
29	HMS 02333	×	R-line 3
30	HMS 02333	×	R-line 17
31	HMS 02333	×	R-line 49
32	HMS 02333	×	ISK-51
33	ICMA 97111	×	R-line 2
34	ICMA 97111	×	R-line 17
35	ICMA 97111	×	R-line 3
36	ICMA 97111	×	R-line 32
37	ICMA 97111	×	R-line 49
38	ICMA 97111	×	ISK-51
39	ICMA 97111	×	78/711

3.4 Experiment 3: Screening of F_1 hybrids for morpho-phenological and biochemical traits and blast disease

3.4.1 Experimental material

In this experiment, the F_1 hybrids developed during *summer*, 2022 and 4 released hybrids (used as check) (HHB 67 improved, HHB 197, HHB 226, HHB 299) were evaluated during *kharif* season 2022.

3.4.2 Experimental design and layout plan

- Experimental design : Randomized Block Design
- Replications : Two
- Number of rows : Three
- Row length : 4 m
- Row to row distance : 0.45 m
- Plant to plant distance : 10-12 cm
- Season : *kharif* 2022
- Location : Research Area, Bajra Section, Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar

3.4.3 Observations recorded in the investigation

The morpho-phenological traits and yield attributes were recorded in F_1 hybrids on five randomly selected competitive plants from each entry similar as done in Experiment **3.2.3.**

3.4.4 Screening and scoring for the blast disease

All the pearl millet hybrids were screened for blast disease under natural field conditions similarly as done in Experiment 3.2.4.

3.4.5 Biochemical traits

Activities of the pathogenesis related enzymes were studied in the F_1 hybrids. For this, leaf samples were collected at 1-, 3-, and 7-days post inoculation of *P. grisea* spore suspension.

3.4.5.1 Phenylalanine Ammonia Lyase (PAL) Activity

PAL activity was estimated following the method of Geetha *et al.* (2005)

a) Reagents

- Phenylalanine
- Sodium borate buffer containing 5mM β-mercaptoethanol
- Trans-cinnamic acid

b) Procedure

i) Extraction

Five hundred mg leaves were homogenized in 5 ml of cold 25 mM borate HCl buffer having pH 8.8 and containing 5 mM β-mercaptoethanol. The homogenate was centrifuged for 15 minutes at 15,000 rpm at 4℃ and supernatant was collected and used as the enzyme source.

ii) Estimation

The assay mixture comprised of 0.1 ml of enzyme extract, 0.5 ml of borate buffer and 1.3 ml water. The reaction was initiated by adding 1 ml of 12 mM L-phenylalanine. The reaction mixture was incubated at 32℃ for 1 hour and the reaction was stopped by adding 0.5 ml of 2N HCl. The absorbance was measured at 290 nm. The estimation of concentration of trans-cinnamic acid was done from the standard curve. The enzyme activity was expressed as µmol trans-cinnamic acid/hour/g fresh weight.

Calculations

PAL (µmole/h/g) = $\frac{c \times D}{MW}$

where,

C = Concentration of trans cinnamic acid established from the calibration curve in µg/ml

D = Dilution factor

MW = Molecular weight of phenylalanine

3.4.5.2 Peroxidase (POX) Activity

POX activity was estimated following the method of Hammerschmidt *et al.* (1982)

a) Reagents

- 100 mM Sodium Phosphate Buffer, pH 7.0
- 10 mM H_2O_2 solution
- 20 mM Guiacol

b) Procedure

i) Extraction

Five hundred mg leaves were homogenized in 1.5 ml of 50 mM sodium phosphate buffer having pH 7.0 and containing 100 µl of 2 mM EDTA, 200 µl of 4% polyvinylpyrrolidone (PVP), 200 µl of 5 mM β-mercaptoethanol and 1 ml of phosphate buffer. The homogenate was centrifuged at 30000 rpm for 30 minutes at 4°C. Supernatant was collected and used as the enzyme source.

ii) Estimation

The assay mixture comprised of 0.4 ml of enzyme extract, 1.5 ml of sodium phosphate buffer and 0.12 ml of H_2O_2 solution. The reaction was initiated by adding 0.480 ml of guaiacol solution. The increase in absorbance was noted at 470 nm for 3 minutes at 1 minute interval. The reaction mixture having the guaiacol solution replaced by the phosphate buffer served as blank. Results were expressed as µmol/min/g.

3.4.5.3 Lipoxygenase (LOX) Activity

LOX activity was determined following the method of Borthakur *et al.* (1987)

a) Reagents

- Linoleic acid
- Tween 20
- 1 M Borate buffer, pH 9.0
- 1 N NaOH
- 0.2 M Sodium phosphate buffer, pH 6.5
- 50 mM Acetate buffer, pH 4.2

b) Procedure

i) Extraction

One gram leaf sample was homogenized with 0.2 M sodium phosphate buffer containing 1% PVP, 0.04% sodium metabisulfite and 0.1% TritonX-100 in a pre-chilled pestle and mortar. The homogenate was centrifuged at 9000 rpm for 20 minutes at 4°C. The supernatant was collected and used as the enzyme source.

ii) Preparation of substrate

Linoleic acid substrate was prepared using the method of Axelrod *et al.* (1981). 0.5 ml of Tween-20 was dissolved in 10 ml of 1 M borate buffer and 0.5 ml of linoleic acid was added drop by drop to the borate buffer containing Tween-20. The contents were slowly and thoroughly mixed to disperse the linoleic acid into a fine emulsion. Then 1.3 ml 1N NaOH was added. The mixture was again agitated until a transparent clear solution was obtained. The resultant solution was then mixed with 90 ml borate buffer. Final volume was made to 200 ml with distilled water.

iii) Estimation

The assay mixture comprised of 60 µl of linoleic acid substrate, 2.5 ml of 50 mM acetate buffer and 0.1 ml of enzyme extract. The reaction was initiated by adding the enzyme. Increase in the absorbance was monitored at 234 nm for 2 minutes. One unit of enzyme activity was defined as the enzyme required to increase 0.1-unit absorbance/min. Results were expressed as units/g.

3.5 Statistical analysis

The mean replicated data recorded for different quantitative characters was subjected to the various statistical procedures using OPSTAT software available at *http://www.hau.ac.in.*

3.5.1 Analysis of variance (ANOVA)

ANOVA was performed for the recorded observations on different traits. The model adopted for variance analysis was:

$$\mathbf{Y_{ij} = \mu + \alpha_i + \beta_j + e_{ij}}$$

where,

Y_{ij} = Observation of ith treatment and jth

µ = General mean

α_i = ith treatment effect

β_j = jth block effect

e_{ij} = random error associated with the ith treatment and jth block

The assumptions of the model are as follows:

a) All the observations should be independent

b) The different effects in the model should be additive

c) Error involved in the population should be normally distributed and independent with mean zero and variance

Source of variation (S.V)	Degree of freedom (d.f)	Sum of square (SS)	Mean sum of square (MS)	Expected mean square (EMS)	F calculated
Replication	(r-1)	SSr	MSr	σ^2e +g σ2 r	MSr/ MSe
Genotype	(g-1)	SSg	MSg	rσ2 g + σ2 e	MSg/ MSe
Error	(r-1) (g-1)	Sse	Mse		
Total	gr-1				

where,

r = Number of replications

g = Number of treatments (genotypes)

MSr = Mean sum of squares due to replications

MSg = Mean sum of squares due to genotypes

MSe = Mean sum of squares due to error

σ^2g = Genotypic variance of character Xi

σ^2r = Variance due to replications

σ^2e = Error variance of character Xi

The genotypic and phenotypic variances were calculated using the following formula:

Genotypic variance (σ^2g) = $\frac{MSg-MSe}{r}$

Phenotypic variance (σ^2p) = σ^2g + σ^2e

Parameters of ANOVA

(i) Mean

Mean was calculated by dividing the sum of all the values by the number of corresponding observations.

$$\bar{X} = \frac{1}{n}\sum_{i=1}^{n} X_i$$

where,

$\bar{X}$ = Simple mean

X_i = Individual value

n = Number of observations

ii) Standard error of difference SE $_{(d)}$

Standard error difference between the two means was calculated with the help of error mean square values from the ANOVA table

$$\boldsymbol{S.E._{(d)}} \pm= \sqrt{\frac{\boldsymbol{2MSe}}{\boldsymbol{r}}}$$

where,

MSe = Error mean square

SE = Standard error

r = number of replications

iii) Critical difference

Critical difference was calculated in order to compare the means of the different 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.

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

iv) Range

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

v) Coefficient of variation (C.V.)

To compare the magnitude of variance among the various traits, the coefficient of variation is suitable as it is a standardized form and was computed by the method of Burton and Devane (1953).

$$\boldsymbol{Genotypic\ coefficient\ of\ variation\ (GCV\%) = \frac{\sqrt{\sigma^2 g}}{\bar{X}} \times 100}$$

$$\boldsymbol{Phenotypic\ coefficient\ of\ variation\ (PCV\%) = \frac{\sqrt{\sigma^2 P}}{\bar{X}} \times 100}$$

where,

$\boldsymbol{\bar{X}}$ = General mean of particular character

$\boldsymbol{\sigma^2 g}$ = Genotypic variance

$\boldsymbol{\sigma^2 P}$ = Phenotypic variance

The phenotypic and genotypic coefficient of variation were categorized according to the methodology of Sivasubramanian and Menon (1973): i.e.,

0-10% = Low **10-20% = Moderate** **>20% = High**

vi) Heritability

Calculation of heritability was done as the ratio of genotypic variance (σ^2g) to the phenotypic variance (σ^2p) and was expressed in form of percentage (Hanson *et al.*, 1956):

$$\textbf{Heritability, } \boldsymbol{h^2} \textbf{ (bs) = } \boldsymbol{\sigma^2 g / \sigma^2 \times 100}$$

The calculated heritability was categorized into three major groups:

0-30 = Low **30-60 % = Moderate** **>60 % = High**

vii) Expected genetic advance (GA)

Genetic advance was worked out by adopting the following formula given by Johanson *et al.* (1955):

$$\textbf{Genetic advance (GA)} = k\times h^2(bs)\times \sigma 2p$$

where,

h^2 (bs) = Heritability in broad sense

k = Selection differential (= 2.06) at 5% intensity of selection

σ^2p = Phenotypic standard deviation

viii) Genetic advance as per cent of mean (GA%M)

Genetic advance as per cent of mean for each trait was calculated by the following formula:

$$\textbf{Genetic Advance as \% of mean} = GA/\bar{X} \times 100$$

where,

GA = Genetic advance

$\bar{X}$ = General mean

The genetic advance as per cent of mean was categorized as below:

0-10 = Low **10-20 % = Moderate** **>20 % = High**

3.5.2 Correlation coefficient analysis

To determine the degree of association in the various characters among the yield parameters and yield itself, the correlation coefficients were calculated.

Both the genotypic and phenotypic coefficients of correlation between the two characters were determined by the methodology of Al-jibouri *et al.* (1958).

$$r_p(xy) = \frac{Cov_p(xy)}{\sqrt{\sigma_p^2(x)\sigma_p^2(y)}}$$

$$r_g(xy) = \frac{Cov_g(xy)}{\sqrt{\sigma_g^2(x)\sigma_g^2(y)}}$$

where,

r_g(xy) and **r_p(xy)** are genotypic and phenotypic correlation coefficients respectively

$\mathbf{Cov_g}$ and $\mathbf{Cov_p}$ are genotypic and phenotypic covariance of x and y respectively

$\boldsymbol{\sigma}^2\mathbf{g}$ and $\boldsymbol{\sigma}^2\mathbf{p}$ are the genotypic and phenotypic variance of x (independent trait) and y (dependent trait) respectively.

The calculated value of **'r'** was compared with table **'r'** value with **(n-2)** degrees of freedom at **5%** and **1%** level of significance, where, n refers to number of pairs of observation.

3.5.3 Principal component analysis

It is a multivariate statistical tool for reducing the data from a huge number of correlated variables into a considerably smaller set of new variables with the help of linear combination of variables which accounts for most of the variations present in the actual variables. The principal component is usually estimated either from the correlation matrix or the covariance matrix. When variables are measured in the different units, scale effects can impact the composition of the derived components. In each condition, it becomes necessary to standardize the variables and the correlations matrix is used to overcome the problem. In this investigation, correlation matrix was used to extract the principal components and was done using the software $\text{GRAPES}_{1.0.0}$ available at *kaugrapes.com.*

Supposing the vector of observations $X = (X_1, X_2 \ldots X_p)$ had correlation matrix R. The determinantal equation was solved to obtain the Eigenvalues and related Eigenvectors of the correlation matrix:

$$|\boldsymbol{R} - \boldsymbol{\lambda}\mathbf{I}|\boldsymbol{v} = \mathbf{0}$$

Being (λ_1, v_1), (λ_2, v_2), ……… (λ_p, v_p) the eigenvalues and eigenvector pairs of R With $\lambda_1 \geq \lambda_2 \geq \ldots\ldots\ldots \geq \lambda_p$, the first principal component -Y_1 was calculated as:

$$\mathbf{Y_1 = v_1'X = v_{11}\, X_1 + v_{12}\, X_2 + \ldots\ldots\ldots\ldots + v_{1p}\, X_P}$$

Such that the variance of Y_1 was maximized subject to the constraint that the sum of squared weights was equal to one (i.e., $\sum v_{1j}^2 = 1$). The second principal component was calculated as:

$$\mathbf{Y_2 = v_2'\, X = v_{21}X_1 + v_{22}X_2 + \ldots\ldots\ldots + v_{2p}X_p}$$

This was not correlated with the first principal component. No principal component was correlated to each other and their variances were equal to the eigen values λ_1, λ_2, ……….,λ_p, i.e.,

$$\mathbf{V\,(Y_j) = \lambda_j (j = 1 \ldots\ldots p\,)}$$

The total variances in P variables were calculated as:

$$\mathbf{\sum^{P} = V\,(\,Yj\,) = tr\,(R) = p = \lambda 1 + \lambda 2 + \ldots\ldots. + \lambda p = \sum \boldsymbol{\lambda}_{\boldsymbol{j=1}}^{\boldsymbol{p}}}$$

Therefore, the proportion of the total variance explained by the j^{th} principal component was calculated as:

$$\lambda j / p \ (j = 1 \ldots\ldots p)$$

In view of the above, the standardized principal components were given by:

$$Y^*1 = Y1 / \sqrt{\lambda 1},\ Y^*2 = Y2 / \sqrt{\lambda 2}, \ldots\ldots\ldots\ Y^*p = Yp / \sqrt{\lambda p}$$

Kaiser's (1958) method of eliminating principal components of correlation matrices with Eigen roots less than one was employed to determine the number of principal components to be retained.

3.5.4 Cluster analysis

It was done using the software IndoStat available at *https://www.indostat.org* and includes following components:

i) Euclidean distance

A convenient measure of dissimilarity between the objects r and s can be derived from the Euclidean distance between the two points. This distance is denoted by d^2_{rs}.

where,

$$d^2_{rs} = \sum (Xij - Xsj)^2$$

The quantity d^2_{rs} is referred as the squared Euclidean distance. For two dimensions (p = 2) the distance between objects r and s can be denoted as:

$$d^2_{rs} = (X_{r1} - X_{s1})^2 + (X_{r2} - X_{s2})^2$$

ii) Euclidean distance in matrix form

The given (n x p) data matrix X with (1x p) row vector $x_1, x_2, \ldots x_n$, the square of the Euclidean distance (d^2_{rs}) between r and s can be written as:

$$d^2_{rs} = (X_r - X_s)\ (X_r - X_s), \quad r \times s = 1, 2, \ldots\ldots n.$$

The (n x p) matrix of d^2_{rs} value is often called as squared Euclidean distance matrix.

iii) Standardized Euclidean distance

A disadvantage of Euclidean distance is a measure of the proximity of its sensitivity to the scale of measurements. It is possible for one or few variables to dominate the distance measure because of big differences in scale. In general, if the scales of measurement are not common for all the p variables, it is preferable to use a weighted distance given by:

$$\sum w_i (x_{rj} - x_{sj})^2$$

where,

The weight (w_j) reflects the importance of the variable

$$\mathbf{j = 1, 2, \ldots, p.}$$

A special case of the weighted Euclidean distance is the standardized Euclidean distance as given by:

$$\mathbf{d^2_{rs} = \sum (1/s^2\, j)\, (X_{rj} - X_{sj})\, 2 = (X_r - X_s)\, B^{-1}\, (X_r – X_s)\ j=1}$$

where,

s^2_j= 1, 2……, p denotes the variance of the variable X_j over the n objects.

B is the diagonal matrix with diagonal elements given by s^2_j, j = 1, 2…., p. The (p x 1) vectors x_r and x_s denote the observations on the two profiles.

iv) Ward's minimum variance method

The clustering method was initially developed by Ward (1963). Several authors independently proposed its names as minimum variance clustering, sum of squares method (Orloci, 1967) and incremental sum of squares method (Burr and Cislak, 1968). The objects, have been grouped based on a specific principle, which is presumed to be measuring the similarity between the objects. Once the clusters are formed, the objects and their respective distances are presented in the form of a tree diagram which is termed as dendrogram. On X-axis, the objects show up in the same order, as they are included in the clusters and the 'stem' or links between the clusters are drawn on Y-axis whose height depends upon the average distance between the clusters. This technique involves fusion of clusters from the matrix of inter year distances provided as under:

The squared distances of objects ½ d^2_{ij} (ij = 1….n) are computed corresponding to all possible pairs of objects. The pair of objects having the least ½ d^2_{ij} is grouped to form initial cluster, say C (1). D_1 represents the increase in sum of square of the distances from the centroids of the C (1) with the other objects, in addition to ½ d^2_{ij} values of the objects, which are not clustered. The search for new clusters is again carried out on the least values of D_1.

The process of revising the matrix D_i [i = 1,…..] based on D (1) and the search of the fusion clusters is continued until fusion of the clusters is combined into one single cluster. The values of D_i in each step of the fusion provides 'links or distances' between the clusters and objects through which the dendrogram can be constructed. In each fusion step, the matrix D is revised in the form of an Index I, which can be calculated as:

$$\mathbf{1\ [C\ (i), C\ (j)] = \frac{n(i)n(j)}{n(i) +\ n(j)}\, [x\ (i) -\ x\ (j)]2}$$

Where, x(i) and x(j) are the centroids of the clusters C (i) and C (j) respectively, which comprises of n(i) 26 and n(j) objects. This clustering method has been used in present study.

v) Cluster mean value

The cluster mean of a particular trait is the summation of mean values of the genotypes included in a cluster, divided by the number of genotypes in the same cluster.

CHAPTER – IV

RESULTS

The present study was undertaken to evaluate the pearl millet genotypes for morpho-phenological traits & blast disease and to study the genetic relatedness among them using microsatellite markers. The observations recorded were interpreted statistically and the results obtained are given below:

Experiment 1: Evaluation of pearl millet genotypes for morpho-phenological traits and blast disease

4.1 Parameters of genetic variation

4.1.1 Analysis of Variance

The mean sum of squares (ANOVA) of the genotypes were found to be significant for all the studied parameters. This shows the prevalence of abundant genetic variability in the genotypes. This was in contrast with the replications, where mean sum of squares were non-significant for all the traits. The fitness of all the parameters under study for further analysis was expressed as significant mean sum of squares due to the treatments and analysis of variance for all the parameters studied is given in Table 4.1.

Table 4.1: Analysis of Variance (ANOVA) for morpho-phenological traits and yield attributes in pearl millet genotypes

Mean sum of squares						
Source of Variation	**Degree of freedom (df)**	**PH (cm)**	**EL (cm)**	**ED (cm)**	**LBW (cm)**	**ETPP**
Treatment	59	795.988**	36.096**	0.481**	1.337**	1.211**
Error	59	79.249	3.796	0.076	0.135	0.083

PH=Plant height, EL= Ear length, ED= Ear diameter, LBW= Leaf blade width, ETPP= Effective number of tillers per plant

Mean sum of squares						
Source of Variation	**Degree of freedom (df)**	**DF**	**DM**	**TW (g)**	**DFPP (g)**	**GYPP (g)**
Treatment	59	52.54**	60.088**	3.479**	2401.74**	365.2**
Error	59	7.031	6.669	0.422	7.41	5.457

DF= Days to 50% flowering, DM= Days to maturity, TW= 1000-Grain weight, DFPP= Dry fodder yield per plant, GYPP= Grain yield per plant

4.1.2 Mean, Range, Genotypic Coefficient of Variation (GCV), Phenotypic Coefficient of Variation (PCV), Heritability (h^2) and Genetic Advance as per cent of mean (GA%M)

The mean (Table 4.2), range, GCV, PCV, h^2 and GA%M of different genotypes taken in the study for various parameters were computed and shown in Table 4.3. Graphical representation of all the different parameters is given in Fig 4.1 and Fig. 4.2. Brief description for them is given as under:

Table 4.2: Mean values for morpho-phenological traits and yield attributes in pearl millet genotypes

S.No.	Genotypes	PH (cm)	EL (cm)	ED (cm)	LBW (cm)	ETPP	DF	DM	TW (g)	DFPP (g)	GYPP (g)
1	R-line 2	104	11.4	2.9	3.8	2.3	66	92	5.60	29.1	10.1
2	R-line 3	159	29.0	3.6	4.3	2.2	60	89	6.70	68.8	25.1
3	R-line 4	143	16.0	2.3	2.3	3.4	57	84	5.55	109.1	42.3
4	R-line 5	154	21.5	3.5	4.0	2.9	68	98	7.75	77.4	27.3
5	R-line 6	145	16.9	3.5	2.8	2.2	61	90	6.55	234.3	91.4
6	R-line 11	157	16.0	2.9	2.5	2.3	53	79	9.80	113.0	45.9
7	R-line 16	127	17.0	2.9	2.8	3.4	53	80	5.50	67.2	25.4
8	R-line 17	127	18.4	3.0	4.9	1.8	52	79	7.45	52.1	19.4
9	R-line 18	142	15.4	2.3	1.9	1.9	51	76	6.35	37.0	12.2
10	R-line 21	140	14.8	2.0	2.7	4.6	52	76	5.65	70.7	29.2
11	R-line 24	143	24.8	2.5	2.9	3.7	56	80	6.30	17.7	6.2
12	R-line 25	152	25.9	2.6	2.9	1.8	55	79	6.95	16.6	19.5
13	R-line 31	162	21.7	2.1	2.9	2.7	54	79	6.55	15.3	5.5
14	R-line 33	125	17.8	3.1	3.4	1.5	57	83	6.70	38.1	14.7
15	R-line 36	145	18.2	2.3	2.6	3.0	56	82	5.55	57.8	23.2
16	R-line 37	165	21.5	2.1	2.8	2.8	56	87	5.05	55.5	16.7
17	R-line 44	123	16.8	2.3	2.6	3.6	51	85	8.30	42.8	12.7
18	R-line 71	126	17.9	2.7	2.4	3.4	52	82	4.90	55.1	21.1
19	R-line 75	129	14.9	1.9	2.9	3.5	50	80	6.60	85.3	31.5
20	R-line 187	173	19.2	3.2	5.2	2.5	65	94	6.80	94.7	20.2
21	R-line 8	160	23.0	2.5	5.4	3.4	68	96	8.25	60.0	17.3

22	R-line 10	144	18.9	3.2	4.5	3.5	56	86	8.65	55.6	21.1
23	R-line 12	117	24.9	3.8	3.8	2.4	55	84	7.45	52.1	13.9
24	R-line 13	152	16.5	2.1	2.7	3.7	52	79	7.45	68.0	24.5
25	R-line 14	163	17.6	2.1	3.1	2.9	52	82	7.80	63.5	23.5
26	R-line 15	156	14.1	2.3	2.2	4.0	60	89	5.15	49.4	10.6
27	R-line 19	124	16.8	2.5	3.6	2.9	50	78	7.40	24.1	9.2
28	R-line 22	135	16.5	2.4	4.0	2.6	52	80	5.70	42.8	15.4
29	R-line 30	167	18.3	2.4	3.5	3.3	59	90	7.55	82.6	33.6
30	R-line 32	149	17.6	2.8	3.5	2.5	54	85	7.85	86.9	31.0
31	R-line 34	138	18.0	2.2	3.0	3.0	50	81	5.95	63.0	29.7
32	R-line 39	161	16.9	2.6	2.9	2.6	69	98	6.40	58.4	26.9
33	R-line 40	169	16.3	2.7	2.9	2.8	60	92	6.95	69.3	19.6
34	R-line 42	159	20.3	2.2	3.1	2.7	54	82	6.60	102.7	40.3
35	R-line 62	141	20.5	2.2	2.5	4.5	51	79	6.50	94.6	38.5
36	R-line 63	156	25.1	3.8	4.0	1.1	65	92	8.85	72.8	17.9
37	R-line 64	144	26.0	3.4	3.4	2.5	55	84	9.75	41.9	13.4
38	R-line 66	137	28.5	1.9	3.7	2.6	51	81	6.95	35.9	15.0
39	R-line 67	122	22.8	2.8	4.7	2.4	57	84	8.25	95.2	34.6
40	R-line 68	146	31.5	2.8	3.9	3.4	52	85	10.20	112.0	33.9
41	ICMB 843-22	107	14.9	2.1	1.9	3.6	54	86	6.95	63.1	20.4
42	ICMB 89111	134	18.8	2.1	2.3	3.9	60	90	7.00	13.0	6.5
43	ICMB 95444	110	15.9	2.1	2.9	2.8	55	84	8.35	26.4	11.9
44	ICMB 04888	84	14.5	2.8	2.8	3.4	53	86	9.60	66.4	19.9
45	ICMB 97111	140	20.5	2.9	2.9	2.6	52	82	6.70	62.1	23.3
46	ICMB 02333	127	16.8	3.2	2.4	1.5	50	79	5.90	47.4	15.5
47	ICMB 04999	114	19.2	2.6	3.2	3.4	55	84	5.90	34.4	10.0
48	H77/833-2-202	138	19.4	2.1	2.0	3.7	51	81	7.30	64.1	23.4
49	HTP 94/54	198	29.2	2.5	3.4	3.0	51	79	10.35	69.5	27.6
50	HBL-11	117	14.1	2.3	3.8	2.3	54	81	5.55	24.9	12.3
51	AC 04/13	145	20.3	2.6	2.7	3.5	52	79	6.95	92.5	42.3
52	G73-107	144	19.3	2.3	2.8	4.3	56	84	7.55	57.1	19.8
53	H77/29-2	146	17.6	2.0	3.0	3.9	52	80	5.95	34.1	13.4

54	H13/0001	119	18.2	2.9	4.4	3.6	63	93	8.00	79.0	24.5
55	H90/4-5	139	17.6	2.4	3.1	4.3	50	79	5.75	64.9	16.6
56	HMS 70B	135	27.0	2.4	2.4	2.8	67	95	5.35	11.2	4.4
57	HMS 23B	139	16.5	2.1	2.9	3.6	52	80	6.20	36.1	15.2
58	HMS 58B	103	21.3	2.1	2.7	2.0	58	83	6.00	21.2	6.8
59	HMS 33B	121	18.9	2.1	2.5	2.7	54	83	8.30	36.4	8.3
60	EBL-12-237	155	22.4	2.9	4.9	4.3	54	82	8.20	91.4	30.9
Grand mean		**140**	**19.4**	**2.5**	**3.2**	**3.0**	**55.0**	**83.0**	**7.0**	**61.0**	**22.2**
C.D. @ 5%		17.9	3.9	0.6	0.7	0.6	5.3	5.2	1.3	5.5	4.7
SE(m)		6.3	1.4	0.2	0.3	0.2	1.9	1.8	0.5	1.9	1.7
SE(d)		8.9	1.9	0.3	0.4	0.3	2.7	2.6	0.6	2.7	2.3
C.V.		6.3	10.0	10.8	11.5	9.7	4.8	3.1	9.2	4.5	10.6

PH= Plant height, EL= Ear length, ED= Ear diameter, LBW= Leaf blade width, ETPP= Effective number of tillers per plant, DF= Days to 50% flowering, DM= Days to maturity, TW= 1000-Grain weight, DFPP= Dry fodder yield per plant, GYPP= Grain yield per plant

Table 4.3: Estimates of genetic variability parameters [mean ± SE (m), range, GCV, PCV, h^2 and genetic advance as percent of mean] for morpho-phenological traits and yield attributes in pearl millet genotypes

Characters	Mean ± SE	Range	GCV (%)	PCV (%)	Heritability (%)	GA%M
PH	140±6.3	84.0-198.0	13.5	14.9	81.8	25.1
EL	19.4±1.4	11.4-31.5	20.6	22.9	80.9	38.3
ED	2.5±0.2	1.9-3.8	17.6	20.6	72.6	30.9
LBW	3.2±0.3	1.9-5.4	24.3	26.9	81.6	45.3
ETPP	3.0±0.2	1.1-4.6	25.2	27.0	87.1	48.5
DF	55.0±1.9	50-69	8.6	9.8	76.3	15.5
DM	83.0±1.8	76-98	6.1	6.8	80.0	11.3
TW	7.0±0.5	4.90-10.35	17.5	19.8	78.3	32.1
DFPP	61.0±1.9	11.2-234.3	56.6	56.8	99.3	116.4
GYPP	22.2±1.7	4.4-91.4	60.9	61.8	97.0	123.6

PH= Plant height, EL= Ear length, ED= Ear diameter, LBW= Leaf blade width, ETPP= Effective number of tillers per plant, DF= Days to 50% flowering, DM= Days to maturity, TW= 1000-Grain weight, DFPP= Dry fodder yield per plant, GYPP= Grain yield per plant

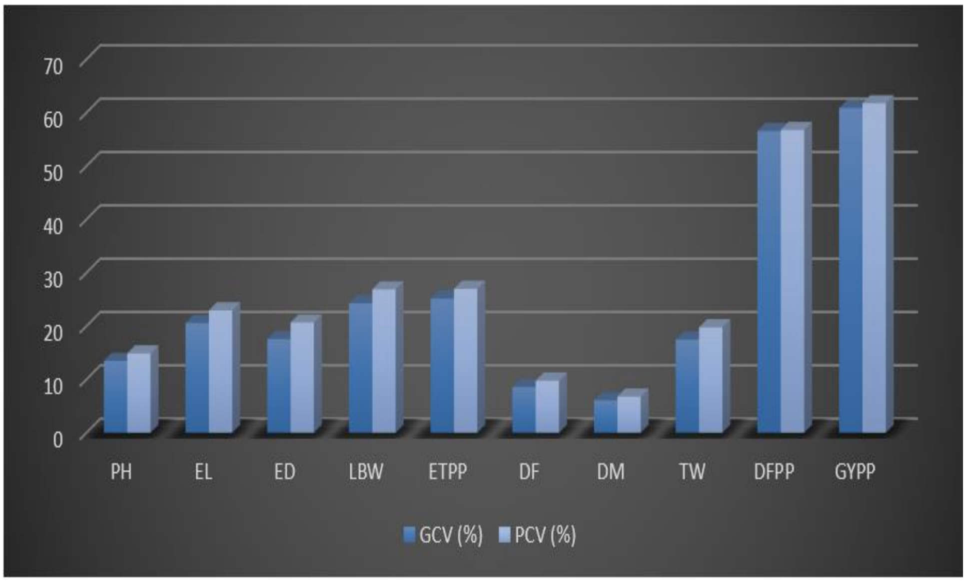

Fig. 4.1: Estimates of GCV (%) and PCV (%) for morpho-phenological traits and yield attributes in pearl millet genotypes

PH= Plant height, EL= Ear length, ED= Ear diameter, LBW= Leaf blade width, ETPP= Effective number of tillers per plant, DF= Days to 50% flowering, DM= Days to maturity, TW= 1000-Grain weight, DFPP= Dry fodder yield per plant, GYPP= Grain yield per plant

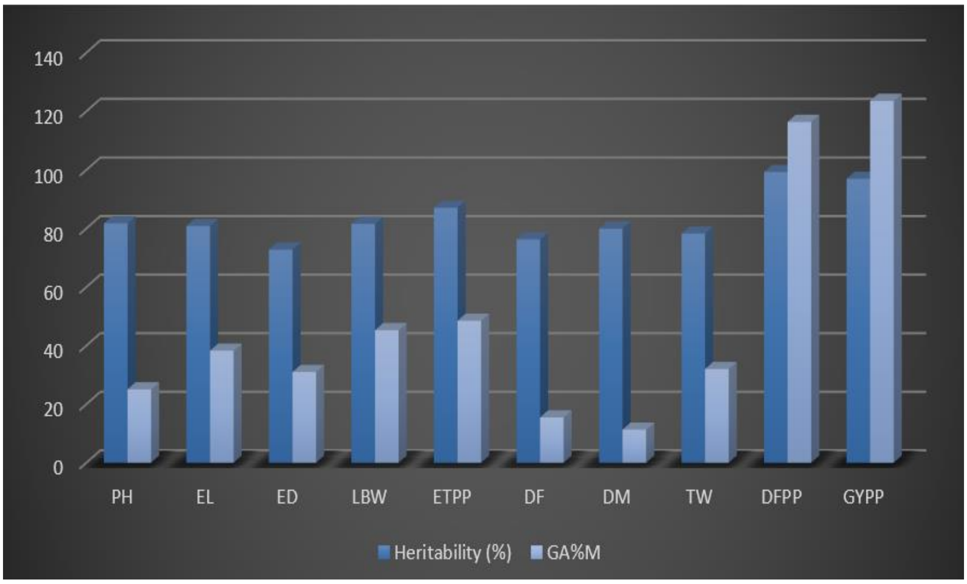

Fig. 4.2: Estimates of h^2 (%) and GA%M for morpho-phenological traits and yield attributes in pearl millet genotypes

PH= Plant height, EL= Ear length, ED= Ear diameter, LBW= Leaf blade width, ETPP= Effective number of tillers per plant, DF= Days to 50% flowering, DM= Days to maturity, TW= 1000-Grain weight, DFPP= Dry fodder yield per plant, GYPP= Grain yield per plant

4.1.2.1 Plant Height (cm)

Plant height of the genotypes under study ranged from 84 cm to 198 cm having mean value of 140±6.3 cm. HTP 94/54 showed the maximum height of 198 cm and the minimum plant height 84 cm was shown by ICMB 04888. Both PCV (14.9%) and GCV (13.5%) estimates were found to be moderate for this trait. High heritability (81.8%) with high genetic advance as per cent of mean (25.1%) was observed (Table 4.3).

4.1.2.2 Ear Length (cm)

The range estimates of this trait varied from 11.4 cm to 31.5 cm with a mean value of 19.4±1.4 cm. The R-line 2 showed the minimum ear length (11.4 cm) whereas maximum length 31.5 cm was recorded by the line R-line 68. GCV (20.6%) and PCV (22.9%) were high for this trait. High heritability (80.9%) with high genetic advance as per cent of mean (38.3%) was observed for this trait which showed the prevalence of additive gene action effects (Table 4.3).

4.1.2.3 Ear Diameter (cm)

Mean value of ear diameter was computed 2.5±0.2 cm ranging from 1.9 cm to 3.8 cm. The R-line 66 and R-line 75 showed minimum ear diameter (1.9 cm) while the lines R-line 12 and R-line 63 showed maximum ear diameter (3.8 cm). PCV (20.6%) was found high whereas, GCV (17.6%) was computed moderate for this trait. High heritability (72.6%) with high genetic advance as per cent of mean (30.9%) was perceived for this trait which showed the importance of additive gene action effects for the selection of this trait (Table 4.3).

4.1.2.4 Leaf Blade Width (cm)

The mean value of leaf blade width was 3.2±0.3 cm ranging from 1.9 cm to 5.4 cm. Estimates of both GCV (24.3%) and PCV (26.9%) were found high. The R-line 18 and ICMB 843-22 showed minimum leaf blade width (1.9 cm) and R-line 8 showed the maximum leaf blade width (5.4 cm). High heritability (81.6%) with high genetic advance as per cent of mean (45.3%) was observed for this trait (Table 4.3).

4.1.2.5 Effective Number of Tillers/Plant

Effective number of tillers per plant ranged from 1.1 to 4.6 with general mean value of 3.0±0.2. The R-line 63 showed minimum number of effective tillers (1.1) and R-line 21 showed the maximum number of effective tillers (4.6). Both GCV (25.2%) and PCV were computed high (27.0%) for this trait. High heritability (87.1%) with high genetic advance as per cent of mean (48.5%) was observed for this trait (Table 4.3).

4.1.2.6 Days to 50% Flowering

The mean value of days to 50% flowering was 55.0±1.9 days ranging from 50 to 69 days. Estimates of both GCV (8.6%) and PCV (9.8%) were found low. The R-line 75, R-line 19, R-line 34, ICMB 02333 and H90/4-5 were the earliest to flower at 50 days, while the line R-line 39 flowered late at 69 days. The trait was having high heritability (76.3%) along with moderate genetic advance as per cent of mean (15.5%) (Table 4.3).

4.1.2.7 Days to Maturity

The mean value of days to maturity was 83.0±1.8 days ranging from 76 to 98 days. Estimates of both GCV (6.1%) and PCV (6.8%) were found low. The R-line 18 and R-line 21 were the earliest to mature in 76 days, while the line R-line 5 and R-line 39 matured late in 98 days. The trait was having high heritability (80.0%) and moderate genetic advance as per cent of mean (11.3%) (Table 4.3).

4.1.2.8 1000-Grain Weight (g)

Wide genetic variation from 4.90 g to 10.35 g with mean 7.0±0.5 was found for this trait. The minimum value was recorded in R-line 71 (4.90 g) while the maximum weight was recorded in the line HTP 94/54 (10.35 g). Moderate GCV and PCV estimates were computed 17.5% and 19.8% respectively. High heritability (78.3%) as well as high genetic advance as per cent of mean (32.1%) was exhibited by this trait (Table 4.3).

4.1.2.9 Dry Fodder Yield/Plant (g)

The trait exhibited significant variation ranging from 11.2 g to 234.3 g with a mean value of 61.0±1.9. The R-line 6 showed maximum dry fodder yield of 234.3 g while the line HMS 70B showed the minimum dry fodder yield of 11.2 g. Both PCV (56.8%) and GCV (56.6%) estimates were high for this trait. High heritability (99.3%) as well as high genetic advance as per cent of mean (116.4%) was found for this character (Table 4.3).

4.1.2.10 Grain Yield/Plant (g)

Mean value of grain yield per plant of selected lines was 22.2±1.7 g ranging from 4.4 g to 91.4 g. The line HMS 70B showed the minimum grain yield of 4.4 g. The maximum grain yield per plant of 91.4 g was recorded by the R-line 6. Both computed PCV (61.8%) and GCV (60.9%) estimates were high for this trait. High heritability (97.0%) as well as high genetic advance as per cent of mean (123.6%) was exhibited by this trait (Table 4.3).

4.2 Correlation coefficient analysis

The relationship between the characteristic (grain yield), its related component traits, and among themselves is critical for any selection programme. Since selection for one character can result in undesirable changes in the other linked traits, the main objective of studying correlation is to ascertain the fitness of distinct characters for indirect selection. Because the yield is a highly complicated quantitative feature that is strongly influenced by environment, any direct selection efforts remain unsuccessful.

There is a significant intrinsic association between the various features, as shown by the magnitudes of the genotypic level correlation coefficients for almost all the traits being higher than the phenotypic level correlation coefficients for the same traits. Table 4.4 contains the calculated phenotypic and genotypic correlation coefficients between grain yield and other component variables as well as among themselves.

Table 4.4: Estimates for Genotypic (above diagonal) and Phenotypic (below diagonal) Correlation Coefficients for morpho-phenological traits and yield attributes in pearl millet genotypes

Traits	PH	EL	ED	LBW	ETPP	DF	DM	TW	DFPP	GYPP
PH	1	0.365^{**}	0.04	0.149	0.025	0.208^{*}	0.166	0.127	0.290^{**}	0.275^{**}
EL	0.357^{**}	1	0.292^{**}	0.329^{**}	-0.174	0.108	0.103	0.398^{**}	-0.032	-0.042
ED	0.063	0.275^{**}	1	0.511^{**}	-0.533^{**}	0.418^{**}	0.423^{**}	0.297^{**}	0.338^{**}	0.240^{**}
LBW	0.167	0.324^{**}	0.479^{**}	1	-0.204^{*}	0.395^{**}	0.388^{**}	0.328^{**}	0.117	0.011
ETPP	0.02	-0.151	-0.420^{**}	-0.180^{*}	1	-0.217^{*}	-0.107	-0.093	0.063	0.045
DF	0.129	0.091	0.337^{**}	0.284^{**}	-0.209^{*}	1	0.927^{**}	0.005	0.065	0.003
DM	0.122	0.089	0.330^{**}	0.258^{**}	-0.107	0.914^{**}	1	0.143	0.156	0.044
TW	0.142	0.335^{**}	0.298^{**}	0.339^{**}	-0.055	-0.057	0.045	1	0.222^{*}	0.147
DFPP	0.260^{**}	-0.025	0.297^{**}	0.113	0.058	0.064	0.147	0.198^{*}	1	0.956^{**}
GYPP	0.256^{**}	-0.043	0.197^{*}	0.014	0.042	-0.025	0.02	0.142	0.939^{*}	1

PH= Plant height, EL= Ear length, ED= Ear diameter, LBW= Leaf blade width, ETPP= Effective number of tillers per plant, DF= Days to 50% flowering, DM= Days to maturity, TW= 1000-Grain weight, DFPP= Dry fodder yield per plant, GYPP= Grain yield per plant

4.2.1 Plant Height (cm)

Plant height was found to be significantly and positively correlated with ear length (rg=0.365, rp=0.357), dry fodder yield per plant (rg= 0.290, rp= 0.260) and grain yield per plant (rg= 0.275, rp= 0.256). It also showed significant correlation with days to 50% flowering at genotypic level (rg= 0.208).

4.2.2 Ear Length (cm)

Ear length was found to be significantly and positively correlated with plant height (rg= 0.365, rp= 0.357), ear diameter (rg= 0.292, rp= 0.275), leaf blade width (rg= 0.329, rp= 0.324) and 1000-grain weight (rg= 0.398, rp= 0.335).

4.2.3 Ear Diameter (cm)

Ear diameter was found to be significant and positively correlated with ear length (rg= 0.292, rp= 0.275), leaf blade width (rg= 0.511, rp= 0.479), days to 50% flowering (rg=0.418, rp=0.337), days to maturity (rg= 0.423, rp= 0.330), 1000-grain weight (rg= 0.297, rp= 0.298), dry fodder yield per plant (rg= 0.338, rp= 0.297) and grain yield per plant (rg=0.240, rp=0.197) but showed negative and significant correlation with effective number of tillers per plant (rg= -0.533, rp= -0.420).

4.2.4 Leaf Blade Width (cm)

Leaf blade width was found to be significantly and positively correlated with ear length (rg= 0.329, rp= 0.324), ear diameter (rg= 0.511, rp= 0.479), days to 50% flowering (rg=0.395, rp=0.284), days to maturity (rg= 0.388, rp= 0.258) and 1000-grain weight (rg= 0.328, rp= 0.339) but showed negative and significant correlation with effective number of tillers per plant (rg= -0.204, rp= -0.180).

4.2.5 Effective Number of Tillers/Plant

Effective number of tillers per plant were found to be significantly and only negatively correlated with ear diameter (rg= -0.533, rp= -0.420), leaf blade width (rg= -0.204, rp= -0.180) and days to 50% flowering (rg= -0.217, rp=-0.209).

4.2.6 Days to 50% Flowering

Days to 50% flowering expressed significant positive correlation with the ear diameter (rg=0.418, rp=0.337), leaf blade width (rg=0.395, rp=0.284) and days to maturity (rg=0.927, rp=0.914) & negative and significant correlation with effective number of tillers per plant (rg=-0.217, rp=-0.209) at both genotypic and phenotypic levels respectively whereas exhibited significant positive correlation with plant height at only genotypic level (rg= 0.208).

4.2.7 Days to Maturity

Days to maturity were found to be significantly and positively correlated with ear diameter (rg= 0.423, rp= 0.330), leaf blade width (rg= 0.388, rp= 0.258) and days to 50% flowering (rg= 0.927, rp= 0.914) at both genotypic and phenotypic levels.

4.2.8 1000-Grain Weight (g)

1000-grain weight was found to be positively and significantly correlated with ear length (rg= 0.398, rp= 0.335), ear diameter (rg= 0.297, rp= 0.298), leaf blade width (rg= 0.328, rp= 0.339) and dry fodder yield per plant (rg= 0.222, rp= 0.198) at both genotypic and phenotypic levels.

4.2.9 Dry Fodder Yield/Plant (g)

Dry fodder yield per plant was found to be positively and significantly correlated with plant height (rg= 0.290, rp= 0.260), ear diameter (rg= 0.338, rp= 0.297), 1000-grain weight (rg= 0.222, rp= 0.198) and grain yield per plant (rg= 0.956, rp= 0.939) at both genotypic and phenotypic levels.

4.2.10 Grain Yield/Plant (g)

Grain yield per plant was found to be positively and significantly correlated with plant height (rg= 0.275, rp= 0.256), ear diameter (rg= 0.240, rp= 0.197), and dry fodder yield per plant (rg= 0.956, rp= 0.939) at both genotypic and phenotypic levels.

4.3 Cluster analysis

The analysis of mean, range, mean sum of squares, skewness, and kurtosis demonstrated substantial variability for all traits, such variation is not a reliable basis for determining the degree of genetic diversity. Non-hierarchical Euclidean cluster analysis was used to solve this issue while also quantifying genetic divergence across groups of genotypes. Progenies were divided into separate clusters based on the minimum Euclidean distance using Ward's minimum variance method (Ward, 1963).

The average distance between and within clusters as well as the cluster mean for various clusters were also calculated. Cluster analysis produced five clusters (Table- 4.5). Cluster V had the maximum number of genotypes i.e., 18 followed by cluster I (17), Cluster III (15), cluster IV (9) and cluster II (1).

Table 4.5: Cluster membership and number of genotypes in each cluster of pearl millet

S.No.	No. of Genotypes	Genotypes
Cluster 1	17	R-line 3, R-line 5, R-line 17, R-line 187, R-line 63, R-line 8, R-line 67, R-line 12, R-line 64, R-line 10, EBL-12-237, H13/001, R-line 68, HTP 94/54, R-line 30, R-line 40, R-line 39
Cluster 2	1	R-line 6
Cluster 3	15	R-line 16, R-line 71, ICMB 04999, R-line 22, HBL-11, R-line 19, HMS 58B, R-line 33, ICMB 02333, R-line 18, R-line 24, R-line 31, R-line 37, R-line 25, R-line 66
Cluster 4	9	R-line 2, ICMB 95444, HMS 33B, R-line 44, ICMB 843-22, ICMB 04888, R-line 15, ICMB 89111, HMS 70B
Cluster 5	18	R-line 36, R-line 21, H90/4-5, H77/29-2, HMS 23B, R-line 75, R-line 34, R-line 13, H77/833-2-202, G73-107, R-line 62, AC 04/13, R-line 4, R-line 14, R-line 42, R-line 32, ICMB 97111, R-line 11

Intra and inter-cluster distance was calculated for the different clusters as shown in the Table 4.6. Intra-cluster distance was maximum in cluster I (4.19) followed by cluster III (3.44), cluster IV (3.31) and cluster V (2.78). Inter-cluster distance was maximum between cluster III and cluster II (9.03) followed by cluster IV and cluster II (8.37). Inter-cluster distance was found minimum between cluster V and cluster III (3.76). Fig. 4.3 demonstrates the cluster diagram showing inter and intra- cluster distances in pearl millet genotypes and Fig. 4.4 shows the dendrogram showing the clustering pattern of pearl millet genotypes.

Table 4.6: Inter and intra-cluster distances in pearl millet genotypes

	Cluster 1	**Cluster 2**	**Cluster 3**	**Cluster 4**	**Cluster 5**
Cluster 1	**4.19**	8.04	5.15	4.99	5.04
Cluster 2		**0.00**	9.03	8.37	7.78
Cluster 3			**3.44**	4.20	3.76
Cluster 4				**3.31**	4.03
Cluster 5					**2.78**

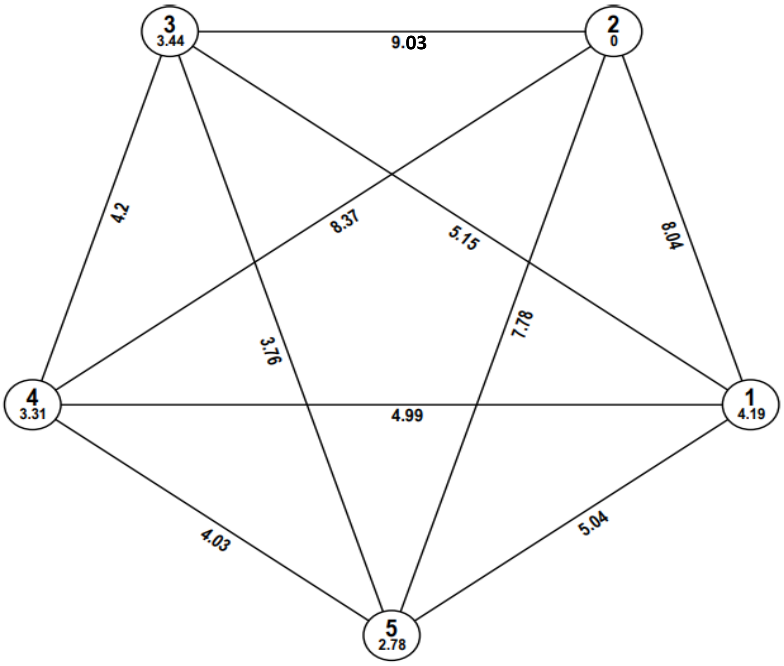

Fig 4.3: **Cluster diagram showing inter and intra-cluster distances in pearl millet genotypes**

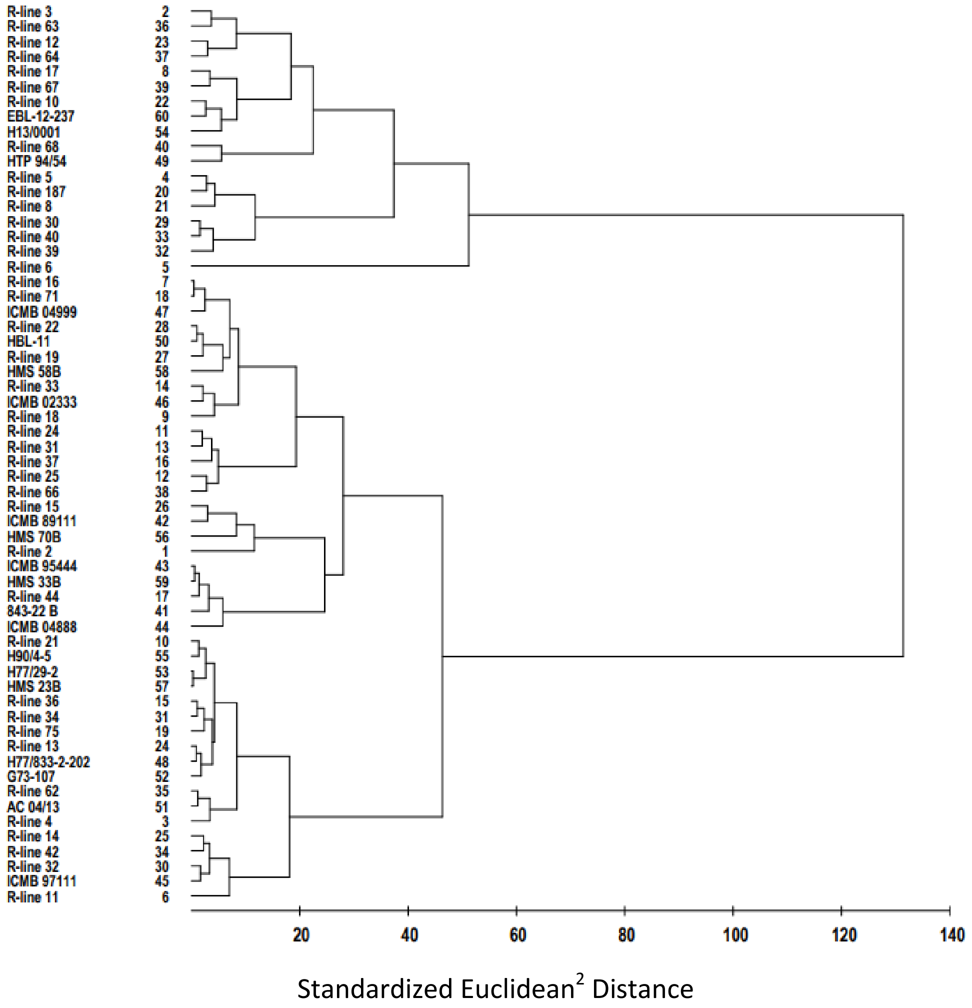

Fig. 4.4: Dendrogram showing the clustering pattern of different pearl millet genotypes

4.4 Principal Component Analysis

Principal component analysis is a technique for determining the relative significance and utility of various variables and genotypes in a data set. It reduces numerous associated variables into a small number of independent principal components that account for the majority of the variations in data. In the present study, principal component analysis was carried out by the method of Sneath *et al.* (1973) and the factors having eigen value more than one were considered (Kaiser, 1958; Jeffers, 1967). The sum of all the eigen values was always equal to the number of variables. In this study, the first four main components had eigen values greater than one and they cumulatively explained 76.44% of the total variation present in original data set (Table 4.7). The first principal component explained 29.99% of the total variability. The second, third and fourth principal components explained 19.91%, 14.94% and 11.59% of the total variability, respectively.

Table 4.7: Total variance explained by different principal components in pearl millet genotypes

Principal components	Eigen value	Variation explained (%)	Cumulative variation explained (%)
1	2.99	29.99	29.99
2	1.99	19.91	49.90
3	1.49	14.94	64.84
4	1.15	11.59	76.44

Varimax rotation was used to perform additional principal component analysis in order to examine character associations with various principal components. Table 4.8 shows the factor loading for different characters after varimax rotation.

Table 4.8: Factor loading after varimax rotation

Variables	PC1	PC2	PC3	PC4
PH (cm)	0.230	-0.192	-0.124	-0.598
EL (cm)	0.258	0.097	-0.516	-0.309
ED (cm)	**0.437**	0.066	-0.084	**0.407**
LBW (cm)	0.374	0.153	-0.213	0.017
ETPP	-0.212	-0.228	0.142	-0.538
DF	0.384	**0.308**	**0.440**	-0.167
DM	0.395	0.250	0.433	-0.192
TW (g)	0.255	-0.100	-0.460	-0.001
DFPP (g)	0.289	-0.577	0.170	0.110
GYPP (g)	0.229	-0.611	0.156	0.121

From Table 4.8, it is clear that first principal component showed maximum positive factor loading for ear diameter (0.437). Principal component 2 showed maximum positive loading for days to 50% flowering (0.308). Principal component 3 also showed high positive loading for days to 50% flowering (0.440). Principal component four showed high loading for ear diameter (0.407). The other major contributors for variations observed in the first principal component were plant height, ear length, leaf blade width, days to 50% flowering, days to maturity, 1000-grain weight, dry fodder yield per plant and grain yield per plant. The variations in PC2 were mainly due to leaf blade width and days to maturity. The variations in PC3 were present mainly through effective number of tillers per plant, days to 50% flowering, dry fodder yield per plant and grain yield per plant. Likewise major contributors to the variations observed in PC4 were dry fodder yield per plant and grain yield per plant.

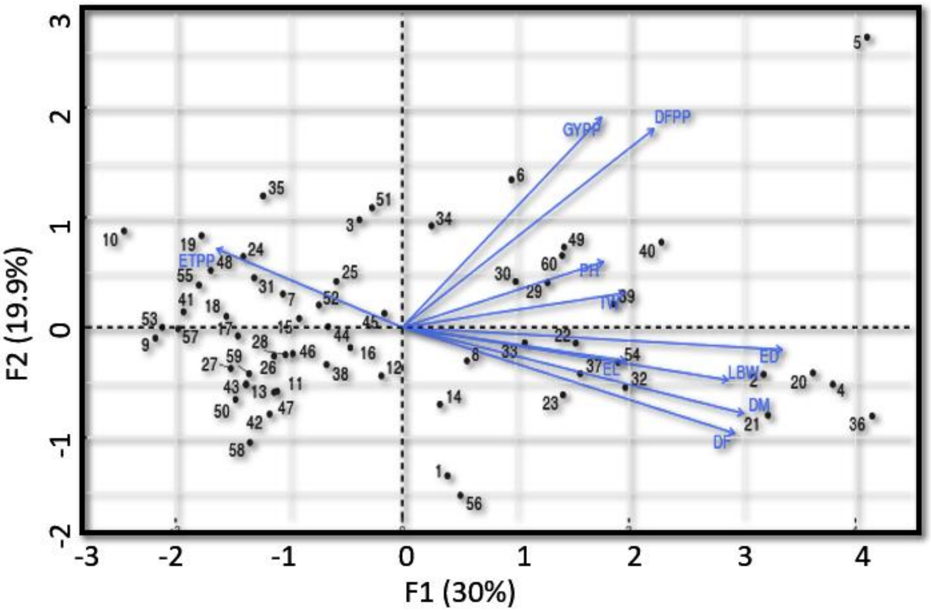

Fig. 4.5: Principal component loading plot of PC-1 and PC-2

A technique for further analysing Principal component data is the PCA biplot, which creates a biplot graph by combining PC scores with loading vectors. The original variable is shown as a vector in the plot (arrow). The more information an arrow offers to the PC, the longer it is, and the higher is the variability it shows. The variables are positively correlated when two vectors form an acute angle whereas, the respective traits are negatively correlated if they form an obtuse angle. The biplot of the genotypes also described their diversity in terms of spatial distance (Figure 4.5). The biplot of genotypes based on PC-1 and PC-2 depicted 49.9% of the total variation. The vector of traits, *viz.* ear diameter, leaf blade width, days to maturity, days to 50% flowering, dry fodder yield per plant, grain yield per plant and effective number of tillers per plant showed longer lengths indicating positive contributions to the components.

The pearl millet genotypes got distributed based on their relative performances with respect to principal component one and two. Principal component one showed high factor loading for plant height, ear length, ear diameter, leaf blade width, days to 50% flowering, days to maturity, 1000-grain weight, dry fodder yield per plant and grain yield per plant. Plant height, 1000-grain weight, dry fodder yield per plant and grain yield per plant were found to be common towards the positive sides of both the principal factors. Genotypes 6, 49, 60, 40, 39, 22, 33, 37, 54, 32, 21, 2 and 20 were clustered towards positive side of PC-1, so they were better in terms of the above-mentioned traits. So, these mentioned genotypes were found superior for the yield parameters.

4.5 Screening studies

The pearl millet genotypes were screened for the blast severity reaction using the 1-9 progressive scale developed by International Rice Research Institute for rice blast. Disease severity was recorded at hard dough stage and the number of genotypes and the estimates of blast severity analysis are given in Table 4.9. The individual blast scores for each genotype are given in Annexure I.

Table 4.9: Estimates of blast severity analysis in pearl millet genotypes

Scale	Disease reaction	Number of genotypes	Genotypes
1.0-2.0	**Highly resistant**	**12**	R-line 4, R-line 21, R-line 44, R-line 75, R-line 10, R-line 12, R-line 15, R-line 32, R-line 34, R-line 42, R-line 62, H13/001
2.1-4.0	**Resistant**	**18**	R-line 2, R-line 3, R-line 17, R-line 8, HMS 23B, HMS 33B, R-line 13, R-line 14, R-line 40, EBL-12-237, R-line 5, R-line 18, R-line 25, R-line 22, R-line 16, ICMB 04888, R-line 19, R-line 63
4.1-6.0	**Moderately resistant**	**13**	R-line 24, R-line 36, R-line 30, AC 04/13, G73-107, H77/29-2, R-line 37, R-line 33, R-line 67, H90/4-5, H77/833-2-202, HBL-11. R-line 11
6.1-8.0	**Susceptible**	**17**	R-line 187, R-line 64, R-line 68, ICMB 843-22, ICMB 04999, R-line 66, ICMB 97111, R-line 6, HTP 94/54, HMS 58B, R-line 31, R-line 71, HMS 70B, R-line 39, ICMB 02333, ICMB 89111, ICMB 95444

Experiment 2: Studying the DNA polymorphism and initiating the crossing program between the selected blast resistant and elite lines

4.6 DNA Polymorphism study in 60 pearl millet genotypes

4.6.1 Isolation of DNA

Genomic DNA was isolated from the young leaves using CTAB extraction method (Saghai-Maroof *et al.*, 1984) with some modifications.

4.6.2 Qualitative and Quantitative estimation of DNA

Quality and quantity of the isolated DNA was estimated using agarose gel electrophoresis and nanodrop. The quantity of DNA obtained from the different plants ranged from 300-800 ng/µl. $A_{260/280}$ ratio ranged from 1.80 to 1.88 indicating that the DNA was free from the contaminants like polysaccharides, polyphenols, RNA, and proteins. The quality of DNA was also estimated by agarose gel electrophoresis. A single high molecular weight band obtained on the gel further confirmed that genomic DNA was intact and free from any kind of enzymatic or mechanical degradation (Plate 4.1).

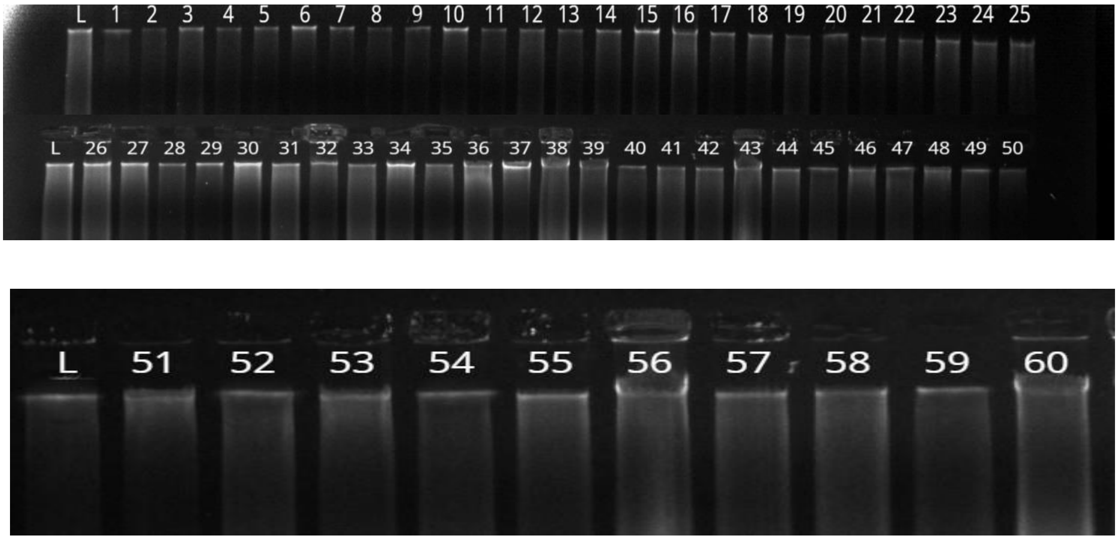

Plate 4.1: Agarose gel (0.8%) showing Genomic DNA; L=Lambda (50 ng), Lane 1-60 represents DNA of 60 pearl millet genotypes.

4.6.3 PCR amplification of genotypes using SSR markers

In the present study, genetic diversity was assessed using 140 SSR markers out of which 38 were found to be polymorphic. They were further used to generate the DNA fingerprint profile of pearl millet genotypes. The SSR banding profile obtained by some of the polymorphic markers are shown in Plate 4.2 and Plate 4.3. Primers amplified a total of 305 alleles which varied from 1 (PSMP 2217) to 16 (Xcump 0016) with a mean of 8.02 alleles per locus. The overall size of the PCR amplified products ranged from 90 bp (PGIRD 46) to 1000 bp (ICMP 3088). Polymorphic Information Content (PIC) values ranged from 0.100 (PSMP 2213) to 0.986 (ICMP 10) (Table 4.10).

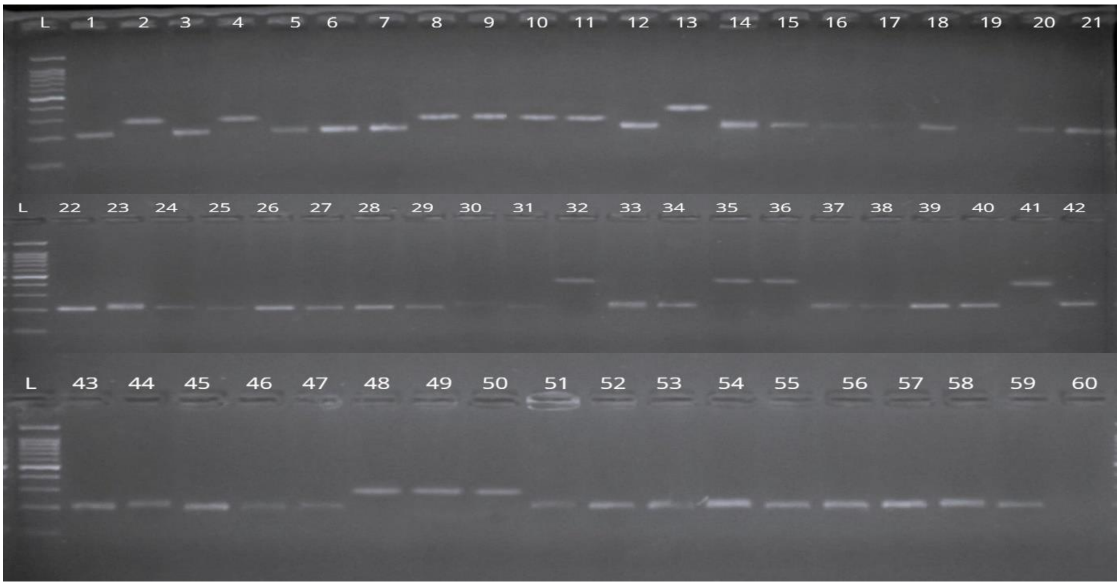

Plate 4.2: SSR banding profile using primer CTM 3; L: 100 bp DNA marker, Lanes 1-60 represents the 60 pearl millet genotypes

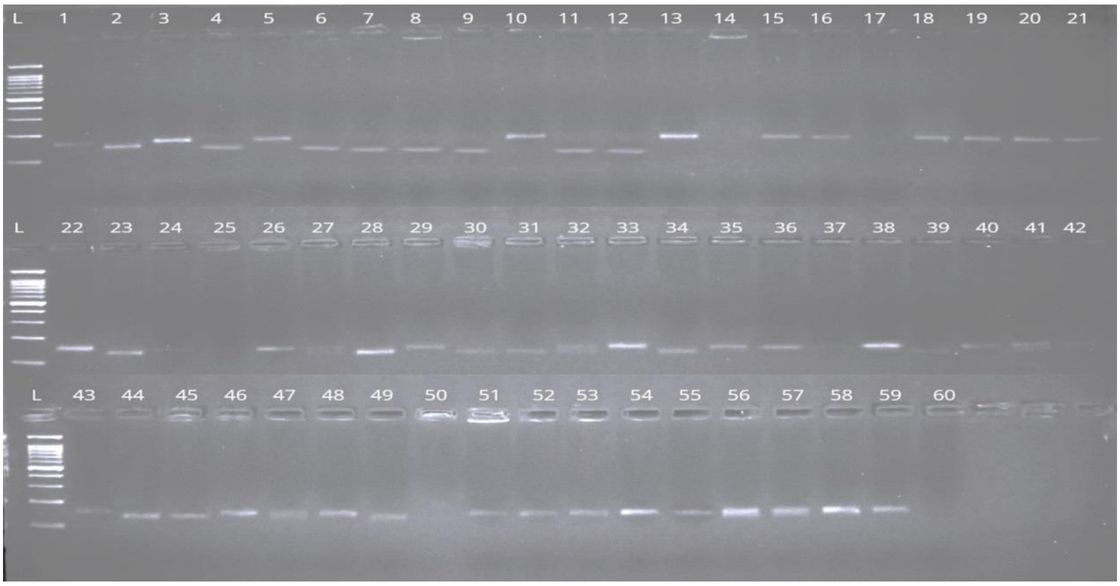

Plate 4.3: SSR banding profile using primer PSMP 20; L: 100 bp DNA marker, Lanes 1-60 represents the 60 pearl millet genotypes

Table 4.10: Amplification results of 38 SSR markers and their PIC values

S.No.	SSR Locus	Amplification Range	PIC
1	PSMP 2209	330-350	0.600
2	PSMP 2008	120-800	0.910
3	PSMP 2027	240-400	0.852
4	PSMP 20	150-200	0.753
5	PSMP 2059	115-125	0.762

6	PSMP 2084	230-500	0.981
7	PSMP 2213	190-200	0.100
8	PSMP 2087	120-140	0.662
9	PSMP 2218	250-800	0.820
10	PSMP 2201	360-510	0.865
11	PSMP 2224	130-180	0.790
12	PSMP 2219	250-300	0.340
13	PSMP 2233	250-900	0.846
14	PSMP 2237	200-500	0.864
15	PSMP 2217	140-150	0.232
16	ICMP 3017	170-240	0.895
17	ICMP 3018	210-225	0.642
18	ICMP 3029	210-250	0.721
19	PSMP 2206	200-600	0.830
20	ICMP 3056	140-160	0.385
21	ICMP3088	140-1000	0.901
22	ICMP 10	300-900	0.986
23	Xcump005	150-200	0.532
24	Xcump 006	150-900	0.839
25	Xcump 009	150-160	0.321
26	Xcump0016	190-900	0.940
27	CTM 3	200-400	0.921
28	CTM 9	180-210	0.345
29	Xcump0018	210-800	0.489
30	CTM-10	160-250	0.782
31	CTM 27	320-600	0.776
32	CTM 55	700-800	0.278
33	CTM 58	280-900	0.354
34	CTM 59	260-265	0.582
35	PGIRD 46	90-100	0.689
36	PGIRD 49	190-400	0.711
37	PGIRD 50	150-220	0.250
38	PGIRD 54	110-170	0.799

4.6.4 Molecular marker based genetic diversity analysis

The SSR diversity data was used for determining the genetic relationship among the 60 pearl millet genotypes using NTSYS-pc software (2.02e). A similarity matrix was made using 'SIMQUAL' subprogram of the software. Then the dendrogram was constructed based on simple matching coefficient using the 'SAHN' sub-program. The 'SAHN' sub-program uses 'UPGMA' algorithm to perform cluster analysis. In this dendrogram (Fig. 4.6), 60 genotypes formed 2 major clusters at a similarity coefficient value of 0.51 which further formed sub clusters and sub-sub clusters. The 1st major cluster consisted of 40 genotypes and the 2nd major cluster comprised of 20 genotypes. First major cluster formed two sub clusters comprising of 38 and 2 genotypes respectively. The second major cluster also formed two sub clusters containing 3 and 17 genotypes respectively. The formation of two major clusters was observed at a similarity coefficient of 0.51 followed by sub-clusters formation at similarity coefficient of 0.57 and so on.

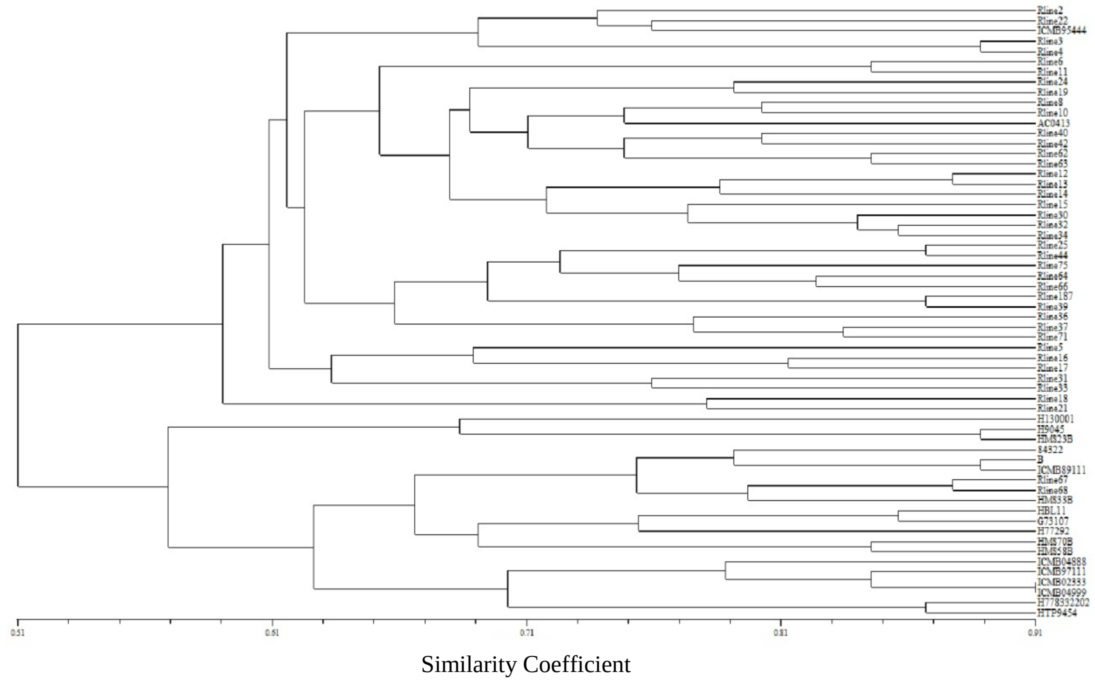

Fig 4.6: UPGMA dendrogram showing relationship among 60 pearl millet genotypes based on similarity matrix data using 38 SSR markers

Furthermore, simple matching matrix was subjected to Principal component analysis (PCA). The groupings of sixty genotypes using PCA analysis in 2-D (Fig. 4.7) and 3-D scaling (Fig. 4.8) followed the same pattern as depicted in the dendrogram.

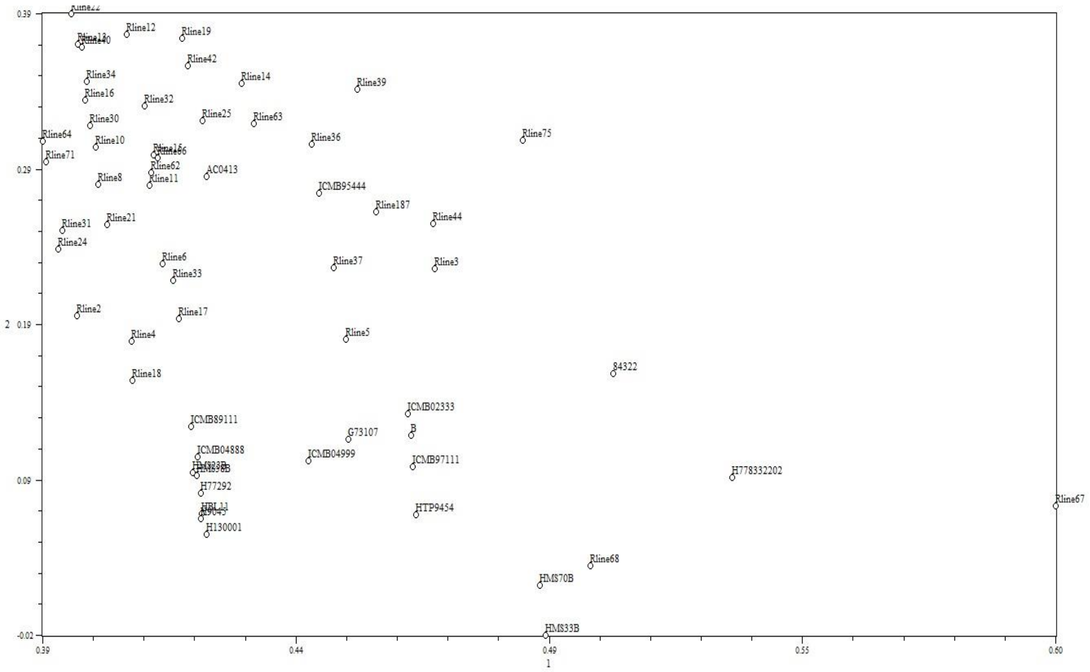

Fig 4.7: Two dimensional PCA (Principal component analysis) scaling of 60 pearl millet genotypes using similarity matrix data of 38 SSR markers

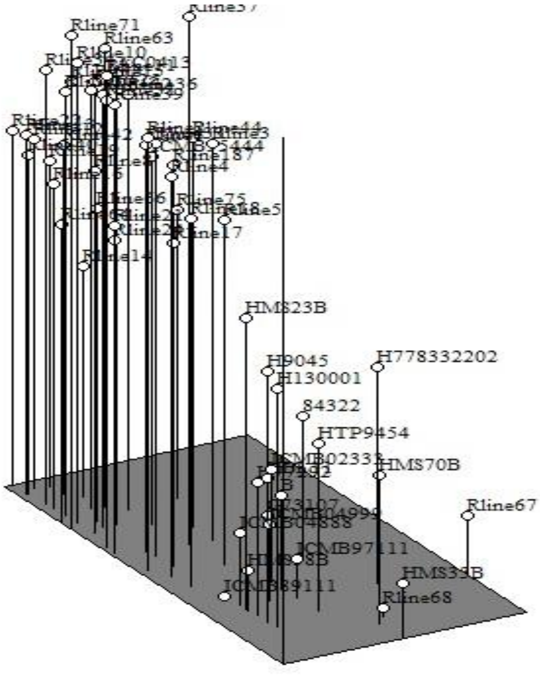

Fig 4.8: Three dimensional PCA scaling of 60 pearl millet genotypes based on 38 SSR markers

4.7 Outcome of the crossing program between the selected blast resistant and elite lines

Out of the crosses initiated, 34 were successful as given in Table 4.11. These F_1 hybrids were further taken as experimental material for Experiment 3.

Table 4.11: List of F_1 hybrids developed

S.No.	♀ parent	×	♂ parent
1	HMS 47A	×	HPT-2-12-32
2	HMS 47A	×	HB15/085
3	HMS 47A	×	R-line 2
4	HMS 47A	×	R-line 10
5	HMS 47A	×	R-line 17
6	HMS 47A	×	R-line 49
7	HMS 47A	×	H78/711
8	HMS 47A	×	SGP-10-107
9	HMS 74A	×	R-line 4
10	HMS 74A	×	78/711
11	ICMA 04999	×	ISK-51
12	ICMA 04999	×	SGP-10-107
13	ICMA 04999	×	EMRL 15/109
14	ICMA 04999	×	R-line 17
15	ICMA 04999	×	78/711
16	ICMA 94555	×	EMRL 15/109
17	HMS 69A	×	SGP-10-107
18	HMS 69A	×	R-line 17
19	HMS 04888A	×	EMRL-15-109
20	HMS 48A	×	R-line 17
21	HMS 48A	×	SGP-10-107
22	HMS 48A	×	78/711
23	HMS 02333	×	R-line 2
24	HMS 02333	×	R-line 3
25	HMS 02333	×	R-line 17
26	HMS 02333	×	R-line 49
27	HMS 02333	×	ISK-51
28	ICMA 97111	×	R-line 2
29	ICMA 97111	×	R-line 17
30	ICMA 97111	×	R-line 3
31	ICMA 97111	×	R-line 32
32	ICMA 97111	×	R-line 49
33	ICMA 97111	×	ISK-51
34	ICMA 97111	×	78/711

Experiment 3: Screening of F_1 hybrids for morpho-phenological and biochemical traits and blast disease

4.8 Parameters of genetic variation:

4.8.1 Analysis of Variance (ANOVA)

For all the investigated parameters, the mean sum of squares (ANOVA) of the hybrids was found significant. This demonstrates the high genetic variability predominance in the hybrids. In contrast, mean sum of squares were not significant for any of the traits in the replications. The fitness of all the parameters under consideration for further analysis was expressed as a significant mean sum of squares due to the treatments, and the analysis of variance for all the parameters investigated is shown in Table 4.12.

Table 4.12: Analysis of Variance (ANOVA) for morpho-phenological traits and yield attributes in pearl millet hybrids

Mean sum of squares						
Source of Variation	**Degree of freedom (df)**	**PH (cm)**	**EL (cm)**	**ED (cm)**	**LBW (cm)**	**ETPP**
Treatment	37	281.414**	11.484**	0.819**	0.341**	4.698**
Error	37	126.859	3.510	0.066	0.062	0.172

PH=Plant height, EL= Ear length, ED= Ear diameter, LBW= Leaf blade width, ETPP= Effective number of tillers per plant

Mean sum of squares						
Source of Variation	**Degree of freedom (df)**	**DF**	**DM**	**TW (g)**	**DFPP (g)**	**GYPP (g)**
Treatment	37	21.79**	28.98**	7.961**	24485.208**	3047.364**
Error	37	2.622	11.023	0.620	372.965	37.627

DF= Days to 50% flowering, DM= Days to maturity, TW= 1000-Grain weight, DFPP= Dry fodder yield per plant, GYPP= Grain yield per plant

4.8.2 Mean, Range, Genotypic Coefficient of Variation (GCV), Phenotypic Coefficient of Variation (PCV), Heritability (h^2) and Genetic Advance as per cent of mean (GA%M)

The mean (Table 4.13), range, GCV, PCV, h^2 and GA%M of the different hybrids taken in the study for various parameters have been computed and shown in Table 4.14. Graphical representation of all the different parameters is given in Fig 4.9 and Fig. 4.10. Brief description for them is given as under:

Table 4.13: Mean values for morpho-phenological traits and yield attributes in pearl millet hybrids

S.No.	**Hybrids**	**PH (cm)**	**EL (cm)**	**ED (cm)**	**LBW (cm)**	**ETPP**	**DF**	**DM**	**TW (g)**	**DFPP (g)**	**GYPP (g)**
1	HMS 47A × HPT-2-12-32	201	25.0	3.2	4.1	3.2	47	73	16.30	206.5	77.0
2	HMS 47A × HB15/085	192	27.0	3.1	4.1	3.3	47	75	10.36	297.4	81.1
3	HMS 47A × R-line 2	195	26.8	3.6	4.5	2.5	46	73	17.46	245.0	88.1
4	HMS 47A × R-line 10	199	30.3	3.1	4.0	2.9	47	78	14.75	222.4	87.5
5	HMS 47A × R-line 17	191	26.0	3.2	3.5	3.3	47	77	15.87	260.0	90.7
6	HMS 47A × R-line 49	202	25.9	3.0	3.5	2.8	48	78	15.80	179.0	65.9
7	HMS 47A × H78/711	205	28.1	3.0	3.2	2.9	47	77	15.61	202.7	78.5
8	HMS 47A × SGP-10-107	206	26.8	3.4	3.8	2.9	48	76	16.83	239.6	92.8
9	HMS 74A × R-line 4	199	32.3	3.5	4.6	5.9	55	86	14.53	179.0	62.4
10	HMS 74A × 78/711	203	27.2	3.2	3.7	4.5	59	89	14.54	240.2	67.7
11	ICMA 04999 × ISK-51	187	25.9	3.0	3.1	3.9	51	82	17.10	201.8	56.0
12	ICMA 04999 × SGP-10-107	182	25.2	3.1	3.5	4.6	48	79	17.22	184.0	59.8
13	ICMA 04999 × EMRL 15/109	188	27.5	3.1	3.2	5.7	54	84	15.38	298.0	105.4
14	ICMA 04999 × R-line 17	181	26.4	3.3	3.7	7.3	50	79	15.39	257.5	91.9
15	ICMA 04999 × 78/711	190	27.3	2.3	3.4	4.8	56	83	15.43	302.5	92.2
16	ICMA 94555 × EMRL 15/109	197	27.6	3.1	3.7	4.4	54	84	15.90	339.0	92.2
17	HMS 69A × SGP-10-107	202	23.9	3.1	3.6	3.3	50	79	16.44	148.0	58.3
18	HMS 69A × R-line 17	189	24.5	3.2	4.3	4.0	48	77	17.45	235.1	69.1
19	HMS 04888A × EMRL-15-109	193	25.4	1.9	3.3	5.7	49	76	15.29	494.5	218.3
20	HMS 48A × R-line 17	195	24.0	1.2	4.6	3.0	49	77	17.90	112.5	36.5
21	HMS 48A × SGP-10-107	195	24.7	3.3	3.7	4.4	47	73	16.19	230.4	77.7
22	HMS 48A × 78/711	188	23.5	1.8	4.3	8.0	50	78	9.25	545.5	175.5
23	HMS 02333 X R-line 2	168	24.6	3.8	4.4	4.3	46	77	17.33	107.3	41.7
24	HMS 02333 × R-line 3	183	25.1	4.0	4.2	4.9	45	75	14.86	214.1	89.0
25	HMS 02333 × R-line 17	188	22.6	3.5	3.9	4.0	48	76	15.59	238.5	72.8
26	HMS 02333 × R-line 49	196	22.0	3.7	3.6	4.1	47	76	17.14	234.6	75.4
27	HMS 02333 × ISK-51	175	24.4	3.2	3.7	3.4	45	76	12.19	290.0	72.5
28	ICMA 97111 × R-line 2	203	25.4	3.7	3.9	4.1	48	76	18.09	206.2	64.3
29	ICMA 97111 × R-line 17	193	24.9	3.2	3.9	5.0	49	78	18.92	182.0	61.9
30	ICMA 97111 × R-line 3	186	23.8	3.4	4.3	7.0	49	78	16.05	638.0	200.1

31	ICMA 97111 × R-line 32	176	22.2	3.1	4.1	4.0	47	74	18.80	189.0	65.4
32	ICMA 97111 × R-line 49	196	24.3	3.4	3.7	5.4	49	79	14.14	181.5	77.6
33	ICMA 97111 × ISK-51	185	24.0	3.0	4.0	8.2	48	76	16.58	243.5	79.3
34	ICMA 97111 × 78/711	193	23.2	3.5	3.5	8.0	48	75	18.50	443.0	154.6
35	HHB 67 improved	200	27.7	2.7	3.1	6.2	45	73	15.96	203.3	66.0
36	HHB 197	166	21.5	1.1	3.4	4.0	50	75	16.45	167.4	126.1
37	HHB 226	155	19.6	2.5	4.3	4.4	48	78	16.04	276.0	79.7
38	HHB 299	167	23.6	3.6	4.2	5.1	56	85	17.00	194.8	83.2
Grand mean		**189.6**	**25.3**	**3.0**	**3.8**	**4.6**	**48.8**	**77.7**	**15.9**	**253.4**	**87.7**
C.D. @ 5%		22.9	3.8	0.5	0.5	0.8	3.3	6.8	1.6	39.3	12.5
SE(m)		8.0	1.3	0.2	0.2	0.3	1.1	2.3	0.6	13.7	4.3
SE(d)		11.3	1.9	0.3	0.2	0.4	1.6	3.3	0.8	19.3	6.1
C.V.		5.9	7.4	8.5	6.5	9.0	3.3	4.3	5.0	7.6	7.0

PH=Plant height, EL= Ear length, ED= Ear diameter, LBW= Leaf blade width, ETPP= Effective number of tillers per plant, DF= Days to 50% flowering, DM= Days to maturity, TW= 1000-Grain weight, DFPP= Dry fodder yield per plant, GYPP= Grain yield per plant

Table 4.14: Estimates of genetic variability parameters [mean ± SE (m), range, GCV, PCV, heritability and genetic advance as percent of mean] for morpho-phenological traits and yield attributes in pearl millet hybrids

Characters	Mean ± SE	Range	GCV (%)	PCV (%)	Heritability (%)	GA%M
PH	189.6±8.0	168-206	4.6	7.5	37.8	5.8
EL	25.3±1.3	22.0-32.3	7.9	10.8	53.1	11.8
ED	3.0±0.2	1.2-4.0	20.2	21.9	85.0	38.3
LBW	3.8±0.2	3.1-4.6	9.7	11.7	69.3	16.7
ETPP	4.6±0.3	2.5-8.2	32.5	33.7	92.9	64.6
DF	48.8±1.1	45-59	6.2	7.0	77.9	11.3
DM	77.7±2.3	73-89	3.8	5.7	44.8	5.3
TW	15.9±0.6	9.25-18.92	12.0	13.0	85.5	22.9
DFPP	253.4±13.7	107.3-638.0	43.3	43.9	96.9	87.9
GYPP	87.7±4.3	36.5-218.3	44.2	44.7	97.5	89.9

PH=Plant height, EL= Ear length, ED= Ear diameter, LBW= Leaf blade width, ETPP= Effective number of tillers per plant, DF= Days to 50% flowering, DM= Days to maturity, TW= 1000-Grain weight, DFPP= Dry fodder yield per plant, GYPP= Grain yield per plant

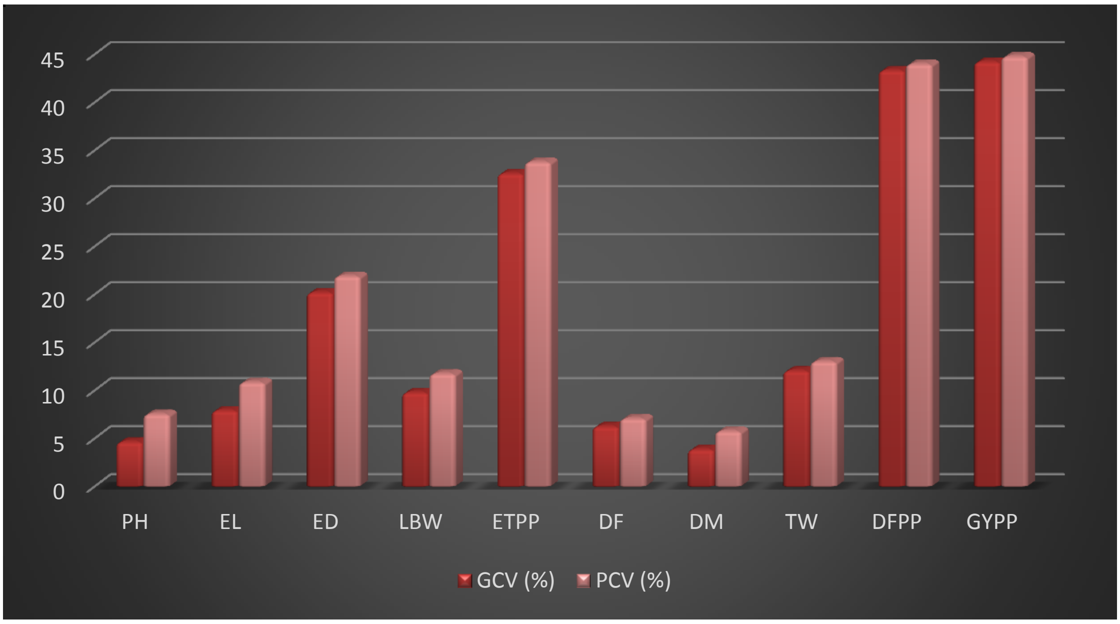

Fig. 4.9: Estimates of GCV (%) and PCV (%) for morpho-phenological traits and yield attributes in pearl millet hybrids

PH=Plant height, EL= Ear length, ED= Ear diameter, LBW= Leaf blade width, ETPP= Effective number of tillers per plant, DF= Days to 50% flowering, DM= Days to maturity, TW= 1000-Grain weight, DFPP= Dry fodder yield per plant, GYPP= Grain yield per plant

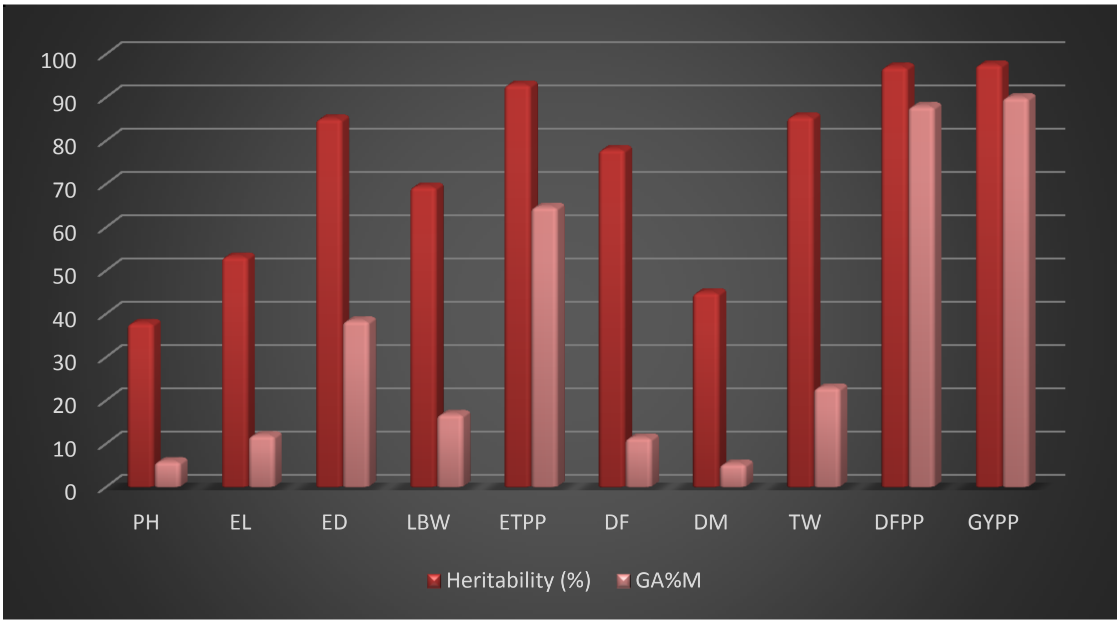

Fig. 4.10: Estimates of h^2 (%) and GA%M for morpho-phenological traits and yield attributes in pearl millet hybrids

PH=Plant height, EL= Ear length, ED= Ear diameter, LBW= Leaf blade width, ETPP= Effective number of tillers per plant, DF= Days to 50% flowering, DM= Days to maturity, TW= 1000-Grain weight, DFPP= Dry fodder yield per plant, GYPP= Grain yield per plant

4.8.2.1 Plant Height (cm)

Plant height of the hybrids under study ranged from 168 cm to 206 cm having mean value of 189.6±8.0 cm. HMS 47A × SGP-10-107 showed the maximum height of 206 cm and the minimum plant height 168 cm was shown by HMS 02333 × R-line 2. PCV (7.5%) was found moderate while GCV (4.6%) was found to be low for this trait. Moderate heritability (37.8%) with low genetic advance as per cent of mean (5.8%) was observed (Table 4.14).

4.8.2.2 Ear Length (cm)

The range estimates of this trait varied from 22.0 cm to 32.3 cm with a mean value of 25.3±1.3 cm. The HMS 02333 × R-line 49 showed the minimum ear length (22.0 cm) whereas maximum length 32.3 cm was recorded by the line HMS 74A × R-line 4. GCV (7.9%) was found low and PCV (10.8%) was found moderate for this trait. Moderate heritability (53.1%) with moderate genetic advance as per cent of mean (11.8%) was observed for this trait (Table 4.14).

4.8.2.3 Ear Diameter (cm)

Mean value of ear diameter was computed 3.0 ±0.2 cm ranging from 1.2 cm to 4.0 cm. The HMS 48A × R-line 17 showed minimum ear diameter (1.2 cm) while the line HMS 02333 × R-line 3 showed maximum ear diameter (4.0 cm). Both the PCV (21.9%) and GCV (20.2%) were computed high for this trait. High heritability (85.0%) with high genetic advance as per cent of mean (38.3%) was perceived for this trait (Table 4.14).

4.8.2.4 Leaf Blade Width (cm)

The mean value of leaf blade width was 3.8±0.2 cm ranging from 3.1 cm to 54.6 cm. GCV (9.7%) were found low whereas, PCV (11.7%) was found moderate. The ICMA 04999 × ISK-51 showed minimum leaf blade width (3.1 cm) and HMS 48A × R-line 17 showed the maximum leaf blade width (54.6 cm). High heritability (69.3%) with moderate genetic advance as per cent of mean (16.7%) was observed for this trait (Table 4.14).

4.8.2.5 Effective Number of Tillers/Plant

Effective number of tillers per plant ranged from 2.5 to 8.2 with general mean value of 4.6±0.3. The HMS 47A × R-line 2 showed minimum number of effective tillers (2.5) and ICMA 97111 × ISK-51 showed the maximum number of effective tillers (8.2). Both GCV (32.5%) and PCV (33.7%) were computed high for this trait. High heritability (92.9%) with high genetic advance as per cent of mean (64.6%) was observed for this trait (Table 4.14).

4.8.2.6 Days to 50% Flowering

The mean value of days to 50% flowering was 48.8±1.1 days ranging from 45 to 59 days. Estimates of both GCV (6.2%) and PCV (7.0%) were found low. HMS 02333 × R-line

3 and HMS 02333 × ISK-51 were the earliest to flower at 45 days, while the line HMS 74A × 78/711 flowered late at 59 days. The trait was having high heritability (77.9%) along with moderate genetic advance as per cent of mean (11.3%) (Table 4.14).

4.8.2.7 Days to Maturity

The mean value of days to maturity was 77.7±2.3 days ranging from 73 to 89 days. Estimates of both GCV (3.8%) and PCV (5.7%) were found low. The HMS 48A × SGP-10-107 were the earliest to mature in 73 days, while the line HMS 74A × 78/711 matured late in 89 days. The trait was having high heritability (44.8%) and low genetic advance as per cent of mean (5.3%) (Table 4.14).

4.8.2.8 1000-Grain Weight (g)

Wide genetic variation from 9.25 g to 18.92 g with mean 15.9±0.6 was found for this trait. The minimum value was recorded in HMS 48A × 78/711 (9.25 g) while the maximum weight was recorded in the line ICMA 97111 × R-line 17 (18.92 g). Moderate GCV and PCV estimates were computed 12.0% and 13.0% respectively. High heritability (85.5%) and high genetic advance as per cent of mean (22.9%) was exhibited by this trait (Table 4.14).

4.8.2.9 Dry Fodder Yield/Plant (g)

The trait exhibited significant variation ranging from 107.3 g to 638.0 g with a mean value of 253.4±13.7. The HMS 02333 × R-line 2 showed maximum dry fodder yield of 638.0 g while the line ICMA 97111 × R-line 3 showed the minimum dry fodder yield of 107.3 g. Both PCV (43.9%) and GCV (43.3%) estimates were high for this trait. High heritability (96.9%) as well as high genetic advance as per cent of mean (87.9%) was found for this character (Table 4.14).

4.8.2.10 Grain Yield/Plant (g)

Mean value of grain yield per plant was 87.7±4.3 g ranging from 36.5 g to 218.3 g. The line HMS 48A × R-line 17 showed the minimum grain yield of 36.5 g. The maximum grain yield per plant of 218.3 g was recorded by the HMS 04888A × EMRL-15-109. Both computed PCV (44.7%) and GCV (44.2%) estimates were high for this trait. High heritability (97.5%) as well as high genetic advance as per cent of mean (89.9%) was exhibited by this trait (Table 4.14).

4.9 Correlation coefficient analysis

Similar to the parents, there was a significant intrinsic association between the various features, as shown by the magnitudes of the genotypic level correlation coefficients for almost all the traits being higher than the phenotypic level correlation coefficients for the same traits. Table 4.15 contains the calculated phenotypic and genotypic correlation coefficients between grain yield and other component variables as well as among themselves.

Table 4.15: Estimates for Genotypic (above diagonal) and Phenotypic (below diagonal) Correlation Coefficients for morpho-phenological traits and yield attributes in pearl millet hybrids

Traits	PH	EL	ED	LBW	ETPP	DF	DM	TW	DFPP	GYPP
PH	1	0.926**	0.200	-0.274*	-0.256*	-0.104	0.017	-0.030	-0.023	-0.075
EL	0.423**	1	0.177	-0.173	-0.126	0.337**	0.594**	-0.270*	-0.111	-0.140
ED	0.129	0.192	1	0.168	-0.030	-0.207	0.021	0.194	-0.125	-0.274*
LBW	-0.213	-0.098	0.109	1	-0.017	-0.114	-0.057	0.012	-0.047	-0.145
ETPP	-0.137	-0.071	-0.032	-0.019	1	0.245*	0.231*	-0.147	0.552**	0.523**
DF	0.094	0.183	-0.132	-0.064	0.210	1	1.106**	-0.074	0.100	0.072
DM	-0.009	0.252*	0.015	-0.058	0.103	0.806**	1	-0.211	0.014	-0.122
TW	-0.017	-0.198	0.155	-0.058	-0.146	-0.070	-0.087	1	-0.396**	-0.293*
DFPP	-0.003	-0.076	-0.137	-0.018	0.531**	0.087	0	-0.352**	1	0.900**
GYPP	-0.049	-0.121	-0.279*	-0.110	0.492**	0.066	-0.052	-0.262*	0.890**	1

PH=Plant height, EL= Ear length, ED= Ear diameter, LBW= Leaf blade width, ETPP= Effective number of tillers per plant, DF= Days to 50% flowering, DM= Days to maturity, TW= 1000-Grain weight, DFPP= Dry fodder yield per plant, GYPP= Grain yield per plant

4.9.1 Plant Height (cm)

Plant height was found to be significantly and positively correlated with ear length (rg=0.926, rp=0.423) at both genotypic and phenotypic levels respectively whereas it showed negative and significant correlation with leaf blade width (rg= -0.274) and effective number of tillers per plant (rg=-0.256) at only genotypic level.

4.9.2 Ear Length (cm)

Ear length was found to be significantly and positively correlated with plant height (rg= 0.926, rp= 0.423) and days to maturity (rg= 0.594, rp= 0.252) at both genotypic and phenotypic levels and with days to 50% flowering (rg=0.337) at only genotypic level whereas it was negatively and significantly correlated with 1000-grain weight (rg=-0.270) at genotypic level.

4.9.3 Ear Diameter (cm)

Ear diameter was found to be significantly and negatively correlated with grain yield per plant (rg= -0.274, rp= -0.279) at both genotypic and phenotypic levels.

4.9.4 Leaf Blade Width (cm)

Leaf blade width was found to be significant and negatively correlated with plant height (rg= -0.274) at only genotypic level.

4.9.5 Effective Number of Tillers/Plant

Effective number of tillers per plant were found to be significantly and positively correlated with dry fodder yield per plant (rg= 0.552, rp= 0.531) and grain yield per plant (rg= 0.523, rp= 0.492) at both genotypic and phenotypic levels and with days to maturity (rg=0.231) at only genotypic level whereas it was negatively and significantly correlated with plant height (rg=-0.256) at genotypic level.

4.9.6 Days to 50% Flowering

Days to 50% flowering expressed significant positive correlation with days to maturity (rg=0.106, rp=0.806) at both genotypic and phenotypic levels respectively whereas exhibited significant positive correlation with ear length (rg= 0.337) and effective number of tillers per plant (rg= 0.245) at only genotypic level.

4.9.7 Days to Maturity

Days to maturity were found to be significantly and positively correlated with ear length (rg= 0.594, rp= 0.252) and days to 50% flowering (rg= 1.106, rp= 0.806) at both genotypic and phenotypic levels and with effective number of tillers per plant at only genotypic level (rg= 0.231).

4.9.8 1000-Grain Weight (g)

1000-grain weight was found to be negatively and significantly correlated with dry fodder yield per plant (rg= -0.396, rp= -0.352) and grain yield per plant (rg= -0.293, rp= -0.262) at both genotypic and phenotypic levels and with ear length at only genotypic level (rg=-0.270).

4.9.9 Dry Fodder Yield/Plant (g)

Dry fodder yield per plant was found to be positively and significantly correlated with effective number of tillers per plant (rg= 0.552, rp= 0.531) and grain yield per plant (rg= 0.900, rp= 0.890) and negatively and significantly correlated with 1000-grain weight (rg=-0.396, rp=-0.352) at both genotypic and phenotypic levels.

4.9.10 Grain Yield/Plant (g)

Grain yield per plant was found to be positively and significantly correlated with effective number of tillers per plant (rg= 0.523, rp= 0.492) and dry fodder yield per plant (rg=

0.900, rp= 0.890) and negatively and significantly correlated with ear diameter (rg=-0.274, rp=-0.279) and 1000-grain weight (rg=-0.293, rp=-0.262) at both genotypic and phenotypic levels.

4.10 Cluster analysis

Progenies were divided into separate clusters based on the minimum Euclidean distance using Ward's minimum variance method (1963).

The average distance between and within clusters as well as the cluster mean for various clusters were also calculated. The results of the cluster analysis produced four clusters (Table- 4.16). Cluster I had the maximum number of genotypes i.e., 26 followed by cluster III (5), Cluster IV (4) and cluster II (3).

Table 4.16: Cluster membership and number of hybrids in each cluster of pearl millet

S.No.	No. of Genotypes	Genotypes
Cluster 1	26	HMS 47A × HPT-2-12-32, HMS 48A × SGP-10-107, HMS 47A × SGP-10-107, ICMA 97111 × R-line 2, HMS 47A × R-line 2, HMS 02333 × R-line 17, HMS 02333 × R-line 49, ICMA 97111 × R-line 49, HMS 02333 × R-line 3, HMS 69A × R-line 17, ICMA 97111 × R-line 17, ICMA 97111 × R-line 32, HMS 02333 × R-line 2, ICMA 04999 × R-line 17, ICMA 97111 × ISK-51, HHB 299, HMS 47A × HB15/085, HMS 02333 × ISK-51, HMS 47A × R-line 17, HMS 47A × H78/711, HMS 47A × R-line 49, HMS 69A × SGP-10-107, HMS 47A × R-line 10, ICMA 04999 × ISK51, ICMA 04999 × SGP-10-107, HHB 67 improved
Cluster 2	3	HHB 226, HHB 197, HMS 48A × R-line 17,
Cluster 3	5	HMS 74A × R-line 4, HMS 74A × 78/711, ICMA 94555 × EMRL 15/109, ICMA 04999 × EMRL 15/109, ICMA 04999 × 78/711
Cluster 4	4	ICMA 97111 × R-line 3, HMS 04888A × EMRL-15-109, HMS 48A × 78/711, ICMA 97111 × 78/711

Intra and inter-cluster distance was calculated for the different clusters as shown in the Table 4.17. Intra-cluster distance was maximum in cluster II (4.49) followed by cluster IV (4.42), cluster I (3.28 and cluster III (3.10). Inter-cluster distance was maximum between cluster II and cluster IV (6.58) followed by cluster II and cluster III (6.22). Inter-cluster distance was found minimum between cluster II and cluster I (4.95). Fig. 4.11 demonstrates the cluster diagram showing inter and intra- cluster distances in pearl millet hybrids and Fig. 4.12 shows the dendrogram showing the clustering pattern of pearl millet hybrids.

Table 4.17: Inter and intra-cluster distances in pearl millet hybrids

	Cluster 1	**Cluster 2**	**Cluster 3**	**Cluster 4**
Cluster 1	**3.28**	4.95	4.74	5.88
Cluster 2		**4.49**	6.22	6.58
Cluster 3			**3.10**	6.18
Cluster 4				**4.42**

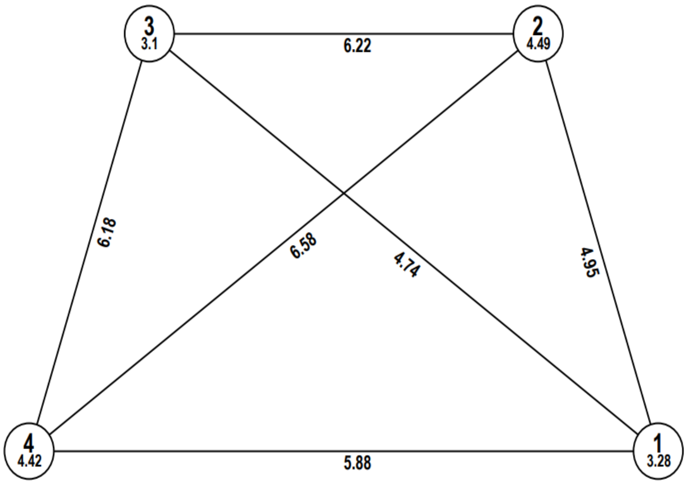

Fig 4.11: Cluster diagram showing inter and intra-cluster distances in pearl millet hybrids

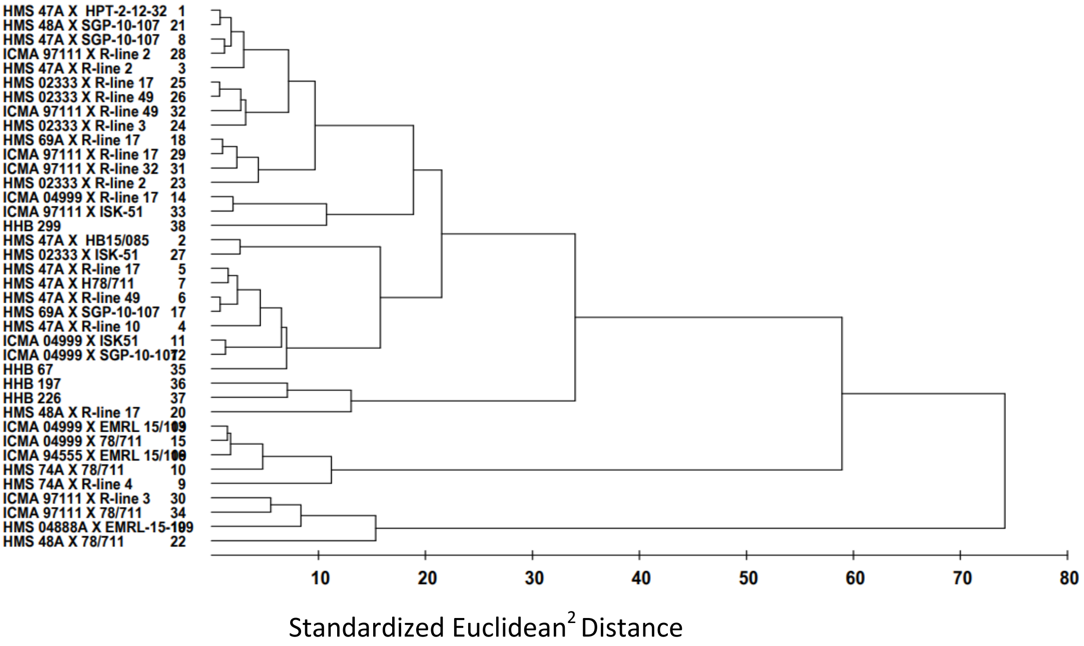

Fig. 4.12: Dendrogram showing the clustering pattern of different pearl millet hybrids

4.11 Principal component analysis

The principal component analysis was done similarly as in experiment 1. The eigen values were used to determine the number of factors to retain. The sum of all the eigen values was always equal to the number of variables. In this study, the first four main components had eigen values greater than one and they cumulatively explained 74.56% of the total variation present in original data set (Table 4.18). The first principal component explained 26.07% of the total variability. The second, third and fourth principal components explained 22.36%, 14.81% and 11.31% of the total variability, respectively.

Table 4.18: Total variance explained by different principal components in pearl millet hybrids

Principal components	Eigen value	Variation explained (%)	Cumulative variation explained (%)
1	2.61	26.07	26.07
2	2.24	22.36	48.43
3	1.48	14.81	63.25
4	1.13	11.31	74.56

Varimax rotation was used to perform additional principal component analysis in order to examine character associations with various principal components. Table 4.19 shows the factor loading for different characters after varimax rotation.

Table 4.19: Factor loading after varimax rotation

Variables	PC1	PC2	PC3	PC4
PH (cm)	-0.095	-0.302	0.61	-0.033
EL (cm)	-0.054	-0.496	**0.387**	0.143
ED (cm)	-0.212	-0.088	0.129	0.659
LBW (cm)	-0.057	0.132	-0.248	**0.672**
ETPP	0.442	0.025	-0.119	0.212
DF	0.203	-0.522	-0.375	-0.089
DM	0.133	-0.566	-0.359	0.032
TW (g)	-0.293	0.108	-0.211	-0.126
DFPP (g)	0.551	0.098	0.194	0.148
GYPP (g)	**0.544**	**0.149**	0.184	-0.029

PH= Plant height, EL= Ear length, ED= Ear diameter, LBW= Leaf blade width, ETPP= Effective number of tillers per plant, DF= Days to 50% flowering, DM= Days to maturity, TW= 1000-Grain weight, DFPP= Dry fodder yield per plant, GYPP= Grain yield per plant

From Table 4.19, it is clear that both the first and second principal factor showed maximum positive factor loading for grain yield per plant i.e., 0.544 and 0.149 respectively. Principal factor three showed high positive loading for ear length (0.387). Principal Factor four showed high loading for leaf blade width (0.672). The other major contributors for variations observed in the first principal component were effective number of tillers per plant, days to 50% flowering, days to maturity, and dry fodder yield per plant. The variations in PC2 were mainly due to leaf blade width and 1000-grain weight. The variations in PC3 were present mainly through plant height, ear diameter, dry fodder yield per plant and grain yield per plant. Likewise major contributors to the variations observed in PC4 were ear length, ear diameter, effective number of tillers per plant and dry fodder yield per plant.

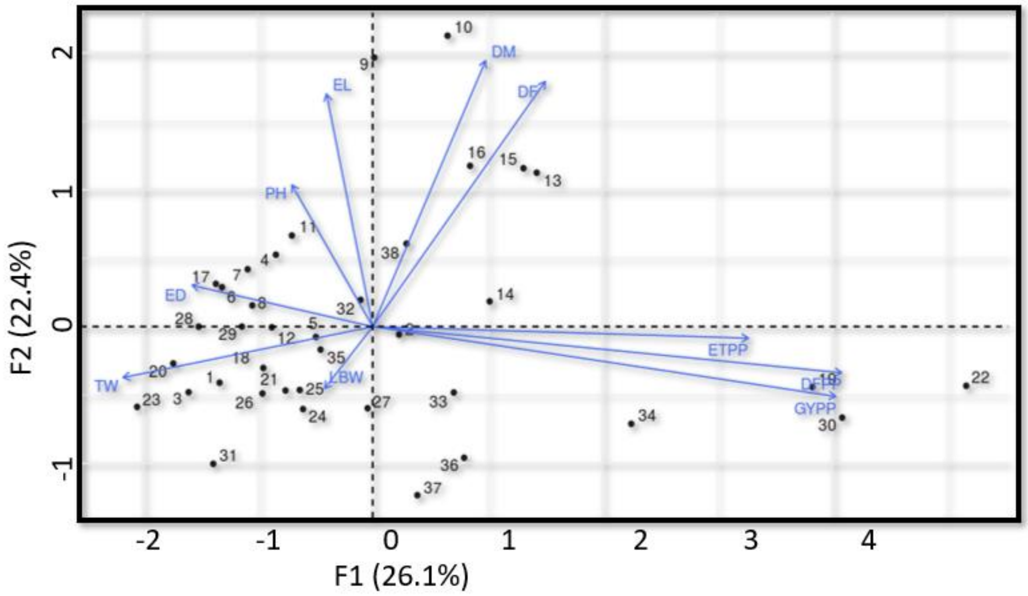

Fig. 4.13: Principal component loading plot of PC-1 and PC-2

PCA Biplot was constructed similar as in experiment 1. The original variable is shown as a vector in the plot (arrow). The biplot of the hybrids also described their diversity in terms of spatial distance (Figure 4.13). The biplot of hybrids based on PC-1 and PC-2 depicted 48.5% of the total variation. The vector of traits, *viz.* ear length, days to maturity, days to 50% flowering, dry fodder yield per plant, grain yield per plant, effective number of tillers per plant and 1000-grain weight showed longer lengths indicating positive contributions to the components.

The pearl millet hybrids distributed based on their relative performances with respect to principal factor one and two. Principal factor one showed high factor loading for effective number of tillers per plant, dry fodder yield per plant, grain yield per plant, days to 50% flowering and days to maturity. Days to 50% flowering and days to maturity were found to be common towards the positive sides of both the principal factors. Hybrids 30, 19 and 22 were clustered towards positive side of PC-1, so they were better in terms of the traits lying in PC-1 (grain yield per plant and dry fodder yield per plant) whereas all the hybrids clustered towards the positive side of PC-II i.e., 11, 4 7, 17, 6, 8 and 32 were better in terms of traits like ear diameter, plant height and ear length. Vectors for the traits leaf blade width and 1000-grain weight were present in the negative side of both the principal components. The hybrids 20, 23, 3, 24, 25 and 35 were better in terms of these traits.

4.12 Screening studies

The pearl millet hybrids were screened for the blast severity reaction using the 1-9 progressive scale developed by International Rice Research Institute for rice blast. Disease severity was recorded at hard dough stage and the number of hybrids and the estimates of blast severity analysis are given in Table 4.20. The individual blast score for each hybrid is given in Annexure II.

Table 4.20: Estimates of blast severity analysis in pearl millet hybrids

Scale	Disease reaction	Number of hybrids	Hybrids
1.0-2.0	**Highly resistant**	**8**	HMS 47A × R-line 10, HMS 47A × R-line 17, HMS 69A × R-line 17, HMS 04888A × EMRL-15-109, ICMA 97111 × R-line 3, ICMA 97111 × R-line 32, ICMA 97111 × 78/711, HHB 299
2.1-4.0	**Resistant**	**18**	HMS 47A × HPT-2-12-32, HMS 47A × HB15/085, HMS 47A × R-line 2, HMS 47A × R-line 49, HMS 47A × H78/711, HMS 74A × R-line 4, ICMA 04999 × EMRL 15/109, ICMA 04999 × R-line 17, ICMA 04999 × 78/711, HMS 02333 × R-line 49, ICMA 97111 × ISK-51, ICMA 97111 × R-line 49, HHB 197, HMS 69A × SGP-10-107, HHB 67 improved, HHB 226, HMS 48A × R-line 17, HMS 48A × SGP-10-107
4.1-6.0	**Moderately resistant**	**10**	HMS 47A × SGP-10-107, HMS 74A × 78/711, ICMA 94555 × EMRL 15/109, HMS 02333 × R-line 3, HMS 02333 × R-line 17, ICMA 97111 × R-line 17, ICMA 04999 × ISK-51, ICMA 04999 × SGP-10-107, HMS 02333 × R-line 2, HMS 48A × 78/711
6.1-8.0	**Susceptible**	**2**	HMS 02333 × ISK-51, ICMA 97111 × R-line 2

4.13 Screening of hybrids for biochemical traits

4.13.1 Phenylalanine Ammonia Lyase (PAL) Activity

PAL activity estimated at different days post inoculation for all the pearl millet hybrids is shown in Fig. 4.14.

On day 1, it ranged from 0.153 to 0.166 µmol t-CA $h^{-1}g^{-1}$ with an overall mean value of 0.160 µmol t-CA $h^{-1}g^{-1}$. On day 2, the values ranged from 0.153 to 0.168 µmol t-CA $h^{-1}g^{-1}$ with an overall mean value of 0.162 µmol t-CA $h^{-1}g^{-1}$. Whereas on day 3, the PAL activity ranged from 0.155 to 0.171 µmol t-CA $h^{-1}g^{-1}$ with an overall mean value of 0.163 µmol t-CA $h^{-1}g^{-1}$. On all three days, the minimum PAL activity found was of the hybrid ICMA 97111 × R-line 2 and the maximum was of HMS 47A × R-line 10. The mean sum of squares (ANOVA) for PAL activity showed significant variations within the hybrids on respective days and on different days of sampling. Also, the value of mean sum of squares for the interaction between hybrids and number of days showed significant values inferring that there was a considerable change in the PAL activity among various hybrids on different sampling days (Table 4.21). This was in contrast with the replications, where mean sum of squares were non-significant for all the traits.

When the enzyme activity of the inoculated resistant and susceptible hybrids was compared, the maximum PAL activity was found in the highly resistant hybrids while in highly susceptible hybrids, least activity was found. Also, the PAL activity was found to increase after the pathogen inoculation as compared to control. Fig. 4.15 shows the relative comparison of PAL activity on Day 3 of sampling of pearl millet hybrids and their blast severity scores.

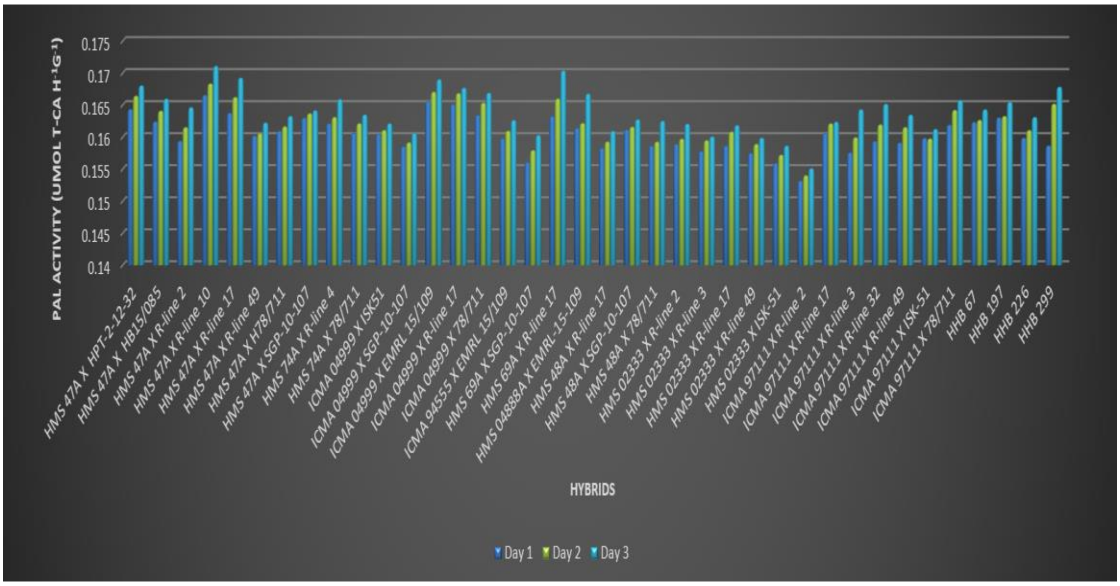

Fig. 4.14: PAL activity (µmol t-CA $h^{-1}g^{-1}$) in pearl millet hybrids on consecutive days of sampling

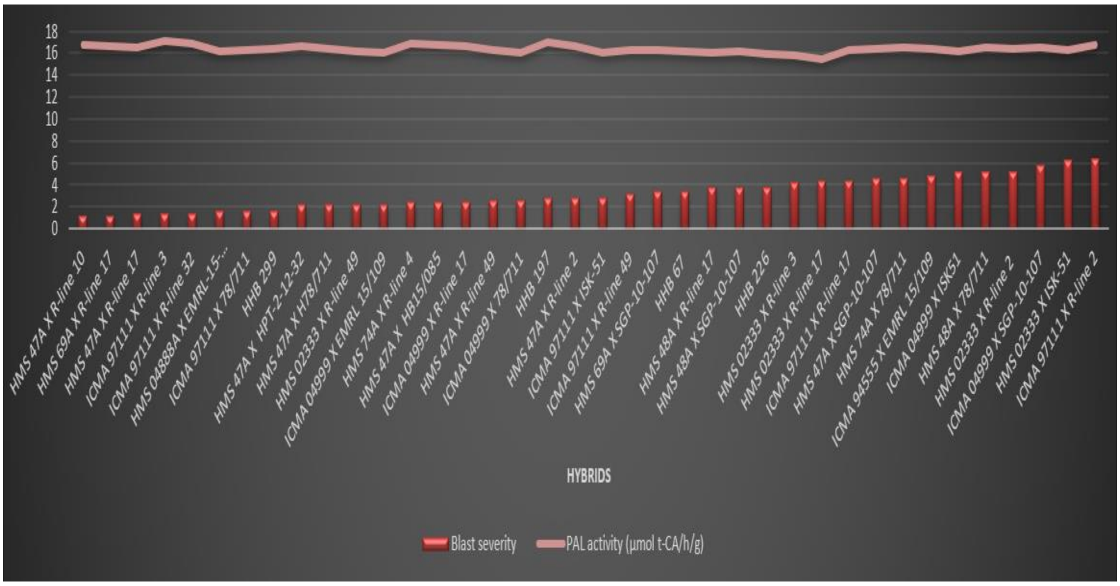

Fig. 4.15: PAL activity in pearl millet hybrids having different degrees of blast severity scores (PAL activity values transformed by multiplying with a common value i.e., 100)

4.13.2 Peroxidase (POX) Activity

POX activity estimated at different days post inoculation for all the pearl millet hybrids is shown in Fig. 4.16.

On day 1, it ranged from 2.10 to 8.77 µmol $min^{-1}g^{-1}$ with an overall mean value of 4.24 µmol $min^{-1}g^{-1}$. On day 2, the values ranged from 2.72 to 9.29 µmol $min^{-1}g^{-1}$ with an overall mean value of 5.79 µmol $min^{-1}g^{-1}$. Whereas on day 3, the POX activity ranged from 3.35 to 12.83 µmol $min^{-1}g^{-1}$ with an overall mean value of 8.27 µmol $min^{-1}g^{-1}$. The maximum POX activity on day 1 and 2 was of the hybrid HMS 47A × R-line 17 while the minimum POX activity was of ICMA 94555 × EMRL 15/109 and ICMA 97111 × R-line 2 respectively. On day 3, maximum activity was found to be of ICMA 97111 × R-line 2 and minimum activity was of HMS 69A × R-line 17. The mean sum of squares (ANOVA) for POX activity showed significant variations within the hybrids on respective days and on different days of sampling and for the interaction between the hybrids & sampling days, similar to PAL activity. (Table 4.21). This was in contrast with the replications, where mean sum of squares were non-significant for all the traits.

When the POX activity of the inoculated resistant and susceptible hybrids was compared, the former was found to have more enzyme activity than the latter while enzyme activity in both the cases was more as compared to control. Fig. 4.17 shows the relative comparison of POX activity on Day 3 of sampling of pearl millet hybrids and their blast severity scores.

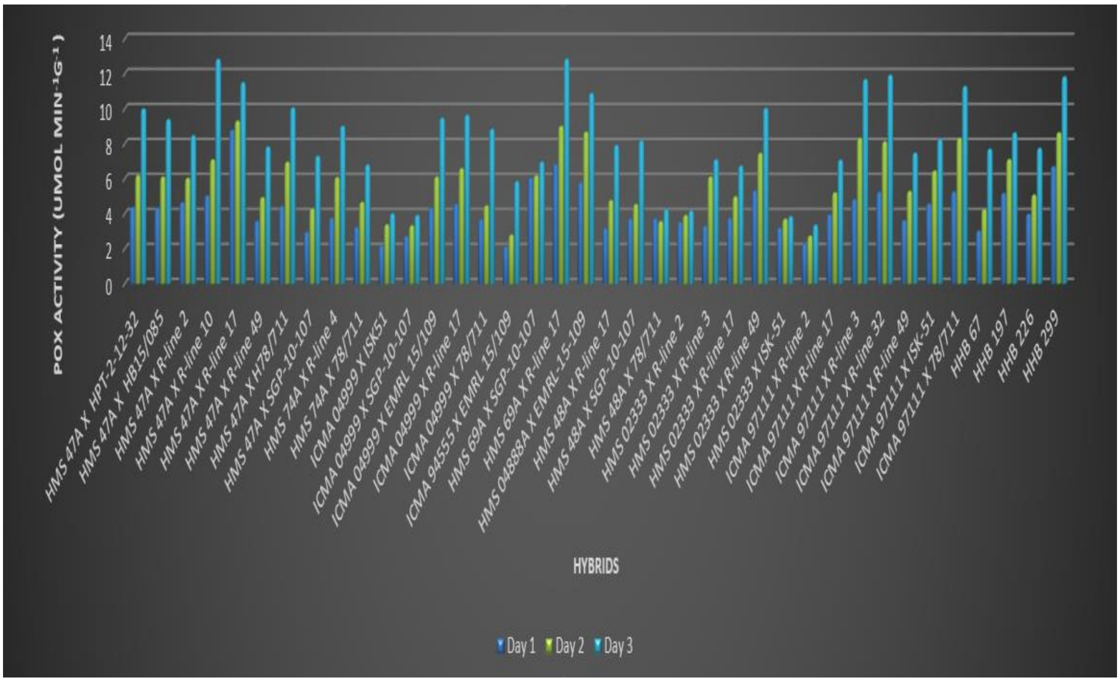

Fig. 4.16: **POX activity (µmol min^{-1}g^{-1}) in pearl millet hybrids on consecutive days of sampling**

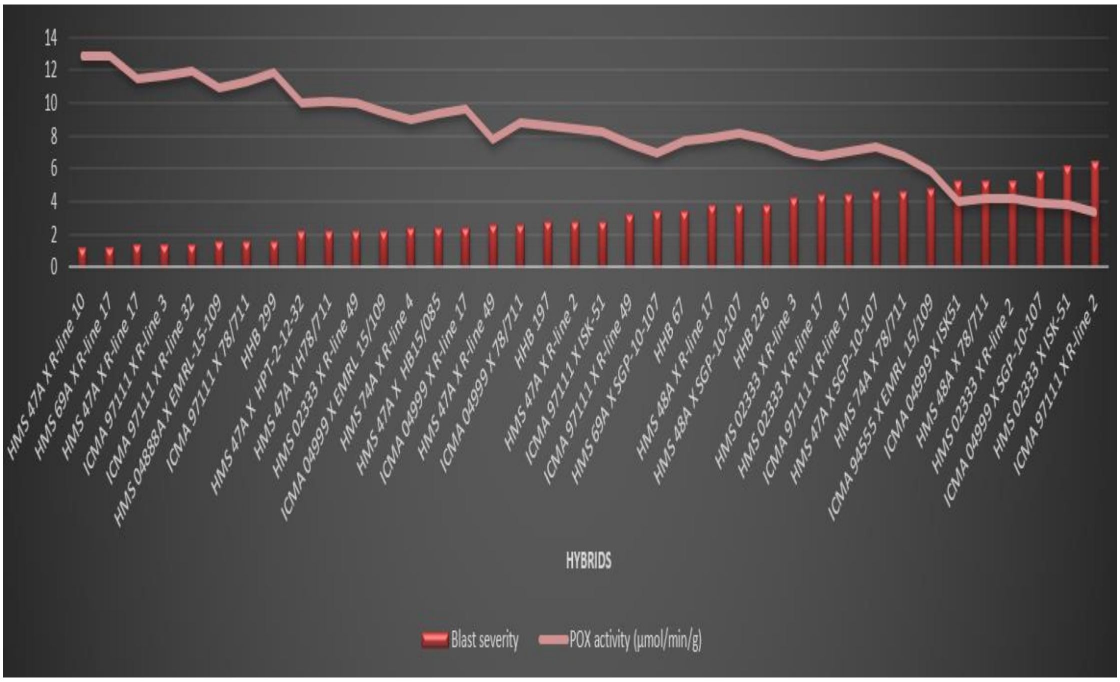

Fig. 4.17: **POX activity in pearl millet hybrids having different degrees of blast severity scores**

4.13.3 Lipoxygenase (LOX) Activity

LOX activity estimated at different days post inoculation for all the pearl millet hybrids is shown in Fig. 4.18.

On day 1, it ranged from 3.80 to 9.38 units g^{-1} with an overall mean value of 5.84 units g^{-1}. On day 2, the values ranged from 4.12 to 13.47 units g^{-1} with an overall mean value of 8.28 units g^{-1}. Whereas on day 3, the LOX activity ranged from 4.57 to 19.90 units g^{-1} with an overall mean value of 12.08 units g^{-1}. The minimum LOX activity on day 1, 2 and 3 was of the hybrid ICMA 97111 × R-line 2, while the maximum LOX activity for day 1, 2 and 3 was found to be of ICMA 97111 × R-line 3, ICMA 04999 × 78/711 and HMS 69A × R-line 17 respectively. The mean sum of squares (ANOVA) for LOX activity showed significant variations within the hybrids on respective days and on different sampling days and also for the interaction between hybrids & number of days similar as PAL & POX activity. (Table 4.21). This was in contrast with the replications, where mean sum of squares were non-significant for all the traits.

When the LOX activity of the resistant and susceptible genotypes was compared, the former was found to have more enzyme activity than the latter. Also, the LOX activity was found to increase after the pathogen inoculation as compared to control. Fig. 4.19 shows the relative comparison of LOX activity on Day 3 of sampling of pearl millet hybrids and their blast severity scores.

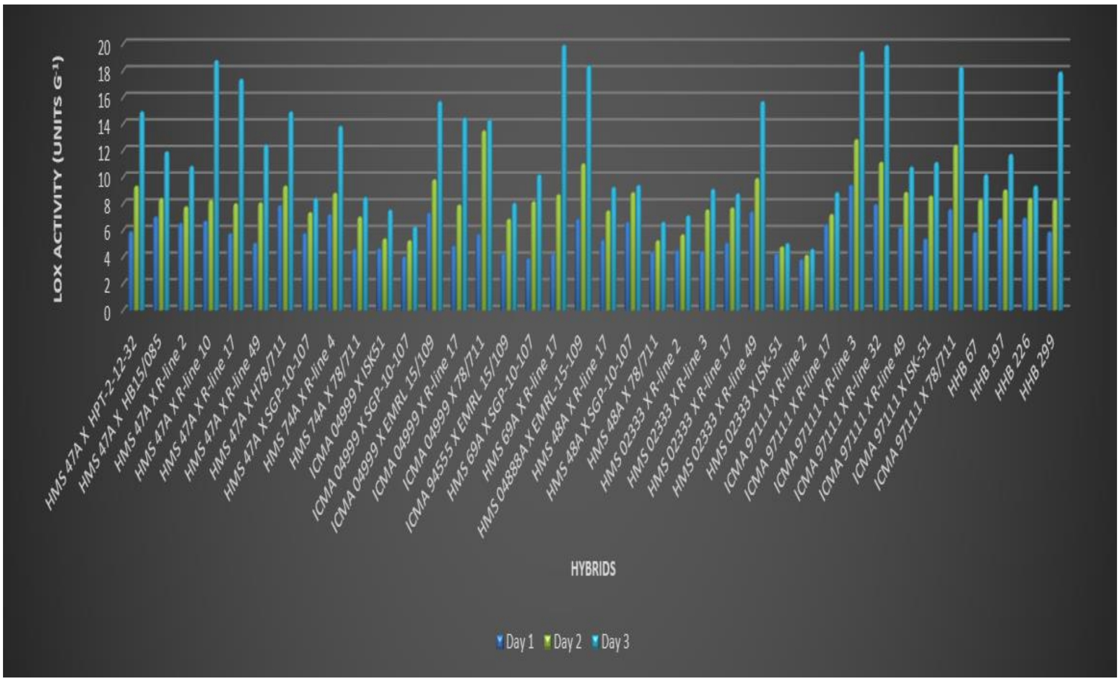

Fig. 4.18: LOX activity (units g^{-1}) in pearl millet hybrids on consecutive days of sampling

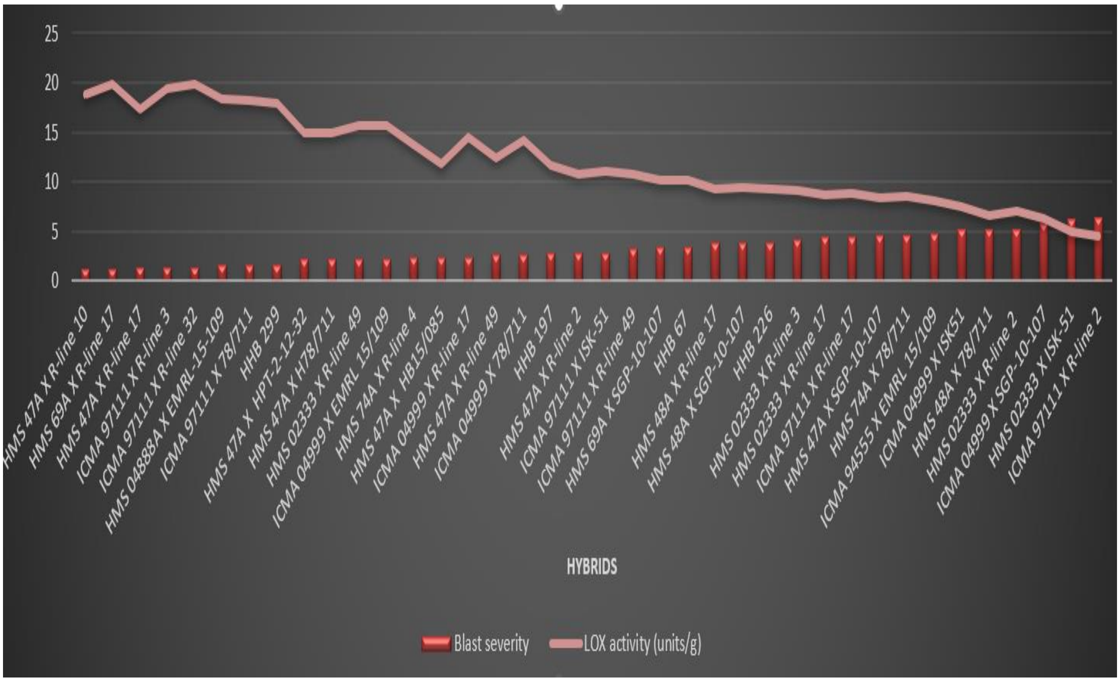

Fig. 4.19: LOX activity in pearl millet hybrids having different degrees of blast severity score

Table 4.21: Analysis of Variance (ANOVA) for enzyme activities in pearl millet hybrids

Mean sum of squares				
Source of Variation	**Degree of freedom (df)**	**PAL (μmol t-CA $h^{-1}g^{-1}$)**	**POX (μmol $min^{-1}g^{-1}$)**	**LOX (units g^{-1})**
Factor A	37	0.541**	20.22**	34.87**
Factor B	2	2.305**	313.84**	751.33**
Interaction AXB	74	0.018**	1.898**	8.716**
Error	113	0.008	0.086	0.264

Factor A=Treatments, Factor B=Sampling Days

CHAPTER – V

DISCUSSION

Pearl millet is a highly allogamous, annual, diploid crop with seven chromosomal pairs. It has a comparatively high nutritional value for a cereal and can be grown in a wide variety of environmental settings. India contributes to 26.6% of the total pearl millet produced in the world (Kumar *et al.*, 2022). Also, to feed India's vast population, high producing cultivars must be developed. Any direct selection is ineffective since yield is a highly complicated quantitative feature that is greatly influenced by environment. As a result, any selection program should include the association between grain yield, its related component traits, and among themselves. To study these components, germplasm is analysed using various biometrical methods, such as ANOVA, principal component, coefficient of variation, and cluster analysis, which further clarifies population structure. Additionally, several biotic and abiotic constraints have a negative impact on the forage and grain yield of crops. One of the biotic constraints is blast disease caused by *Pyricularia grisea* Sacc. (Teleomorph: *Magnaporthe grisea*), which was previously thought to be one of the minor diseases of pearl millet, has recently become a very major disease, especially for the commercial hybrids of India. This has indicated the necessity for a tactical breeding program which incorporates disease resistance with the enhancement in the yield to develop improved cultivars which are acceptable to the farmers.

The most important factor in the success of any breeding programme is the presence of genetic variability in the population. Characterization of germplasm can be done using morphological descriptors and/or molecular markers. Compared to molecular markers, morphological descriptors are easy and simple to investigate, but they have some drawbacks, such as limited polymorphism and environmental influences on phenotype that have an impact on the evaluation method (Wang *et al.*, 2018). Significant advancements have been made recently in the application of molecular techniques to crop improvement. Although several different molecular marker types have been used to assess the genetic diversity of pearl millet, microsatellites, or simple sequence repeats (SSRs) are particularly useful for detecting allele frequency within populations and determining population structure due to their high rate of polymorphism, selective neutrality, co-dominant character, distribution across the genome, and cost and labour efficiency (Ozkan *et al.*, 2022; Zwyrtkova *et al.*, 2022).

Thus, considering the above-mentioned facts, in the present study, along with the study of morphological components of grain yield and blast severity analysis; genetic diversity analysis was also done in the light of the following objectives: (i) To evaluate pearl millet germplasm lines and hybrids for morpho-phenological traits and blast disease, (ii) To study DNA polymorphism and initiate the crossing program between selected blast resistant and elite lines and (iii) To screen F_1 hybrids for morphological and biochemical traits and blast disease.

Key elements of the result obtained have been discussed under the following headings:

5.1 Analysis of variance

5.2 Parameters of genetic variation

5.3 Correlation coefficient analysis

5.4 Cluster analysis

5.5 Principal component analysis

5.6 Screening studies

5.7 Microsatellite marker analysis

5.8 Enzyme activity analysis

5.1 Analysis of variance

The analysis of variance revealed highly significant differences among different pearl millet genotypes evaluated for all the ten morphological characters studied, indicating the presence of sufficient variability. Large variation among the genotypes was found for the traits like plant height, ear length, ear diameter, leaf blade width, effective number of tillers per plant, days to 50% flowering, days to maturity, 1000-grain weight, dry fodder yield per plant and grain yield per plant. The F_1 hybrids also showed considerable variations for these morphological characters. The results were found to be in collaboration with Singh *et al.* (2015). They also found considerable amount of genetic variability in the material under study for the above-mentioned traits.

Similar studies were also conducted by Dhedhi *et al.* (2016) in which they investigated 29 fodder pearl millet hybrids and found significant variations for all the investigated characters. Ramya *et al.* (2018) also investigated the genetic variability for yield and its contributing factors in 376 pearl millet genotypes and found a high range of variance for all the characters studied i.e., Plant height, days to 50% flowering, plant height, number of tillers per plant, leaf blade length, leaf blade width, spike diameter, spike length, grain yield/plant and 1000-seed weight. Similar investigation was carried by Rani *et al.* (2022) to examine the genetic diversity in 30 inbred lines of pearl millet for dry fodder yield and its corresponding features. All the characters showed a significant variance, indicating a lot of variation.

5.2 Parameters of genetic variation

The genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability, and genetic advance as a percentage of mean are first evaluated for the improvement of any specific characteristic in a population, which further determines the suitable method of selection. The findings of the current study suggested that high heritability for a trait does not essentially couple with high genetic advance as found in the studied genotypes and developed F_1 hybrids. However, for the traits like plant height, ear length, ear diameter, leaf blade width, effective number of tillers per plant, 1000-grain weight, dry fodder yield per plant and grain yield per plant, high heritability was found coupled with high genetic advance as percent of mean in the genotypes. These results are found in coordination with Chauhan *et al.* (2020) in their study of different pearl millet genotypes where they found that all the characters under study exhibited high heritability as well as high genetic advance whereas Dhedhi *et al.* (2016) found that all the investigated traits showed strong heritability and high to moderate genetic progress as percent of mean in 29 fodder pearl millet hybrids. Similar results were reported by Rani *et al.* (2022) in their study on 30 inbred lines of pearl millet.

For all of the characteristics, it was discovered that the PCV was consistently larger than the GCV, indicating the effect of environmental factors on trait expression and similar conclusion was made by the investigation of Ramya *et al.* (2018) and Abubakar *et al.* (2019) who obtained high PCV than the corresponding GCV with a moderate to high heritability for the traits like height, number of internodes per plant, panicle length, number of tillers per plant, stem diameter, day until anthesis, panicle diameter, weight of panicle, and weight of seed/panicle. Kumar *et al.* (2020) also reported the similar results.

5.3 Correlation coefficient analysis

The analysis of correlation is crucial in order to determine whether different characters are suitable for indirect selection because the selection for any one-character results in unfavourable changes in a number of other associated traits. Any direct selection is ineffective since yield is a highly complicated quantitative feature that is greatly influenced by environment. As a result, any selection program should include the association between the characteristic (grain yield), its related component qualities, and among themselves.

There is a strong intrinsic relationship between different attributes, as evidenced by the magnitudes of correlation coefficients for all the traits at the genotypic level being higher than their corresponding correlation coefficients at the phenotypic level for both the germplasm lines and F_1 hybrids. It was found that the traits *viz.* plant height, ear diameter and dry fodder yield per plant exhibited significant positive correlation with grain yield per plant. Dapke *et al.* (2014) and Kumar *et al.* (2015) reported similar results and found significant positive correlation of the above-mentioned traits with the grain yield per plant. Findings of Harrer and Karad (1998) and Kulkarni *et al.* (2000) also closely agree with the present investigation indicating the positive correlation of dry fodder yield per plant with grain yield per plant. On the other hand, Sankar *et al.* (2013) and Shanthi *et al.* (2014) found positive correlation of plant height with grain yield per plant & Arya *et al.* (2009) and Singh *et al.* (2015) reported significant positive correlation of 1000-grain weight with grain yield per plant. Significant positive correlation of dry fodder yield with grain yield per plant as found in the present investigation is inspiring for the future development of dual-purpose hybrids.

5.4 Cluster analysis

For the preservation of germplasm resources and the selection of eligible divergent parents for hybridization programmes, the grouping and classification of germplasm stock is essential. In the present investigation, the pearl millet genotypes grouped into five clusters. Intra and inter cluster distances between different clusters revealed that cluster II and IV were most distant, whereas cluster V and III were found to be least distant. According to the results of the cluster analysis, the genotypes that are in the most distant clusters would be more advantageous in the selection of parents for a subsequent breeding program since diverse parents would generate the most heterotic combinations.

Kumar *et al.* (2020) also performed cluster analysis to study the phenotypic diversity among the 48 pearl millet genotypes for agro-morphological traits and they too obtained five clusters with varied number of genotypes in each cluster. Shashibhushan *et al.* (2022) also performed cluster analysis in 40 pearl millet genotypes and obtained seven clusters which indicated the presence of diversity among the lines.

5.5 Principal component analysis

Principal component analysis is essentially a data reduction approach that was first developed by Pearson (1901) and later updated by Hotelling (1933). Principal component analysis solves the challenging issue of huge and unmanageable data by breaking down the initial set of inter-correlated variables into a smaller number of linear combinations that account for most of the variability in the original set. The first principal component absorbs and accounts for the majority of the overall variability in the set of all variables, whereas the subsequent components account for progressively less variability.

In the present investigation, the first four principal components having eigen values more than one cumulatively explained 76.442% of the total variation present in original data set for ten variables. The relative contribution to the variability by different principal components was found proportional to their eigen values and progressively decreased. The first principal component explained 29.99% of the total variability. The second, third and fourth principal components explained 19.92%, 14.94% and 11.59% of the total variability, respectively. In the developed F_1 hybrids also, four principal components were found having eigen values more than one and cumulatively explaining 74.56% of the variability. Gupta and Khandelwal (2022) performed similar study in 31 pearl millet genotypes and found three principal components with eigen values more than one, explaining 73.35% of the total variability. Similar investigations were carried out by Jain and Diwan (2021); Kalagare *et al.* (2022) and Kumar *et al.* (2020) as well.

5.6 Screening studies

The most practical and cost-effective method for managing diseases in the crops has been host plant resistance. The easiest, most efficient, and cost-effective way to handle diseases is to use highly disease resistant cultivars in farming systems. These highly resistant cultivars also conserve natural resources and use less energy, time, and money in addition to these benefits. Comparative to other approaches to manage the diseases, use of resistant cultivars is an environment friendly strategy.

The growth and production of the pearl millet crop are generally hampered by the presence of pests and diseases. Blast has recently grown in significance among other diseases during the past several years. The fungus severely affects all growth stages of the plant, from seedling to adult, and has an impact on both grain yield and the dry fodder yield, consequently restricting the overall yield. Therefore, the development of hybrids with blast resistance acquires major relevance in order to raise crop productivity and production. Therefore, the present investigation was carried out to screen 60 pearl genotypes against blast disease and develop the blast resistant hybrids using the selected resistant lines as parents or as one of the parents.

The results revealed that 12 genotypes were found to be highly resistant (1.0-2.0), 18 genotypes were found resistant (2.1-4.0) and 13 were found moderately resistant (4.1-6.0). In the past, several researchers in India have made an effort to identify potential sources of resistance to the pathogen that causes pearl millet blast. 123 designated B-lines of pearl millet were examined by Goud *et al.* (2016) against five pathotypes of blast disease and found that nine lines were highly resistant to all five pathotypes. Sharma *et al.* (2013), Sharma *et al.* (2021) and Dagar *et al.* (2022) also did similar studies.

In the present investigation, the F_1 hybrids developed showed dominance for the resistance towards the blast infection. Similar outcomes have been reported by Gupta *et al.* (2012), Pawar *et al.* (2016) and Boratkar and Bhivgade (2022) in their studies concluding that if one of the parents is resistant to the blast disease, then the hybrid obtained is also resistant, which further indicates that blast resistance is governed by a single dominant gene.

5.7 Microsatellite marker analysis

To ascertain if visually based taxonomic classifications show patterns of genomic differentiation, a thorough analysis of the molecular genetic variation in germplasm is helpful. Additionally, this can give breeders knowledge about the characteristics of germplasm's population structure, allelic richness, and diversity, enabling them to utilize genetic resources for cultivar development more efficiently and with fewer pre-breeding steps. Due to the quick and high-quality data produced, germplasm characterization based on molecular markers has now become more significant. SSRs have been identified as the preferred molecular markers among all others because of their high interlaboratory reproducibility, low cost, and user-friendly design. Their popularity has increased as a result of their co-dominant nature.

In the present study, 38 SSR markers were found to show polymorphism among the pearl millet genotypes taken in the study. The primers amplified a total of 305 alleles which varied from 1 (PSMP 2217) to 16 (Xcump 0016) with a mean of 8.02 alleles per locus. This value is found to be much higher than the mean alleles per locus as reported by Budak *et al.* (2003) i.e., 5.7, Kapila *et al.* (2008) i.e., 6.26, Singh *et al.* (2013) i.e., 3.0, Sumanth *et al.* (2013) i.e., 3.07, Kumar *et al.* (2020) i.e., 4.62 and Priya *et al.* (2022) i.e., 5.37, but lesser than the value obtained by Stich *et al.* (2010) i.e., 16.4 and Bashir *et al.* (2015) i.e., 13.33, and comparable with the value obtained by Nepolean *et al.* (2012) i.e., 8.1.

The overall size of the PCR amplified products ranged from 90 bp (PGIRD 46) to 1000 bp (ICMP 3088) and the Polymorphic Information Content (PIC) values ranged from 0.100 (PSMP 2213) to 0.986 (ICMP 10) with an average of 0.66, which is higher than the value obtained by Kapila *et al.* (2008) i.e., 0.58 and Singh *et al.* (2013) i.e., 0.44. Many of the similarities between the genotypes were explained by the UPGMA dendrogram generated using the NTSYS-pc software. The 60 genotypes were clustered into 2 major clusters at similarity coefficient value of 0.51 which further formed sub clusters and sub-sub clusters. The similarity coefficient varied from 0.51 to 0.91 which shows a vast range of diversity between the genotypes similarly as presented by earlier work of Budak *et al.* (2003), Kapila *et al.* (2008) and Kumar *et al.* (2020). The dendrogram tree also revealed that the blast susceptible and resistant genotypes separated into different groups within the clusters with a few exceptions.

5.8 Enzyme activity analysis

A biochemical screening is necessary to determine the presence of enzymes, osmolytes, and antioxidants, all of which are crucial under stress situations. Genotypes showing more content of pathogenesis related defense components have more potential in resisting the stress conditions. In the present study, activities of the pathogenesis related enzymes were studied in the F_1 hybrids i.e., Phenylalanine ammonia lyase (PAL), Peroxidase (POX) and Lipoxygenase (LOX) on three consecutive days post inoculation. The mean sum of squares (ANOVA) for all the three enzymes showed significant variations within the hybrids on the respective days and on different days of sampling. Also, the value of mean sum of squares for the interaction between hybrids and number of days showed significant values inferring that there was a considerable change in the enzyme activity among various hybrids on different sampling days.

Likewise, when compared to the blast severity data, it was found that highly resistant hybrids had maximum enzyme activity whereas, highly susceptible hybrids had minimum activity. Basavaraj *et al.* (2018), in their study on the efficiency of abiotic elicitors in inducing resistance against blast disease of pearl millet found increased activities of PAL, POX and β-1,3-glucanase in resistant and inoculated seedlings after challenged with the pathogen. Similarly, Basavaraj *et al.* (2019), Lavanya *et al.* (2022), Nagarathna *et al.* (1992) and Nagarathna *et al.* (1993) also found the increased activities of defense related enzymes after challenging the material under study with the pathogen in their respective investigations.

CHAPTER – VI

SUMMARY AND CONCLUSION

The present study was conducted to screen the pearl millet genotypes for morpho-phenological traits & blast disease and to study the genetic relatedness among them using microsatellite markers. It comprised of 60 pearl millet genotypes which have been maintained by Chaudhary Charan Singh Haryana Agricultural University over the years. The experiment was carried out in two replications in randomized block design at the experimental field of Bajra section, CCS HAU, Hisar during *kharif,* 2021. The data was recorded on 5 representative plants for 10 morpho-phenological traits and yield attributes *viz.*, plant height (cm), ear length (cm), ear diameter (cm), leaf blade width (cm), effective number of tillers per plant, days to 50% flowering, days to maturity, 1000-grain weight (g), dry fodder yield per plant (g) and grain yield per plant (g).

The salient features of the findings of this investigation have been summarized below:

- The presence of substantial genetic diversity among the genotypes chosen for examination for further improvement was clearly demonstrated by mean sum of squares, which were highly significant due to genotypes for all the analysed features.
- The traits *viz.*, plant height, ear length, ear diameter, leaf blade width, effective number of tillers per plant, 1000-grain weight, dry fodder yield per plant and grain yield per plant exhibited high estimates of heritability and genetic advance as per cent of mean indicating the scope of adequate chances of selection in the germplasm lines of pearl millet for these traits.
- The genotypic level correlation coefficients for nearly all traits were larger than their corresponding phenotypic level correlation coefficients which revealed a robust inherent association between the traits.
- High significant positive correlation coefficients of the grain yield with traits like plant height, ear diameter and dry fodder yield per plant and the high direct positive effect of these traits suggest that these can directly be selected for the breeding programs.
- Principal component analysis reduced the original 10 variables into 4 major principal components explaining 76.442% of the total variation in the data. First principal component contributed 29.99% of the total variability. The second, third and fourth

principal components contributed 19.919%, 14.94% and 11.594% of total variability in the data, respectively.

- According to the factor loading after varimax rotation, it can be concluded that PF-1 can be designated as the yield factor as it showed high loadings for dry fodder yield per plant and grain yield per plant respectively.
- The promising genotypes for grain yield was found to be R-line 6, having highest grain yield per plant (91.4 g) followed by R-line 11 (45.9 g).
- In terms of blast scores, R-line 4, R-line 21, R-line 44, R-line 75, R-line 10, R-line 12, R-line 15, R-line 32, R-line 34, R-line 42, R-line 62, H13/001 were found to be highly resistant. Hence, these can be can be used as parents to develop superior hybrids in the future pearl millet breeding programmes.
- In case of F_1 hybrids, HMS 47A × R-line 10, HMS 47A × R-line 17, HMS 69A × R-line 17, HMS 04888A × EMRL-15-109, ICMA 97111 × R-line 3, ICMA 97111 × R-line 32, ICMA 97111 × 78/711, HHB 299 were found to be highly resistant and the disease reaction showed dominance.
- Among the hybrids, the traits *viz*., ear diameter, leaf blade width, effective number of tillers per plant, days to 50% flowering, days to maturity, 1000-grain weight (g), dry fodder yield per plant (g) and grain yield per plant (g) showed high heritability but not always coupled with high genetic advance as percent of mean.
- Similar to the parents, the genotypic level correlation coefficients for nearly all traits were larger than their corresponding phenotypic level correlation coefficients.
- Principal component analysis of the hybrids reduced the original 10 variables into 4 major principal components explaining 74.559% of the total variation in the data. First principal component contributed 26.07% of the total variability. The second, third and fourth principal components contributed 22.361%, 14.814% and 11.311% of total variability in the data, respectively.
- The promising hybrids for grain yield was found to be HMS 69A × R-line 17, having highest grain yield per plant (218.3 g) followed by ICMA 97111 × R-line 3 (200.1 g).
- In the molecular marker analysis, it was found that a total of 305 alleles were amplified by 38 SSR markers, which varied from 1 (PSMP 2217) to 16 (Xcump 0016) with a mean of 8.02 alleles per locus.
- The overall size of the PCR amplified products ranged from 90 bp (PGIRD 46) to 1000 bp (ICMP 3088).

- Polymorphic Information Content (PIC) values ranged from 0.100 (PSMP 2213) to 0.986 (ICMP 10)
- Estimates of genetic distance on the basis of simple matching coefficient varied from 0.51 to 0.91.
- The 60 genotypes clustered into 2 major clusters similarity coefficient value of 0.51 which further formed sub clusters and sub-sub clusters.
- R-line 2 and HTP 94/54 were found to be most diverse. The highest similarity % was shared by ICMB 02333 and ICMB 04999 (91%) followed by R-line 3 and R-line 4, H 90/4-5 and HMS 23 B & ICMB 89111 and HMS 58B i.e., 89%.

By exposing the vast range of variance for the characteristics among the pearl millet genotypes, the current work amply indicates the possibility for the large genetic gain through appropriate selection or hybridization. Given that grain yield positively correlated with a number of morphological traits, it is possible to enhance grain yield by improving one or more of these traits. Direct selection for plant height and ear diameter suggested rapid improvement in the grain yield in pearl millet genotypes.

However, since the prevalence of pests and diseases has a significant impact on grain yield, it is important to conduct a more thorough analysis of the blast severity reaction involving the current germplasm lines. The present study also demonstrates the presence of sufficient variability among R lines and B lines for blast severity reaction and a total of 43 genotypes were found resistant towards the disease & 12 among them were best performing on the basis of biochemical analysis and blast severity screening, which can be can be used in the future disease resistance breeding programs.

The molecular clustering results revealed that considerable genetic diversity exists among the selected parents, which can be used in breeding programs This study showed that the germplasm lines maintained at CCS HAU over a period contains enough variability for several traits.

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Annexure-I

Table: Blast scores of the pearl millet genotypes

S.No.	Genotypes	Blast score
1	R-line 2	2.2
2	R-line 3	2.4
3	R-line 4	1.4
4	R-line 5	3.2
5	R-line 6	6.8
6	R-line 11	6.0
7	R-line 16	3.4
8	R-line 17	2.6
9	R-line 18	3.2
10	R-line 21	1.4
11	R-line 24	4.8
12	R-line 25	3.2
13	R-line 31	7.2
14	R-line 33	5.4
15	R-line 36	4.6
16	R-line 37	5.2
17	R-line 44	1.2
18	R-line 71	7.4
19	R-line 75	1.4
20	R-line 187	6.2
21	R-line 8	2.6
22	R-line 10	1.2
23	R-line 12	1.2
24	R-line 13	2.8
25	R-line 14	2.8
26	R-line 15	1.2
27	R-line 19	3.8
28	R-line 22	3.2
29	R-line 30	4.4
30	R-line 32	1.8

31	R-line 34	1.4
32	R-line 39	7.6
33	R-line 40	2.8
34	R-line 42	1.2
35	R-line 62	1.4
36	R-line 63	4.0
37	R-line 64	6.2
38	R-line 66	6.4
39	R-line 67	5.4
40	R-line 68	6.2
41	ICMB 843-22	6.2
42	ICMB 89111	7.8
43	ICMB 95444	8.0
44	ICMB 04888	3.6
45	ICMB 97111	6.4
46	ICMB 02333	7.6
47	ICMB 04999	6.2
48	H77/833-2-202	5.6
49	HTP 94/54	6.8
50	HBL-11	5.8
51	AC 04/13	4.8
52	G73-107	4.4
53	H77/29-2	4.8
54	H13/0001	1.2
55	H90/4-5	5.4
56	HMS 70B	7.4
57	HMS 23B	2.6
58	HMS 58B	6.8
59	HMS 33B	2.4
60	EBL-12-237	2.2

Annexure-II

Table: Blast scores of the pearl millet hybrids

S.No.	Hybrids	Blast score
1	HMS 47A × HPT-2-12-32	2.2
2	HMS 47A × HB15/085	2.4
3	HMS 47A × R-line 2	2.8
4	HMS 47A × R-line 10	1.2
5	HMS 47A × R-line 17	1.4
6	HMS 47A × R-line 49	2.6
7	HMS 47A × H78/711	2.2
8	HMS 47A × SGP-10-107	4.6
9	HMS 74A × R-line 4	2.4
10	HMS 74A × 78/711	4.6
11	ICMA 04999 × ISK-51	5.2
12	ICMA 04999 × SGP-10-107	5.8
13	ICMA 04999 × EMRL 15/109	2.2
14	ICMA 04999 × R-line 17	2.4
15	ICMA 04999 × 78/711	2.6
16	ICMA 94555 × EMRL 15/109	4.8
17	HMS 69A × SGP-10-107	3.4
18	HMS 69A × R-line 17	1.2
19	HMS 04888A × EMRL-15-109	1.6
20	HMS 48A × R-line 17	3.8
21	HMS 48A × SGP-10-107	3.8
22	HMS 48A × 78/711	5.2
23	HMS 02333 × R-line 2	5.2
24	HMS 02333 × R-line 3	4.2
25	HMS 02333 × R-line 17	4.4
26	HMS 02333 × R-line 49	2.2
27	HMS 02333 × ISK-51	6.2

28	ICMA 97111 × R-line 2	6.4
29	ICMA 97111 × R-line 17	4.4
30	ICMA 97111 × R-line 3	1.4
31	ICMA 97111 × R-line 32	1.4
32	ICMA 97111 × R-line 49	3.2
33	ICMA 97111 × ISK-51	2.8
34	ICMA 97111 × 78/711	1.6
35	HHB 67 improved	3.4
36	HHB 197	2.8
37	HHB 226	3.8
38	HHB 299	1.6

ABSTRACT

Title of Thesis	:	Molecular characterization for targeting foliar blast disease in pearl millet [*Pennisetum glaucum* (L.) R. Br.]
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Admission No.	:	2019BS32D
Title of Degree	:	Doctor of Philosophy
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Year of Award of degree	:	2023
Major subject	:	Molecular Biology & Biotechnology
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Keywords: Pearl millet, blast disease, genetic variability, SSR markers

In the present investigation, 60 pearl millet genotypes were evaluated at experimental field of Bajra Section, CCS HAU, Hisar during *kharif* season, 2021 for morpho-phenological traits & blast disease and the study of genetic relatedness among them using microsatellite markers. Highly significant mean sum of squares were obtained due to the genotypes for all the traits studied. The traits *viz.*, plant height, ear length, ear diameter, leaf blade width, effective number of tillers per plant, 1000-grain weight, dry fodder yield per plant and grain yield per plant exhibited high heritability (>60%) and high genetic advance as percent of mean (GA%M) (>20%) whereas, the traits ear length, leaf blade width, effective number of tillers per plant, dry fodder yield per plant and grain yield per plant exhibited high Genotypic Coefficient of Variation (GCV) (>20%) and Phenotypic Coefficient of Variation (PCV) (>20%). Correlation coefficient analysis revealed high significant positive correlation coefficients of grain yield with traits *viz.*, plant height, ear diameter and dry fodder yield per plant. As per blast screening, 43 genotypes were found resistant to the disease while 17 genotypes were found susceptible. In order to develop blast resistant hybrids, selected resistant lines were crossed with elite lines of pearl millet and out of the 34 hybrids developed, 32 were found resistant to blast. The increased activities of Phenylalanine Ammonia Lyase (PAL), Peroxidase (POX) and Lipoxygenase (LOX) in the resistant hybrids further confirmed the disease scoring results.

MAJOR ADVISOR **DEGREE HOLDER**

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Symposium/Conference:

- Kamboj, A., Yashveer, S., Yadav, D.V. and Kumar, V. (2022). Screening of pearl millet genotypes against foliar blast disease through SSR markers and field scoring. In: National symposium on 'Crop protection through bio-rational approaches – current trends and future perspective' & annual meeting of Indian Phyto-pathological society (NZ), March 10, 2022, Hisar, India. (Abstract book: S-II-MAP-021).
- Kamboj, A., Yashveer, S., Yadav, D.V. and Kumar, V. (2023). Molecular characterization for screening of pearl millet genotypes against foliar blast disease. In: National conference on 'Sustainable Development through Agriculture Production, Protection & Policy Landscape for crop care, January 18-19, 2023, MVN University, Palwal, Haryana.

(Aarti Kamboj)

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