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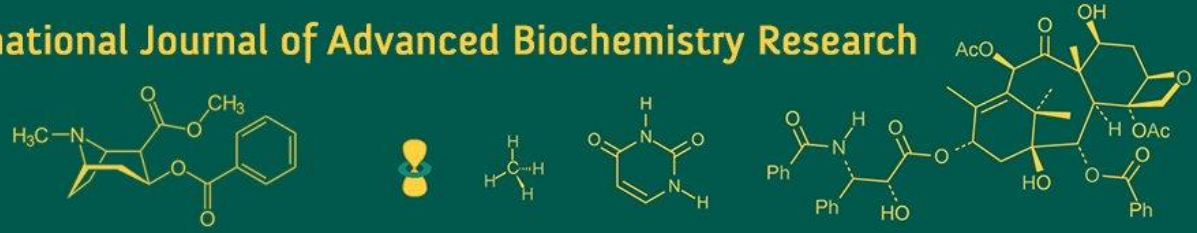

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Genetic studies for blast disease in a biparental RIL population of pearl millet

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Abstract
Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is a vital cereal crop widely cultivated in arid and semi-arid regions due to its adaptability and high nutritional value. In this study, two hundred and ninety recombinant inbred lines of pearl millet were evaluated to investigate quantitative traits and blast resistance across two environments, Patancheru (ENV-I) and Vizianagaram (ENV-II). Principal Component Analysis identified 14 Principal Components (PCs), with the first five explaining 65.50%, 59.00%, and 65.35% of the total variance under ENV-I, ENV-II and pooled conditions respectively. The analysis revealed that the Blast Score positively contributed to the first Principal Component (PC1), while Stem Girth showed a positive contribution exclusively in ENV-II. Conversely, traits such as Days to 50% Flowering, Days to Maturity, Panicle Length, Panicle Diameter, Plant Stand, Plant Height, Number of Leaves, Harvest Index, Seed Yield, Thousand Seed Weight, Number of Tillers, and Number of Productive Tillers negatively influenced the PC1 across ENV-I, ENV-II, and pooled conditions, with Stem Girth being the exception in ENV-II. Additionally, the observed 1:1 ratio of resistant to susceptible lines under glasshouse conditions suggests that resistance to blast disease in the pearl millet population is likely controlled by a single monogenic factor. This comprehensive analysis enhances our understanding of the genetic and agronomic interactions in pearl millet, providing valuable insights for breeding programs aimed at improving both disease resistance and crop performance.

Keywords: Principal component analysis (PCA), chi-square (χ^2), blast resistance

Introduction
Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is a highly cross-pollinated, diploid (2n=14) annual C4 crop indigenous to central tropical Africa. Its cultivation is widespread across the arid and semi-arid regions of Africa and India, where it is valued for its resilience and minimal agricultural input requirements. It exhibits tremendous genetic diversity due to its wide distribution across the world, its adaptability to harsh environmental conditions, and its cross-pollination mechanism with protogynous flowering (Satyavathi *et al*. 2013; Singh *et al*. 2013) [14, 17]. The crop's inherent biodiversity and high productivity make it a preferred choice for farmers in India (Krishnan and Meera, 2018) [7]. As a staple food, pearl millet is crucial for meeting the nutritional needs of large populations, especially in developing and underdeveloped regions of Asia and Africa (Annor *et al*., 2017) [2].

The primary goal of plant breeding programs is to enhance crop productivity, typically measured as yield per unit area. In pearl millet, one of the significant constraints to achieving high yields is blast disease, caused by the fungal pathogen *Pyricularia grisea* (Cooke) Sacc., with its teleomorph *Magnaporthe grisea*. This disease can lead to severe yield losses, making the development of blast-resistant varieties essential for sustainable cultivation. Understanding the genetic mechanisms underlying blast resistance is crucial for breeding programs aimed at reducing the disease's impact. Evaluating genotypes against various fungal pathotypes under controlled and field conditions is key to uncovering resistance inheritance patterns and selecting robust lines for breeding.

To advance the development of blast-resistant pearl millet varieties, it is essential to analyze the relationship between agronomic traits and resistance mechanisms. Understanding the genetic diversity within the population is crucial for effective population grouping and selection (Nachimuthu *et al*., 2014) [11].

One of the most effective approaches for this analysis is employing advanced analytical methods, and Principal Component Analysis (PCA) is an especially powerful tool. PCA helps identify the minimum number of components that explain the maximum variability within the dataset (Anderson, 1972; Morrison, 1978) [1, 10]. In this study, PCA is employed to assess and quantify the relationships among traits in an F_7 recombinant inbred line population selected for blast resistance, aiming to identify the principal components that significantly influence both agronomic performance and blast resistance.

In addition to PCA, the Chi-square test is employed to determine the inheritance patterns of blast resistance. This statistical analysis helps in understanding the genetic basis of resistance and its segregation in the RIL population. By integrating PCA and Chi-square test results, the study aims to offer a comprehensive approach to breeding for improved blast resistance in pearl millet, ultimately contributing to the development of varieties with enhanced resistance and better yield stability.

Materials and Methods

The research material for this study on blast resistance against *Magnaporthe grisea* (anamorph: *Pyricularia grisea*) comprised 288 F_7 recombinant inbred lines (RILs). This population was derived from a cross between two parental lines: ICMR 100844, known for its resistance to blast, and ICMB 95444, which is susceptible. These parents were chosen for their diverse genetic backgrounds in blast resistance and were crossed to produce the F_7 generation using the single seed descent method at ICRISAT, Patancheru.

For phenotypic evaluation, 290 RILs, including the two parental lines, were assessed at two locations: ICRISAT, Patancheru, Hyderabad (ENV-I) and the Agricultural Research Station, Vizianagaram, Andhra Pradesh (ENV-II) during the kharif season of 2023. The experiment followed an Alpha Lattice Design with two replications. Each RIL was planted in two rows, each 2 meters long, with a spacing of 60 × 10 cm between plants. Standard agronomic practices were implemented to ensure optimal crop growth. Data were collected on five plants per plot for various traits, including Days to 50% Flowering (DFF), Days to Maturity (DM), Plant Stand (PS), Plant Height (PH), Number of Tillers (NT), Number of Productive Tillers (NPT), Number of Leaves (NL), Panicle Length (PL), Panicle Diameter (PD), Stem Girth (SG), Thousand Seed Weight (TSW), Seed Yield per plant (SY), Harvest Index (HI), and Blast Score (BS).

To assess the resistance of this population in glass house conditions, two diverse, highly virulent pathotypes of *Magnaporthe grisea* were utilized: Pg 293, collected from Jaipur (Rajasthan), and Pg 289, from Alwar (Rajasthan). Phenotyping was conducted at ICRISAT, Hyderabad, under controlled glasshouse conditions in 2023. The inoculum for these pathotypes was prepared following the method described by Sharma *et al*. (2013) [15]. The experimental design was a Completely Randomized Design (CRD) with two replicates, each consisting of one pot with 12 seedlings. The seeds were sown in 10 cm diameter pots filled with a sterilized soil mixture (soil: sand: farmyard manure in a 2:1:1 ratio) and kept at a temperature of 30±1 °C for 12 days. Prior to inoculation, spore suspensions were adjusted to 1×10^5 spore/mL, with Tween 20 (0.02% v/v) added for uniform spore dispersal. Fourteen-day-old seedlings were inoculated via spray and then covered with polythene bags to prevent cross-contamination. After 24 hours at 25 °C, the pots were transferred to a glasshouse with high humidity (>90%) maintained through mist irrigation for 4 days. Foliar blast severity was assessed 6 days post-inoculation using a 1-9 scale, where lines with a score ≤3.0 were classified as resistant, 3.1-5.0 as moderately resistant, 5.1-7.0 as susceptible, and >7.0 as highly susceptible as per Sharma *et al*. (2013) [15].

Principal component analysis was performed to assess the complex statistical variables as described by Hostelling (1993) [4]. The chi-square test (P≤0.05) was used to compare the ratio of observed resistant to susceptible plants in the segregating populations under both field conditions and artificial epiphytotic conditions. Best Linear Unbiased Predictors (BLUPs) were estimated for all the traits from individual and pooled environments. Principal Component Analysis (PCA) were performed in R 4.4.0 with" "prcomp" function. This comprehensive study includes a Principal Component Analysis (PCA) of all evaluated traits to elucidate the underlying patterns of variability and correlation among them. Additionally, chi-square tests were employed to assess the inheritance patterns of blast resistance, providing insights into the genetic architecture and inheritance mechanisms of the trait.

Results and Discussion

Principal Component Analysis (PCA)

Principal Component Analysis is a multivariate technique that reduces the dimensionality of a dataset by transforming a large number of potentially correlated variables into a smaller number of uncorrelated variables, called principal components (PCs), which capture the majority of the variability in the original data set (Hostelling, 1933) [4]. The Eigen values, percent variance, percent cumulative variance and factor loading of different characters studied are presented in (Table 1). In this study, PCA was performed on the dataset, generating a total of fourteen principal components. The first five PCs accounted for 65.50%, 59.00%, and 65.35% of the total variance with Eigenvalue greater than 1.00 under ENV-I, ENV-II, and Pooled conditions, respectively, indicating that these components effectively represent the essential features of the dataset (Table 1), making them essential in understanding the trait interactions related to blast resistance in pearl millet.

In Principal Component Analysis (PCA), eigenvalues represent the amount of variance explained by each principal component, with higher eigenvalues indicating components that capture more variability in the data (Figure 3).

PC1 (x-axis) explained the greatest proportion of variance, accounting for 19.90%, 17.10%, and 19.90% of the total variance in ENV-I, ENV-II, and Pooled, respectively (Fig. 1a, 1b, 1c). Under ENV-I, ENV-II, and Pooled, Blast Score (0.37, 0.26, 0.32) contributed positively to PC1, whereas Stem Girth (0.01) also had a positive contribution in ENV-II. Conversely, Days to 50% Flower (-0.06, -0.24, -0.16), Days to Maturity (-0.11, -0.27, -0.23), Panicle Length (-0.02, -0.12, -0.08), Panicle Diameter (-0.39, -0.20, -0.34), Plant Stand (-0.16, -0.14, -0.17), Plant Height (-0.40, -0.34, -0.38), Number of Leaves (-0.18, -0.18, -0.21), Harvest Index (-0.19, -0.38, -0.28), Seed Yield (-0.47, -0.44, -0.46), Stem Girth(-0.18, -0.12), Thousand Seed Weight (-0.28, -

0.17, -0.23), Number of Tillers (-0.21, -0.33, -0.25), and Number of Productive Tillers (-0.24, -0.31, -0.25) contributed negatively to PC1 in ENV-I and Pooled, with SG (0.01) being an exception in ENV-II.

PC2 (y-axis) accounted for 17.70%, 13.20%, and 17.10% of the total variance in ENV-I, ENV-II, and Pooled, respectively (Table 1). TSW (-0.07, -0.07, -0.10), SY (-0.08, -0.11, -0.13), HI (-0.18, -0.03, -0.11), NT (-0.31, -0.35, -0.35), and NPT (-0.42, -0.46, -0.45) contributed negatively to PC2, while DFF (0.49, 0.52, 0.46), DM (0.42, 0.43, 0.37), PL (0.35, 0.36, 0.40), SG (0.27, 0.16, 0.27), PD (0.18, 0.08, 0.14), PH (0.14, 0.12, 0.13), NL (0.11, 0.04, 0.07), BS (0.03, 0.09, 0.06) and PS (0.09, 0.02) (except ENV-I, -0.04) had positive contributions across ENV-I, ENV-II, and Pooled environment.

PC3 showed variability in contributions across environments. In ENV-I, DFF, DM, NT, NPT, NL, PL, and SY were positively associated with PC3, whereas PS, PH, PD, SG, TSW, HI, and BS were negatively associated. In ENV-II, DFF, DM, PH, NT, NPT, NL, SG, TSW, and BS had positive contributions, while PS, PL, PD, SY, and HI were negatively associated. For the Pooled data, PS, PH, PD, SG, TSW, HI, SY, and BS were positively associated with PC3, whereas DFF, DM, NT, NPT, NL, and PL were negatively associated (Table 1).

PC4 highlighted different variables as significant in each environment. In ENV-I, DFF, PH, NT, NPT, NL, PD, and TSW contributed positively, while DM, PS, PL, SG, SY, HI, and BS contributed negatively. In ENV-II, DFF, DM, PS, NT, NPT, NL, PL, SY, and HI were positively associated, whereas PH, PD, SG, TSW, and BS were negatively associated. In the Pooled dataset, DFF, PH, NT, NPT, NL, PD, SG and TSW were positively associated with PC4, while DM, PS, PL, SY, HI, and BS were negatively associated (Table 1).

PC5 revealed a mixed pattern of contributions. In ENV-I, DFF, DM, PS, PH, and NL contributed negatively, while NT, NPT, PL, PD, SG, TSW, SY, HI, and BS were positively associated. In ENV-II, DFF, DM, NT, NPT, PL, PD, SG, TSW, SY, HI, and BS were positively associated, with PS, PH, and NL contributing negatively. For the Pooled dataset, DM, PH, NT, NPT, PL, PD, SG, TSW, SY, HI, and BS were positively associated with PC5, whereas DFF, PS, and NL were negatively associated (Table 1).

The positive contribution of Blast Score (BS) to the first principal component (PC1) across all environments suggests that blast resistance is a primary factor influencing overall variation within the population. The unique positive contribution of Stem Girth (SG) in ENV-II to PC1, in contrast to its negative impact in other environments, underscores the environmental specificity of this trait's relationship to blast resistance.

Conversely, agronomic traits such as Days to 50% Flowering (DFF), Days to Maturity (DM), Panicle Length (PL), Panicle Diameter (PD), Plant Stand (PS), Plant Height (PH), Number of Leaves (NL), Harvest Index (HI), Seed Yield (SY), Thousand Seed Weight (TSW), Number of Tillers (NT), and Number of Productive Tillers (NPT) negatively influenced PC1 in most conditions (Fig. 2a, 2b, 2c). This negative association suggests that a higher score of blast (Susceptible) is often coupled with a reduction in these agronomic traits, highlighting a potential trade-off between yield components and blast resistance in the selection process.

In the second principal component (PC2), traits like TSW, SY, HI, NT, and NPT contributed positively, indicating their significant role in contributing to yield, while the negative contribution of traits such as BS, DFF, and DM reflects their inverse relationship with these yield components.

In the PCA analysis, Blast Score (BS) showed positive contributions to both PC1 and PC2, aligning with the findings of Kandel *et al*. (2020) [6] and Suman *et al*. (2019) [18]. For Days to First Flower (DFF) and Days to Maturity (DM), positive contributions to different principal components were consistent with the results of Pujar *et al*. (2020) [13] and Shashibhushan *et al*. (2022) [16]. Similarly, Panicle Length (PL), Panicle Diameter (PD), Plant Height (PH), Number of Tillers (NT), and Thousand Seed Weight (TSW) displayed positive associations in various principal components, as reported by Pujar *et al*. (2020) [13], Shashibhushan *et al*. (2022) [16], Gupta *et al*. (2022) [3] and Kumar *et al*. (2022) [8]. Conversely, the negative contributions of DFF, DM, and PH to PC1, and Seed Yield (SY) to PC2, are supported by Pujar *et al*. (2020) [13] and Shashibhushan *et al*. (2022) [16]. Additionally, the negative contributions of PL and NT are corroborated by Gupta *et al*. (2022) [3].

Table 1: PCA estimates, eigen value and the percent variance contribution of agronomic traits and blast disease score

	PCs	Eigen Values	Variance %	Cumulative Variance	DFF	DM	PS	PH	NT	NPT	NL	PL	PD	SG	TSW	SY	HI	BS
ENV-I	1	2.79	19.92	19.92	-0.06	-0.11	-0.16	-0.40	-0.21	-0.24	-0.18	-0.02	-0.39	-0.18	-0.28	-0.47	-0.19	0.37
	2	2.48	17.69	37.60	0.49	0.42	-0.04	0.14	-0.31	-0.42	0.11	0.35	0.18	0.27	-0.07	-0.08	-0.18	0.03
	3	1.53	10.90	48.51	0.33	0.38	-0.22	-0.14	0.52	0.38	0.05	0.22	-0.30	-0.15	-0.30	0.04	-0.04	-0.08
	4	1.33	9.47	57.98	0.01	-0.07	-0.30	0.24	0.16	0.07	0.21	-0.20	0.12	-0.15	0.33	-0.30	-0.70	-0.11
	5	1.05	7.52	65.50	-0.15	-0.19	-0.63	-0.07	0.08	0.11	-0.39	0.35	0.19	0.40	0.13	0.10	0.02	0.14
ENV-II	1	2.40	17.13	17.13	-0.24	-0.27	-0.14	-0.34	-0.33	-0.31	-0.18	-0.12	-0.20	0.01	-0.17	-0.44	-0.38	0.26
	2	1.84	13.15	30.28	0.52	0.43	0.09	0.12	-0.35	-0.46	0.04	0.36	0.08	0.16	-0.07	-0.11	-0.03	0.09
	3	1.57	11.25	41.53	0.29	0.25	-0.03	0.01	0.42	0.35	0.20	-0.09	-0.01	0.06	0.10	-0.47	-0.52	0.08
	4	1.33	9.50	51.03	0.19	0.24	0.01	-0.48	0.10	0.14	0.07	0.01	-0.60	-0.09	-0.45	0.13	0.20	-0.09
	5	1.12	7.97	59.00	0.03	0.15	-0.69	-0.05	0.14	0.10	-0.47	0.10	0.02	0.45	0.06	0.09	0.10	0.09
POOLED	1	2.78	19.88	19.88	-0.16	-0.23	-0.17	-0.38	-0.25	-0.25	-0.21	-0.08	-0.34	-0.12	-0.23	-0.46	-0.28	0.32
	2	2.39	17.10	36.98	0.46	0.37	0.02	0.13	-0.35	-0.45	0.07	0.40	0.14	0.27	-0.10	-0.13	-0.11	0.06
	3	1.51	10.79	47.77	-0.35	-0.36	0.15	0.23	-0.45	-0.34	-0.07	-0.21	0.40	0.11	0.35	0.06	0.07	0.09
	4	1.37	9.79	57.55	0.09	-0.03	-0.22	0.25	0.22	0.12	0.22	-0.12	0.18	0.01	0.36	-0.37	-0.67	-0.09
	5	1.09	7.80	65.35	-0.08	0.002	-0.72	0.002	0.08	0.06	-0.52	0.27	0.10	0.25	0.18	0.14	0.08	0.04

DFF - Days to 50% Flowering, DM - Days to Maturity, PS - Plant Stand, PH - Plant Height, NT - Number of Tillers, NPT - Number of Productive Tillers, NL - Number of Leaves, PL - Panicle Length, PD - Panicle Diameter, SG - Stem Girth, TSW - Thousand Seed Weight, SY - Seed Yield, HI - Harvest Index, BS - Blast Score

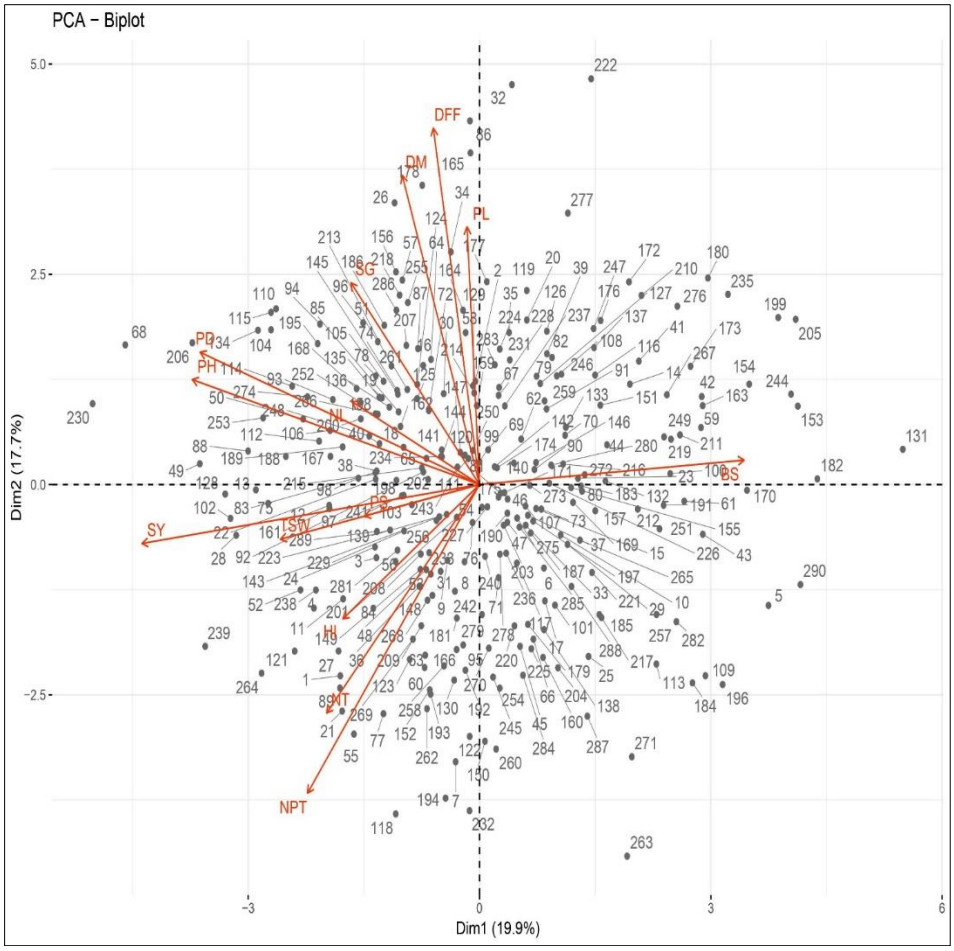


Fig 1a: Biplot showing the relative position of RIL population based on PCA scores under ENV-I

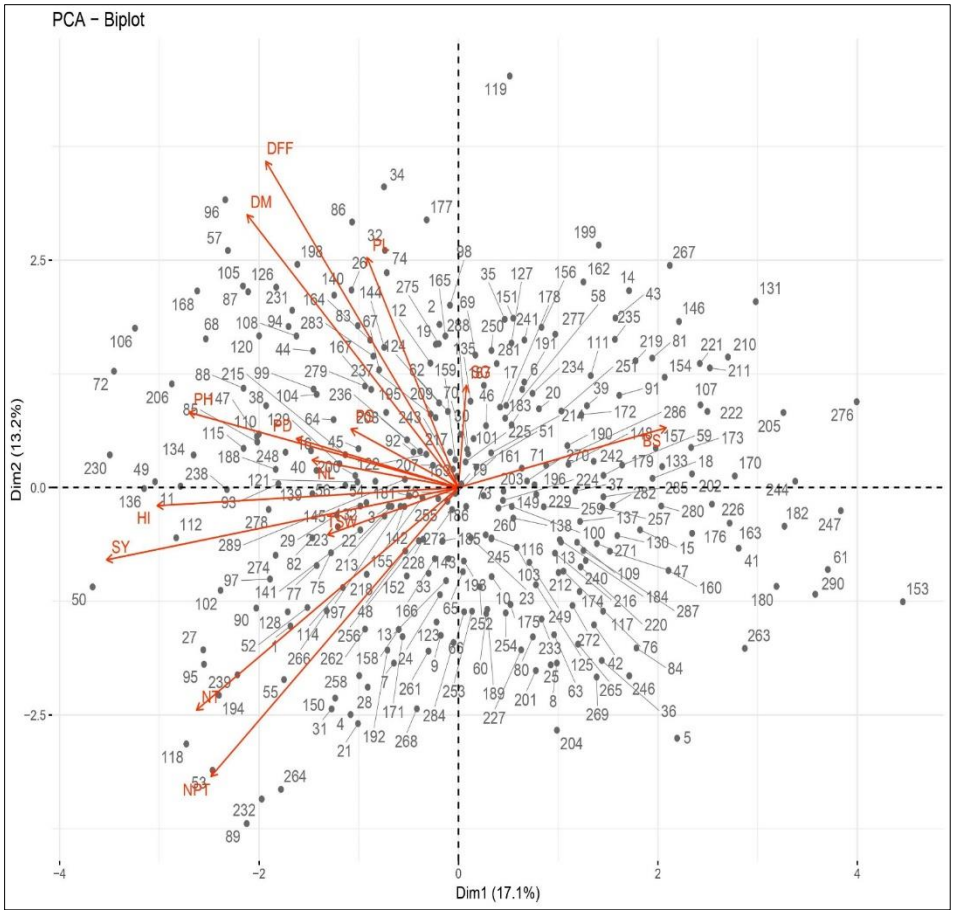
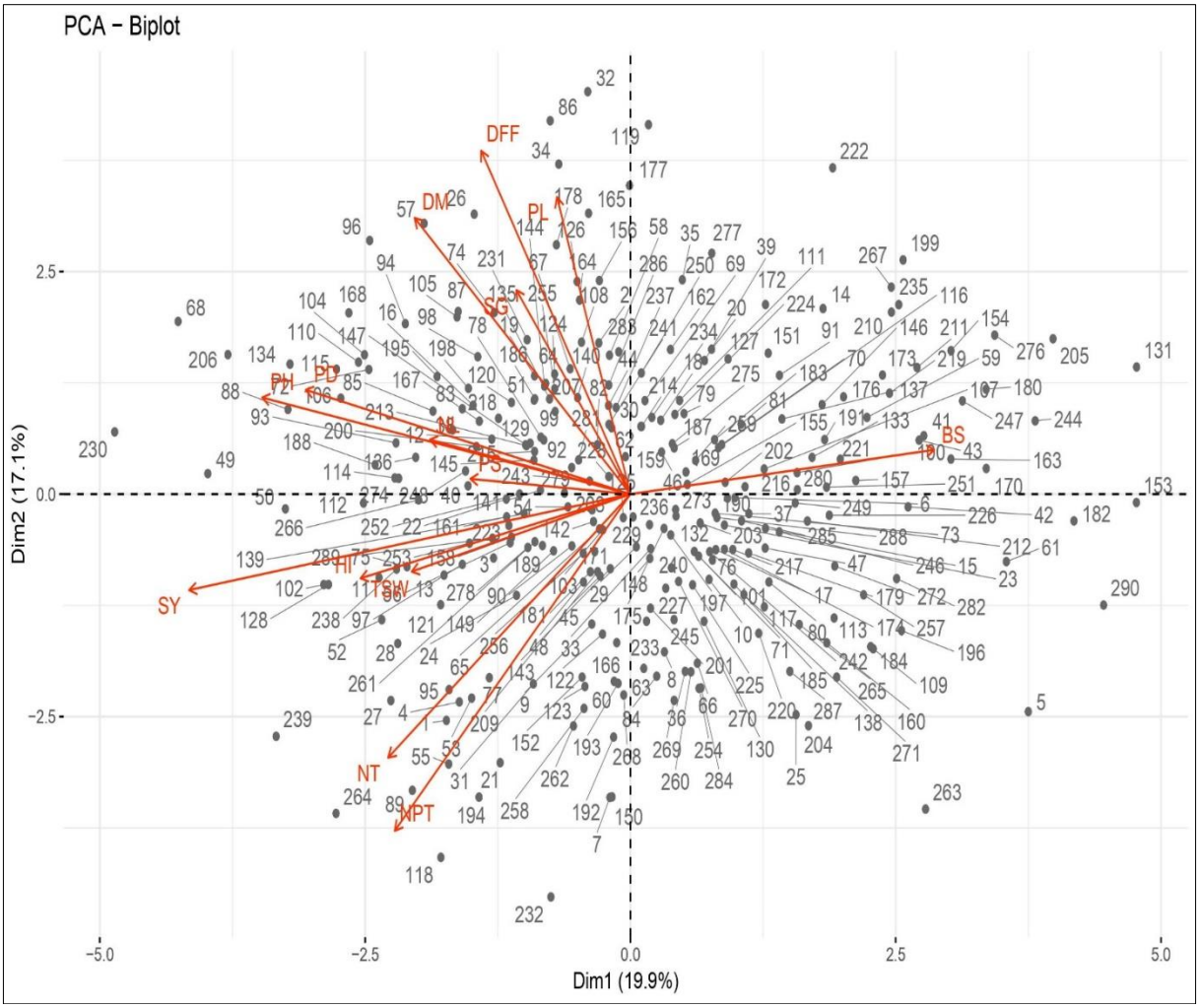
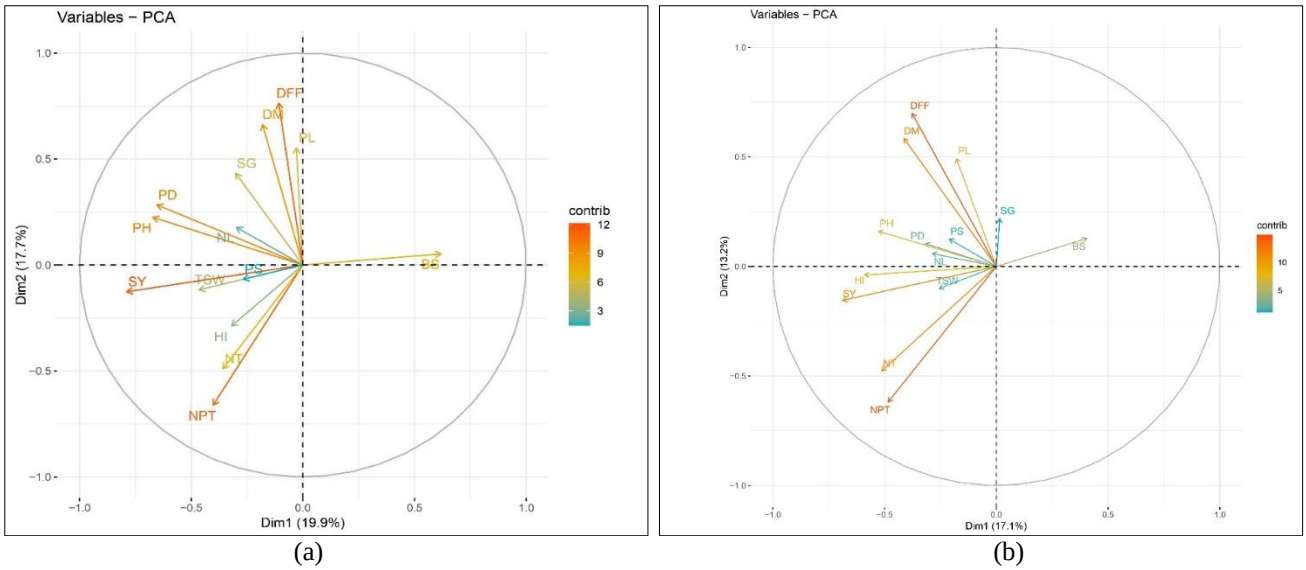


Fig 1b: Biplot showing the relative position RIL population based on PCA scores under ENV-II

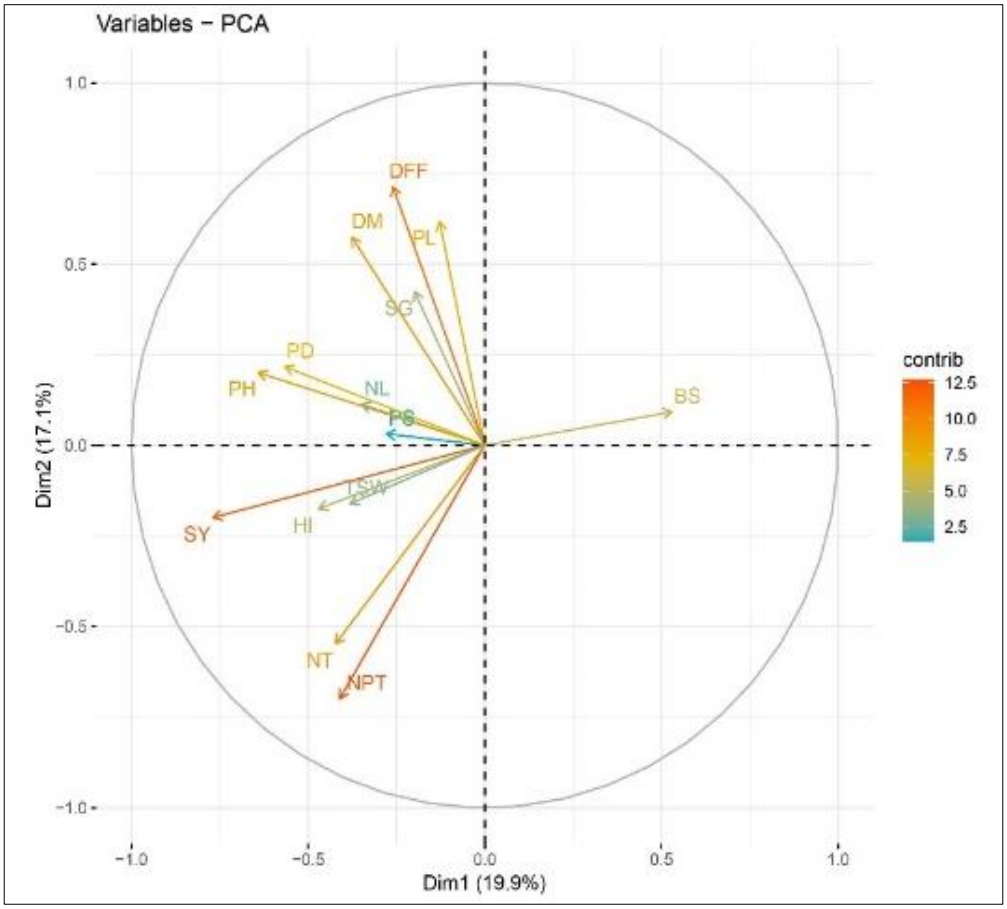


DFF - Days to 50% Flowering, DM - Days to Maturity, PS - Plant Stand, PH - Plant Height, NT - Number of Tillers, NPT - Number of Productive Tillers, NL - Number of Leaves, PL - Panicle Length, PD - Panicle Diameter, SG - Stem Girth, TSW - Thousand Seed Weight, HI - Harvest Index, BS - Blast Score

Fig 1c: Biplot showing the relative position of RIL population based on PCA scores under pooled environment



(a) (b)



(c)

Fig 2a, 2b, 2c: PCA for 14 agronomic traits and blast score in ENV-I, ENV-II and pooled environment

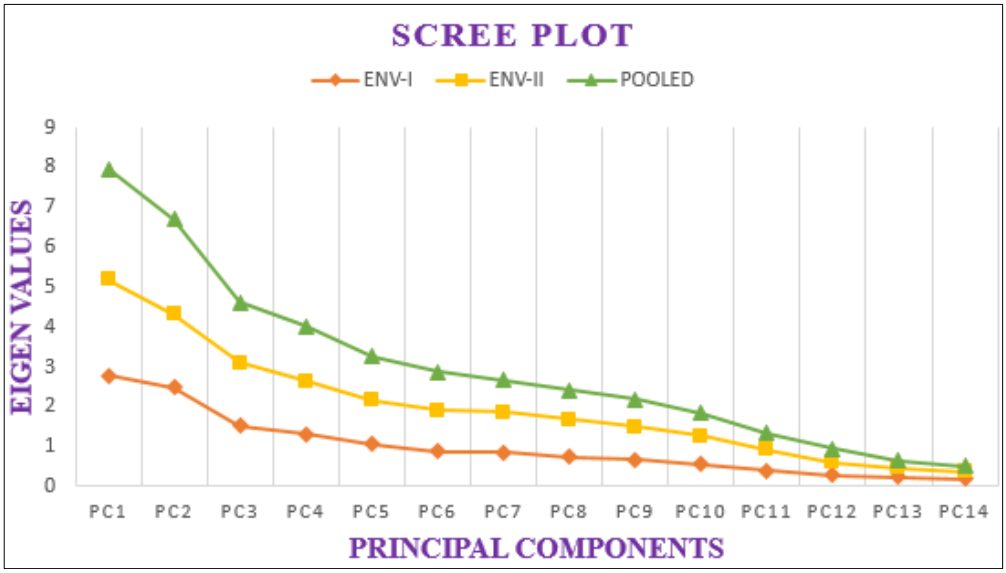


Fig 3: Scree plot showing the eigenvalue variation against respective principal components

Chi-Square (χ^2) Test

Table 2: Genetic analysis of blast resistance from the F_7 RIL Population

LOCATION	Observed (O) Resistant	Observed (O) Susceptible	Expected (E) Resistant	Expected (E) Susceptible	$\chi^2 = ((O-E)^2/E)_R + (O-E)^2/E)_S)$
Pg 293 (GH)	154	134	144	144	1.388
Pg 289 (GH)	137	151	144	144	0.680
Field-I	182	106	144	144	20.055
Field-II	176	112	144	144	14.222

The degrees of freedom (Number of Categories -1) is 2-1=1 (since there is one category) at a significance level of 0.05, the critical value is 3.84

The Chi-square (chi^2) analysis provides insight into the distribution of resistance and susceptibility across different locations.

1. Field-I exhibited a high Chi-square value of 20.055 (Table 2), indicating a significant deviation from the expected distribution of resistant and susceptible individuals. This suggests a substantial variation in the observed resistance compared to what was anticipated based on the expected numbers.
2. Filed-II also showed a notable Chi-square value of 14.222 (Table 2), which reflects a considerable discrepancy between observed and expected values. Similar to Filed-I, this indicates that Filed-II has significant factors affecting resistance or susceptibility.
3. Pg 293 (GH) and Pg 289 (GH) displayed lower Chi-square values of 1.388 and 0.680, respectively (Table 2). These values are indicating that the observed frequencies of resistant and susceptible individuals align more closely with the expected frequencies. This suggests that the resistance and susceptibility distribution at these locations is more consistent with what was anticipated, with fewer deviations from the expected outcomes.

Null Hypothesis (H_0): Resistance to blast disease in pearl millet is equally distributed in the RIL population with a 1:1 ratio of resistant to susceptible individuals, i.e., 144 resistant and 144 susceptible.

Alternative Hypothesis (H_1): Disease resistance is not equally distributed among the RILs.

Rejection of H_0 indicates a significant deviation from the expected 1:1 ratio, with observed data showing substantial differences in resistance levels at specific locations i.e.,182 resistant and 106 susceptible at Field-I; 176 resistant and 112 susceptible at Field-II. So, the observed distribution of resistance is significantly different from the expected equal distribution, suggesting that resistance is not uniformly distributed across the RIL population.

Acceptance of H_0 indicates the observed counts of resistant and susceptible RILs (154 resistant and 134 susceptible at Pg 293; 137 resistant and 151 susceptible at Pg 289 are close to the expected 1:1 ratio (144 resistant and 144 susceptible), with differences likely attributable to random variation. The Chi-square values (1.388 for Pg 293 and 0.680 for Pg 289) are below the critical value of 3.84, indicating that the differences between observed and expected counts are not statistically significant. Therefore, the null hypothesis, which posits that resistance is equally distributed, is accepted.

The observed 1:1 ratio of resistant to susceptible RILs under glasshouse conditions suggests that resistance to blast disease in the pearl millet RIL population is likely controlled by a single monogenic factor. This indicates Mendelian inheritance, with the trait segregating into an equal number of resistant and susceptible individuals. The consistent 1:1 ratio aligns with the expectation of monogenic control, supporting the hypothesis that the resistance gene is either dominant or recessive. Minor deviations are likely due to random variation rather than complex genetic or environmental factors. This monogenic resistance is corroborated by Jia *et al*. (2009) [5], Zheng *et al*. (2016) [19], and Mallik *et al*. (2021) [9].

The observed deviation from a 1:1 ratio of resistant to susceptible RILs in field conditions, with most RILs showing moderate resistance, may be attributed to the lower pathogen virulence in the field compared to the highly virulent pathogen isolates used in glasshouse conditions. In the field, reduced pathogen virulence could result in less pronounced disease expression, allowing more RILs to show moderate resistance rather than a clear resistance-susceptibility dichotomy. This contrasts with the glasshouse, where the high virulence of the pathogen isolates likely produces a more defined 1:1 distribution of resistance, reflecting a more straightforward monogenic inheritance pattern. In field settings, environmental variables such as temperature, humidity, and soil conditions may vary significantly, influencing disease prevalence and the expression of resistance. Furthermore, genotype-environment interactions and differences in management practices between field and glasshouse conditions may impact the observed distribution of resistance.

Conclusion

The comprehensive analysis of the F_7 recombinant inbred line (RIL) population of pearl millet revealed critical insights into the genetic and environmental factors influencing blast resistance and associated agronomic traits. The Principal Component Analysis (PCA) demonstrated that the first five principal components captured a significant proportion of the total variance, with Blast Score (BS) being a key contributor to the first principal component (PC1) across all environments, emphasizing its importance in assessing disease resistance. The positive contribution of Stem Girth (SG) to PC1 in ENV-II further highlights the complex interaction between traits under different environmental conditions.

The study's findings indicate that blast resistance in this pearl millet population is likely controlled by a single monogenic factor, as suggested by the observed 1:1 ratio of resistant to susceptible RILs under controlled glasshouse conditions. However, the deviation from this ratio in field conditions, where most RILs exhibited moderate resistance, underscores the influence of environmental factors and pathogen virulence on resistance expression.

Genetically, these results suggest that while major gene(s) may govern blast resistance, their expression can be significantly affected by environmental conditions, which also modulate the expression of other agronomic traits. Therefore, breeding programs aiming to develop blast-resistant pearl millet varieties should consider both the genetic and environmental components of resistance. By focusing on RILs that combine strong blast resistance with favorable agronomic traits, breeders can develop robust, high-yielding varieties capable of thriving across diverse growing conditions, ultimately contributing to the sustainable cultivation of pearl millet.

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