# Original Research Article

**Integrative Evaluation of Pearl Millet Restorer Lines for Blast Resistance Using Phenotypic Screening and Gene Specific SSR Markers**

**Abstract**

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is a climate-resilient cereal crop cultivated extensively in arid and semi-arid regions, particularly in India and sub-Saharan Africa. Despite its tolerance to harsh abiotic stresses, productivity is limited by an array of biotic factors, especially blast disease caused by *Magnaporthe grisea,* leading to significant yield losses by affecting leaves, stems and panicles. The present investigation aimed to evaluate 77 diverse pearl millet restorer lines for putative resistance against blast disease by screening under field conditions and employing gene-specific Simple Sequence Repeat (SSR) markers. The field experiment was conducted during the *Kharif*, 2023 at RVSKVV, Gwalior, in a randomized complete block design with two replications. Blast resistance screening was carried out under natural epiphytotic conditions, and disease severity was rated using a modified 1–9 scale. Among the evaluated restorer lines, 13 genotypes found to be highly resistant, including R-20876, R-23774, R-24665 and R-20212 owing to their negligible blast symptoms. Concurrently, molecular analysis was performed employing 20 SSR markers. Of the 77 genotypes, several exhibited high to moderate resistance, while others ranged between susceptible to highly susceptible. Five SSR markers revealed moderate to high polymorphism, with major allele frequency ranging from 0.402 to 0.571, gene diversity from 0.557 to 0.701, and PIC values from 0.479 to 0.646. Marker Xpgird 49 was proved the most informative. The study highlights the potential of integrating phenotypic and molecular screening to identify resistant and genetically diverse genotypes. These findings can be instrumental in breeding programmes aimed to develop high-yielding, blast-resistant pearl millet cultivars for sustainable agriculture in disease-prone regions.

**Keywords:** Blast disease, Disease resistance, Field screening, *Magnaporthe grisea*, Molecular characterization, Pearl millet (*Pennisetum glaucum*)

1. **Introduction**

*Pennisetum glaucum* (L.) R. Br. is a crucial staple cereal crop cultivated predominantly in the semi-arid and arid regions of the world (Satyavathi et al., 2021; Reddy et al., 2021; Makwana et al., 2022). Belonging to the Poaceae family, it is a hardy, climate-resilient species capable of withstanding extreme environmental conditions such as high temperatures, prolonged drought, and nutrient-deficient soils (Choudhary et al., 2021a; Rajpoot et al.,2023a; Karthik et al., 2024; Khandelwal et al., 2024). It is rich in carbohydrates and serves as an excellent source of energy and resistant starch (RS), containing approximately 92.5% dry matter. The composition includes 5–7% fat, 2.1% ash, and 1.2 g dietary fibre per 100 g. It also provides 13.6% crude protein, with quality protein ranging between 8–19%, and a significant starch content of 63.2%. Additionally, it exhibits α-amylase activity and is a valuable source of essential minerals (2.3 mg/100 g), vitamins A and B, and antioxidant compounds such as coumaric acid and ferulic acid (Goswami et al., 2020; Rajpoot et al., 2023b; Patel et al., 2023a). Due to these unique adaptive traits, pearl millet plays a vital role in the food, fodder, and nutritional security of millions of smallholder farmers, especially in South Asia and sub-Saharan Africa (Rajpoot et al., 2024; Riahi et al., 2024; Ramashia et al., 2025). In India, it is predominantly cultivated during the *Kharif* season in states like Rajasthan, Gujarat, Maharashtra, Haryana, and Uttar Pradesh, often under rainfed conditions (Yadav & Rai, 2013; Patel et al., 2023b; Garin et al., 2023).

Despite its remarkable tolerance to abiotic stresses, the productivity of pearl millet remains suboptimal, largely due to biotic constraints, among which blast disease caused by the fungal pathogen *Magnaporthe grisea* (syn. *Pyricularia grisea*) is of significant concern (Parihar et al., 2022; Parmar et al., 2022; Makwana et al., 2023; Poonacha et al., 2023).This pathogen has emerged as one of the most destructive foliar diseases in pearl millet, especially under warm and humid conditions that favour its proliferation (Adhikari et al., 2020; Singh et al., 2021a; Parihar et al., 2023; Patel et al., 2024a). The disease primarily affects the leaves but can also extend to nodes, stems, and panicles, severely impacting plant health and grain yield (Patel et al., 2024b; Andargie et al., 2025). Initial symptoms appear as small, water-soaked lesions that rapidly enlarge into elliptical or spindle-shaped spots with greyish centres and brown margins (Singh et al., 2021). As the infection progresses, these lesions coalesce, causing extensive leaf blighting, premature senescence, and in severe cases, complete defoliation. When the pathogen infects the panicle, it leads to peduncle lesions, reduced grain filling, and in extreme cases, panicle sterility (Islam et al., 2020; Verma et al., 2021; Poonacha et al., 2023). Such widespread tissue damage disrupts photosynthesis and nutrient translocation, culminating in significant yield losses. The rapid evolution of the pathogen and limited availability of resistant cultivars pose challenges for disease management (Chauhan et al., 2023; McLaughlin et al., 2023).

To address these challenges, the identification and deployment of blast-resistant genotypes are crucial for sustainable crop production (Mbinda et al., 2021; Ojha et al., 2024). Molecular markers, particularly Simple Sequence Repeats (SSRs), provide a powerful tool for assessing genetic diversity and identifying resistance sources within germplasm collections (Mishra et al., 2020; Rajpoot et al., 2020; Shyam et al., 2020; Upadhyayet al., 2020; Choudhary et al., 2021b; Mishra et al., 2021). SSR markers offer high polymorphism, co-dominant inheritance and genome-wide distribution, making them suitable for genetic diversity studies and marker-assisted selection in breeding programmes (Nadeem et al., 2018; Bhattarai et al., 2021; Liu et al., 2025). The present study aimed to evaluate 77 pearl millet restorer lines for their putative resistance against blast disease screening under field conditions and employing gene-specific SSR markers. The integration of phenotypic screening with molecular characterization helps in understanding the relationship between genetic makeup and disease resistance, and facilitates the identification of promising restorer line (s) for utilizing them in future breeding programmes to develop blast disease resistance. The generated information will aid in broadening the genetic base of pearl millet and contribute to the development of high-yielding, blast-resistant cultivar (s).

**2. Material & Methods**

**2.1 Experimental Site**

The field experiment was conducted at the experimental field, College of Agriculture, Rajmata Vijayaraje Scindia Agricultural University, Gwalior M.P., India during *kharif*, 2023, while molecular work was carried out at Plant Molecular Biology Laboratory, Department of Plant Molecular Biology and Biotechnology, College of Agriculture, Rajmata Vijayaraje Scindia Agricultural University, Gwalior, Madhya Pradesh, India. The weather conditions were normal during the crop season with an average maximum and minimum temperature during growth remaining at 35.2°C and 24.5°C, respectively. The total rainfall received during the crop growing period from July to October 2022 was 907.7 mm. Rainfall was observed scanty and unevenly distributed during the crop growing period. The field exhibited uniform topography and comprised medium-black soil, devoid of waterlogging circumstances, thereby offering a favourable agro-ecological environment for the successful cultivation of pearl millet.

**2.2 Experimental Details**

The experiment was conducted using a Randomized Block Design (RBD) with two replications. A total of 77 restorer lines (Table1) of *Pennisetum glaucum* (L.) R. Br. with divergent reactions to blast disease *viz*., susceptible, tolerant, and resistant obtained from ICRISAT, Hyderabad, Telangana, India. Each genotype was sown in a single row with an inter-row spacing of 40 cm and intra-row spacing of 10 cm, allowing sufficient space for optimal plant growth, light interception, and aeration. The length of each row was maintained at 4 meters, ensuring an adequate plot size for the reliable assessment of disease incidence. Uniform agronomic practices, including weeding, pest control and fertilization, were meticulously followed across all experimental plots to ensure proper crop establishment and to facilitate the accurate evaluation of the genetic potential of each genotype against blast disease.

**Table 1 List of pearl millet restorer lines used in the investigation**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **S. No.** | **Genotype** | **S. No.** | **Genotype** | **S. No.** | **Genotype** | **S. No.** | **Genotype** | **S. No.** | **Genotype** | **S. No.** | **Genotype** |
| 1 | R-20002 | 14 | R-20206 | 27 | R-20846 | 40 | R-24657 | 53 | R-25511 | 66 | R-21283 |
| 2 | R-20012 | 15 | R-20208 | 28 | R-20871 | 41 | R-24658 | 54 | R-25850 | 67 | R-21355 |
| 3 | R-20057 | 16 | R-20212 | 29 | R-23906 | 42 | R-24659 | 55 | R-25865 | 68 | R-21370 |
| 4 | R-20079 | 17 | R-20218 | 30 | R-23956 | 43 | R-24661 | 56 | R-25910 | 69 | R-21930 |
| 5 | R-20090 | 18 | R-20443 | 31 | R-23565 | 44 | R-24662 | 57 | R-26462 | 70 | R-21970 |
| 6 | R-20103 | 19 | R-20444 | 32 | R-24610 | 45 | R-24663 | 58 | R-26482 | 71 | R-22013 |
| 7 | R-20105 | 20 | R-20671 | 33 | R-24650 | 46 | R-24664 | 59 | R-26492 | 72 | R-22713 |
| 8 | R-20108 | 21 | R-20706 | 34 | R-24651 | 47 | R-24665 | 60 | R-26607 | 73 | R-22768 |
| 9 | R-20111 | 22 | R-20710 | 35 | R-24652 | 48 | R-24666 | 61 | R-27738 | 74 | R-22908 |
| 10 | R-20113 | 23 | R-20810 | 36 | R-24653 | 49 | R-24667 | 62 | R-20876 | 75 | R-22918 |
| 11 | R-20115 | 24 | R-20822 | 37 | R-24654 | 50 | R-25046 | 63 | R-21111 | 76 | R-22968 |
| 12 | R-20204 | 25 | R-20823 | 38 | R-24655 | 51 | R-25056 | 64 | R-21121 | 77 | R-23774 |
| 13 | R-20205 | 26 | R-20826 | 39 | R-24656 | 52 | R-25086 | 65 | R-21136 |  |  |

**2.3 Disease Screening under Field conditions**

Field screening for blast disease incidence was conducted under natural epiphytotic conditions, following the protocol standardized by ICRISAT (Wilson et al., 1989). Each genotype was sown in four central rows, while a highly susceptible check line was planted in the first row and every fifth row to serve as infector/indicator rows. To enhance disease pressure, seedlings were spray-inoculated at both pre-tillering and pre-flowering stages with an aqueous spore suspension until run-off. High humidity conditions (>90% RH), essential for disease development, were maintained using sprinkler irrigation twice daily-once in the morning (10:00–11:00 AM) and once in the late afternoon (5:00–6:00 PM) on non-rainy days. Disease severity was assessed at the hard-dough stage using a modified 1–9 progressive scale originally developed by the International Rice Research Institute for rice blast. Based on this scale, genotypes were categorized into different disease reaction classes (Table 2):

**Table 2 Scale for scoring of blast disease of pearl millet restorer lines (Wilson et al., 1989)**

|  |  |  |
| --- | --- | --- |
| **Rating**  **scale** | **Symptomsandlesions** | **Diseasereaction** |
| 1 | No lesion too small brown specks of a pin head size. | Highly resistant |
| 2-3 | Large brown specks.  Small, roundish to slightly elongated, necrotic grey lesion.  Spots are about1-2 mm in diameter with a brown margin. | Resistant |
| 4-5 | Typical blast lesions, elliptical,1-2cm long, usually confined to the area between main veins, covering <2%of the leaf area.  Typical blast lesions cover <10% of the leaf area. | Moderately resistant |
| 6-7 | Typical blast covering10-25%of the leaf area. Typical blasts cover 26-50% of the leaf area. | Susceptible |
| 8-9 | Typical blast lesions cover 51-75% of the leaf area and are many leaves dead.  >75%leaf area covered with lesions and most leaves dead. | Highly Susceptible |

**2.4 Molecular analysis**

A total of 20 gene-specific Simple Sequence Repeat (SSR) markers were employed for screening 77 pearl millet restorer lines for blast (Table 3). Genomic DNA was extracted from fresh, young leaves using the modified CTAB method (Thompson & Murray, 1980) with minor modification as suggested by Tiwari et al. (2017). DNA quality and quantity were assessed *via* Nanodrop spectrophotometry and 0.8% agarose gel electrophoresis. The DNA samples were diluted to a working concentration of 25 ng/µl using TE buffer or nuclease-free water. PCR amplification was performed in a 10 µl reaction volume containing 30 ng of template DNA, 10 pmol each of forward and reverse primers, 1X PCR buffer, 0.1 µl of Taq DNA polymerase, and 0.1 mM dNTPs. The thermal cycling conditions were optimized per primer annealing temperature. Amplified products were resolved on 3% agarose gels prepared in 1X TBE buffer, stained with ethidium bromide (10 mg/ml), and visualized under UV light using a gel documentation system (Syngene, USA). A 100 bp DNA ladder was used as a molecular weight marker. Electrophoresis was carried out at 110 V for 2 hours to ensure proper resolution of alleles.

**2.5 Statistical analysis**

The PCR products generated by SSR were investigated by scoring qualitatively for presence or absence of bands. Distance matrix was computed based on shared alleles. With the help of the power marker v3.25 programme (Liu & Mouse, 2025), the major allelic frequency, polymorphism information content and genetic distance-based clustering were carried out. The dendrogram was constructed based on unweighted pair group method for arithmetic average (UPGMA).

**Table 3 Gene-specific Simple Sequence Repeat (SSRs*)* molecular markers employed to screen out the pearl millet restorer lines against blast (Terensan *et al.,* 2021)**

|  |  |  |  |
| --- | --- | --- | --- |
| **S. No.** | **Marker name (SSR)** | **Forward sequence** | **Reverse sequence** |
| 1 | **Xpsmp2001** | CATGAAGCCAATTAGGTCTC | ACCATCTGACTTGTTCTTATCCCTCA |
| 2 | **Xpsmp2008** | GATCATGTTGTCATGAATCACC | ACACTACACCTACATACGCTCC |
| 3 | **Xpsmp2030** | ACCAGAGCTTGGAAATCAGCAC | CATAATGCTTCAAATCTGCCCACACCAGA |
| 4 | **Xpsmp2043** | TCATATTCTCCTGTCTAAAACGTC | ACAAATCGTACAAGTTCCACTCCAGA |
| 5 | **Xpsmp2063** | GAGCACATGAAATAGGAAG | AAGGTAGTTATAGTTAGCTTGATC |
| 6 | **Xpsmp2071** | TTGCAGTCCCACGAATTATTTG | CTATGAATTTATAATCCTGATACT |
| 7 | **Xpsmp2076** | GGAATAGTATATTGGCAAAATGTG | ATACTACACCTGTAAGCATTGTC |
| 8 | **Xpsmp2267** | GGAAGGCGTAGGGATCAATCTCAC | ATCCACCCGACGAAGGAAACGA |
| 9 | **Xpsmp2090** | AGCAGCCCAGTAATACCTCAGCTC | AGCCCTAGCGCACAACACAAACTC |
| 10 | **Xpsmp2248** | TCTGTTTGTTTGGGTCAGGTCCTTC | CGAATACGTATGGAGAACTGCGCATC |
| 11 | **Xpsmp2237** | TGGCCTTGGCCTTTCCACGCTT | CAATCAGTCCGTAGTCCACACCCCA |
| 12 | **Xpsmp2231** | TTGCCTGAAGACGTGCAATCGTCC | AACGGACTTCTGCACTTAGCAGG |
| 13 | **Xpsmp2027** | AGCAATCCGATAACAAGGAC | AGCTTTGGAAAAGGTGATCC |
| 14 | **Xpsmp2236** | ATAAGTGGGACCCACATGCAGCAC | CGAAAGACTAGCAAAATTGCGCCTTCTGT |
| 15 | **Xpsmp2261** | AATGAAAATCCATCCCATTTCGCC | CGAGGACGAGGAGGGCGATT |
| 16 | **Xpsmp2263** | AAAGTGAATACGATACAGGAGCTGA | GCATTTCAGCCGTTAAGTGAGACAA |
| 17 | **Xpsmp2266** | CAAGGATGGCTGAAGGGCTATG | TTTCCAGCCCACACCAGTAATC |
| 18 | **Xpsmp2273** | AACCCCACCAGTAAGTTGTGCTGC | GATGACGACAAGACCTTCTCTCC |
| 19 | **Xpgird13** | CAGCAGCGAGAAGTTTAGCA | GCGTAGACGGCGTAGATGAT |
| 20 | **Xpgird49** | AGCTCCTCGACGGAGAAAGT | GACGGTGTCGACGAAGATG |

**3. Results & Discussion**

**3.1 Field Evaluation**

A total of 77 pearl millet restorer lines were evaluated under field conditions to assess their reaction to blast disease using a 1–9 rating scale. Based on disease severity, the genotypes were categorized into five distinct classes (Table 4; Table5; Fig.1). Thirteen genotypes, including R-20876, R-23774, R-24665, R-20212, R-25511, R-20204, R-26607, R-20218, R-20002, R-22968, R-21355, R-21121 and R-20822, exhibited no or minimal symptoms and were classified as highly resistant. Genotypes categorized as highly resistant exhibited negligible or no disease symptoms, suggesting the presence of strong, stable resistance mechanisms that effectively hinder pathogen establishment and progression (Das et al., 2021; Kumar et al., 2021; Parihar et al., 2022; Parmar et al., 2022). These genotypes likely possess major resistance genes or effective gene combinations conferring broad-spectrum resistance, making them promising candidates for use in resistance breeding programmes (Ning et al., 2020; Pedrozo et al., 2025).

Thirteen genotypes such as R-20111, R-26462, R-24667, R-20057, R-20103, R-20443, R-21283, R-20012, R-24650, R-20115, R-21370, R-20113, and R-20108 were found to be resistant, showing limited lesion development. While moderate resistance was observed in nineteen genotypes, including R-23956, R-24654, R-25850, R-23906, R-25086, R-24655, R-21111, R-20706, R-20105, R-20079, R-26492, R-22768, R-20206, R-20205, R-20090, R-24657, R-21136, R-24659, and R-24658, which exhibited typical blast lesions covering less than 10% of the leaf area. Genotypes with resistant and moderately resistant reactions demonstrated reduced lesion development and limited disease spread, indicating partial or polygenic resistance. This form of resistance, often governed by quantitative trait loci (QTLs), tends to be more durable and stable across diverse environments. Such genotypes can play a pivotal role in pyramiding resistance genes to develop cultivars with long-lasting resistance (Fukuoka et al., 2015; Pilet-Nayel et al., 2017; Jiang et al., 2020; Ontoy et al., 2023).

Sixteen genotypes, *viz*., R-24663, R-25046, R-24653, R-20810, R-27738, R-25865, R-24666, R-20671, R-24651, R-22713, R-20871, R-20826, R-20208, R-24652, R-22013 and R-21930, were categorized as susceptible with moderate to severe lesion coverage (10–50% of leaf area). While sixteen genotypes, namely: R-23565, R-21970, R-20846, R-25910, R-20710, R-24610, R-24664, R-22908, R-26482, R-25056, R-24661, R-24656, R-24662, R-20444, R-22918, and R-20823, were found to be highly susceptible, exhibiting severe disease symptoms with more than 50% of the leaf area affected and widespread leaf necrosis.

Susceptible and highly susceptible genotypes showed extensive blast symptoms, including widespread foliar lesions and necrosis, indicating either the absence of effective resistance genes or the breakdown of resistance under high inoculum pressure (Devanna et al., 2022; Jeevan et al., 2023; Vasquez-Teuber et al., 2024). These genotypes are less desirable for cultivation in blast-prone areas but may still serve as useful checks or parental lines in studies focused on resistance gene discovery or functional validation (Hafees et al., 2021; Simon et al., 2023). Similar studies have also been conducted by Verma et al. (2021), Kumar et al. (2022) and Moghariya et al. (2024). The observed variability in disease response among the genotypes underscores the importance of field-based screening for reliable identification of resistant lines. Moreover, integration of phenotypic screening with molecular characterization can enhance the precision and efficiency of resistance breeding strategies in pearl millet, ultimately contributing to the development of resilient cultivars for sustainable production in blast-endemic regions (Yadav & Rai, 2013; Bidyananda et al., 2024; Mishra et al., 2024; Singh et al., 2024).

**Table 4 Categorization of pearl millet genotypes based on disease scoring**

|  |  |  |
| --- | --- | --- |
| **Rating Scale** | **Restorerlines** | **Reaction to blast disease** |
| 1 | R-20876, R-23774, R-24665, R-20212, R-25511, R-20204, R-26607, R-20218, R-20002, R-22968, R-21355, R-21121 and R-20822 | Highly resistance |
| 2-3 | R-20111, R-26462, R-24667, R-20057, R-20103, R-20443, R-21283, R-20012, R-24650, R-20115, R-21370, R-20113 and R-20108 | Resistant |
| 4-5 | R-23956, R-24654, R-25850, R-23906, R-25086, R-24655, R-21111, R-20706, R-20105, R-20079, R-26492, R-22768, R-20206, R-20205, R-20090, R-24657, R-21136, R-24659 and R-24658 | Moderate resistance |
| 6-7 | R-24663, R-25046, R-24653, R-20810, R-27738, R-25865, R-24666, R-20671, R-24651, R-22713, R-20871, R-20826, R-20208, R-24652, R-22013 and R-21930 | Susceptible |
| 8-9 | R-23565, R-21970, R-20846, R-25910, R-20710, R-24610, R-24664, R-22908, R-26482, R-25056, R-24661, R-24656, R-24662, R-20444, R-22918 and R-20823 | Highly susceptible |

|  |  |
| --- | --- |
| **RESISTANT.jpeg** | **MODERATELY RESISTANT.jpeg** |
| **Resistant** | **Moderately Resistant** |
| **SUSCEPTIBLE.jpeg** | **HOGHLY SUSCEPTIBLE.jpeg** |
| **Susceptible** | **Highly Susceptible** |
| **Fig.1: Severity symptoms of blast disease on pearl millet leaves** | |

**Table 5 Classification of pearl millet restorer lines against foliar blast based on disease severity**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Genotypes** | **Category** | **Genotypes** | **Category** | **Genotypes** | **Category** |
| R-20002 | HR | R-23956 | MR | R-26492 | MR |
| R-20012 | R | R-23565 | HS | R-26607 | HR |
| R-20057 | R | R-24610 | HS | R-27738 | S |
| R-20079 | MR | R-24650 | R | R-20876 | HR |
| R-20090 | MR | R-24651 | S | R-21111 | MR |
| R-20103 | R | R-24652 | S | R-21121 | HR |
| R-20105 | MR | R-24653 | S | R-21136 | MR |
| R-20108 | R | R-24654 | MR | R-21283 | R |
| R-20111 | R | R-24655 | MR | R-21355 | HR |
| R-20113 | R | R-24656 | HS | R-21370 | R |
| R-20115 | R | R-24657 | MR | R-21930 | S |
| R-20204 | HR | R-24658 | MR | R-21970 | HS |
| R-20205 | MR | R-24659 | MR | R-22013 | S |
| R-20206 | MR | R-24661 | HS | R-22713 | S |
| R-20208 | S | R-24662 | HS | R-22768 | MR |
| R-20212 | HR | R-24663 | S | R-22908 | HS |
| R-20218 | HR | R-24664 | HS | R-22918 | HS |
| R-20443 | R | R-24665 | HR | R-22968 | HR |
| R-20444 | HS | R-24666 | S | R-23774 | HR |
| R-20671 | S | R-24667 | R |  |  |
| R-20706 | MR | R-25046 | S |  |  |
| R-20710 | HS | R-25056 | HS |  |  |
| R-20810 | S | R-25086 | MR |  |  |
| R-20822 | HR | R-25511 | HR |  |  |
| R-20823 | HS | R-25850 | MR |  |  |
| R-20826 | S | R-25865 | S |  |  |
| R-20846 | HS | R-25910 | HS |  |  |
| R-20871 | S | R-26462 | R |  |  |
| R-23906 | MR | R-26482 | HS |  |  |

**3.2 Disease Screening Employing SSR Molecular Markers**

A total of 20 allele-specific SSR markers were employed to assess genetic diversity among pearl millet genotypes. Only 5 SSR proved to be polymorphic (Table 6; Fig. 2). The major allele frequency across the markers ranged between 0.402 to 0.571, with a mean value of 0.454, indicating moderate allele distribution among the genotypes. The numbers of alleles per marker varied from 3 to 7, with an average of 4.2 alleles, suggesting a moderate level of polymorphism. Gene diversity, which reflects the probability that two randomly chosen alleles are different in the population, arrayedbetween 0.557 (Xpsmp 2267) to 0.701 (Xpgird 49), with a mean worth of 0.650. This indicates considerable genetic variability captured by the selected markers. The polymorphic information content (PIC), an indicator of the informativeness of a marker, ranged from 0.479 to 0.646, with a mean PIC value of 0.586. Among the markers, Xpgird 49 showed the highest gene diversity (0.701) and PIC (0.646), making it the most informative marker in this study. These findings highlight the utility of SSR markers in evaluating genetic variability among pearl millet genotypes. The moderate to high levels of gene diversity and PIC values observed suggested that the selected markers are robust and informative (Das & Baisakh, 2023; Jorben et al., 2024; Ambawat et al., 2025). The genetic variation uncovered through these markers can be utilized for identifying diverse parental lines, constructing genetic linkage maps, and performing marker-assisted selection (MAS) for desirable traits, such as disease resistance and stress tolerance (Salgotra & Stewart, 2020; Hasan et al., 2021; Kushanov et al., 2021). The informative nature of markers makes them particularly valuable for future molecular breeding efforts in pearl millet (Yadav et al., 2021; Srivastava et al., 2022; Gunguniya et al., 2023). Similar studies have also been reported by Verma et al. (2021), Singh et al. (2021b), Rajput et al. (2023) and Parihar et al. (2023). Overall, the SSR markers used were effective in detecting genetic diversity among pearl millet genotypes, and the observed variability can be exploited in marker-assisted breeding programmes for trait improvement (Kapadia et al., 2014; Jorben et al., 2024; Kumar et al., 2024).

**3.3 Phylogenetic cluster analysis**

The genetic similarity among pearl millet genotypes was examined based on molecular investigation using UPGMA tree constructed with the application of power Marker v3.25 software (Fig. 3). All the genotypes were divided into 2 main clusters *i.e.,* one major and one minor cluster. Minor cluster contained 21 genotypes while major cluster had 56 pearl millet genotypes. Minor cluster was further divided into two sub clusters. Major sub cluster had 17 genotypes namely: R-20002, R-20090, R-24655, R-20208, R-20710, R-20671, R-24653, R-24661, R-20111, R-20113, R-20871, R-22013, R-23565, R-24610 and R-26482. However, minor sub cluster had only four genotypes including R-21136, R-24662, R-21355 and R-24654. The major cluster was further divided into two sub clusters. Minor sub cluster had only one genotype *viz*., R-27738 and remaining 55 genotypes were grouped into major sub cluster. Separation of R-27738 indicates higher genetic diversity of this particular genotype from rest of the genotypes at DNA level. The major sub cluster was further divided into two groups. The major group had 51 genotypes and minor group had only two genotypes including R-25850 and R-26607. Similar to the present investigation, Budak et al. (2003) utilized the pair group approach with arithmetic averages (UPGMA) to analyze the genetic diversity of 53 millet lines. The results showed that UPGMA could be used to easily distinguish between the millet germplasm lines, revealing two major and eight smaller clusters. In a study, Ambawat et al. (2020) used a cluster analysis based on SSR markers to classify the genotypes into nine clusters, with similarity coefficients ranging from 0.58 to 0.73. In the study of Verma et al. (2021), the genotypes were separated into seven distinct clusters.

Many agronomically important qualities are known to be caused by polygenic traits, which restrict breeding at the molecular level (Choudhary et al., 2021b). The limited success of molecular plant breeding of traits with genes with major effects can be attributed to a number of factors, including the inability to use a wider variety of crop genetic resources, linkage drag associated with larger chromosomal regions selection, and challenges using molecular markers with lower genome coverage. Therefore, theoretical and technological advancements are required to improve the breeding system. Crop breeding has been transformed since the beginning of time by the introduction of innovative molecular tools including genome editing, NGS, and high throughput phenomics.

**Table 6 Allele specific SSR markers presenting major allele frequency, numbers of alleles, gene diversity and Polymorphic Information Content (PIC)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Marker** | **Major Allele Frequency** | **AlleleNo** | **Gene Diversity** | **PIC** |
| Xpsmp 2063 | 0.454545 | 7 | 0.660145 | 0.602488 |
| Xpsmp 2267 | 0.571429 | 3 | 0.557261 | 0.479094 |
| Xpsmp 2027 | 0.402597 | 4 | 0.684432 | 0.62671 |
| Xpgird 13 | 0.441558 | 3 | 0.648339 | 0.5752 |
| Xpgird 49 | 0.402597 | 4 | 0.700624 | 0.645856 |
| **Mean** | **0.454545** | **4.2** | **0.65016** | **0.58587** |

|  |
| --- |
| MOLECULAR MARKER 1.jpeg |
| **Fig. 2: Allelic variation using gene-based markers Xpsmp 2063 showing polymorphism among pearl millet restorer lines** |

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**Fig. 3: Dendrogram of 77 pearl millet restorer lines based on banding pattern analysis of gene based SSR markers using MEGA 6.0 software**

**Conclusion**

The present investigation successfully identified significant variability among 77 pearl millet restorer lines in their response to blast disease under natural field conditions. A subset of genotypes displayed high to moderate levels of resistance, indicating their potential as valuable sources of resistance for future breeding efforts. Molecular characterization using gene-based SSR markers further revealed moderate to high genetic diversity, with markers such as Xpgird 49 proved highly informative. The integration of phenotypic screening with molecular marker analysis not only validated the resistance levels but also provided insights into the underlying genetic diversity among the restorer lines. These findings can be effectively utilized in marker-assisted selection (MAS) and resistance breeding programme aimed to develop durable, high-yielding, and blast-resistant pearl millet cultivars. This approach may contribute to improving crop productivity and resilience, especially in blast-endemic and climate-stressed areas.

**Disclaimer (Artificial Intelligence)**

Author (s) hereby declares that No generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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