



Unraveling stability in rice genotypes for resistance against leaffolder (*Cnaphalocrocis medinalis*) under varied environmental conditions

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Abstract

Rice, serving as a crucial staple food for more than half of the global population, confronts significant challenges from insect pests, particularly the rice leaf folder (*Cnaphalocrocis medinalis*), which leads to substantial yield losses. This study meticulously evaluates 94 rice landraces for leaffolder resistance under diverse environmental conditions using genotype \times environment (G \times E) interaction analysis. The additive main effects and multiplicative interaction model and genotype and genotype \times environment interaction (GGE) biplot are employed to assess stability and resistance traits. The findings reveal that 44 genotypes demonstrate stable resistance across three test environments (*Kharif*, 2021, *Rabi*, 2021–2022 and *Kharif*, 2022). Resistance is primarily governed by genotypic factors (89.93%), with minimal influence from the environment (5.22%). The GGE biplot highlights distinct mega-environments, identifying *Kharif*, 2021, followed by *Kharif*, 2022, as the ideal test environments. This study identifies specific stable resistant genotypes that could prove valuable for sustainable pest management and contribute to further crop improvement programs.

Keywords AMMI model · GGE biplot · Landrace · Leaffolder · Rice · Stable resistance

Introduction

Rice holds a paramount position in global agriculture, serving as a vital staple food for more than half of the world's population, particularly in Asia. According to the Food and Agriculture Organization (FAO) of the United Nations, rice is a primary source of calories for over 3.5 billion people, contributing to food security and nutritional well-being (FAO 2022). Insect pests pose a formidable challenge to rice cultivation, exerting a direct and often detrimental influence on yield up to 25–30% loss (Salim et al. 2001). Rice leaf

folder (*Cnaphalocrocis medinalis*) stands out as a prominent insect pest that significantly contributes to 63–80% yield losses in rice cultivation (Rajendaran et al. 1986; Murugesan and Chelliah 1987). The larvae of this species exhibit a distinctive behavior of rolling and folding rice leaves, creating protective shelters as they feed on the leaf tissues. This feeding habit disrupts the normal physiological processes of the rice plant, impairing photosynthesis and nutrient assimilation. The resultant damage leads to reduced leaf area and reduced grain-filling capacity (Murugesan and Chelliah 1983; Padmavathi et al. 2013). The economic consequences of rice leaf folder infestations underscore the importance of effective pest management strategies, including the use of resistant rice varieties, biological control agents and application of chemical pesticides, to mitigate the pest. Using chemical insecticides for rice leaffolder control raises environmental and food safety concerns, with the potential to disrupt ecological balance. While biological control agents could be an alternative option, but encounter challenges like continual field application, mass rearing, field application and ability of the biocontrol agents to locate the host. Hence, Host Plant Resistance (HPR) emerges as a sustainable substitute, involving cultivating resistant rice varieties to

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address leaffolder issues without the drawbacks of chemical and biological methods (Khus and Brar 1991). The importance of resistance varieties in managing leaffolder cannot be overstated. Developing and cultivating rice varieties with inherent resistance to this insect pest is a key strategy to mitigate yield losses and promote sustainable agriculture.

The process of screening for insect resistance in natural field conditions is intricate, given the fluctuations in insect populations across both time and space. Understanding the genotype \times environment (G \times E) interaction is essential for identifying stable resistance genotypes. Valuable tools for exploring G \times E interactions include the additive main effects and multiplicative interaction (AMMI) model and the Genotype and genotype \times environment interaction (GGE) biplot. The AMMI model, as proposed by Gauch (2006), treats environment (E), genotype (G) and their interaction (G \times E) as distinct parameters for effective evaluation. In contrast, the GGE biplot method developed by Yan and Kang (2003) assesses genotype (G) and genotype-environment interaction (GE). The AMMI model enhances data precision by mitigating structural variations among genotypes in different environments. Concurrently, the GGE parameter offers comprehensive insights for genotype selection, facilitating the choice of location- or season-specific genotypes and the assessment of general adaptation (Gauch 2006).

Keeping these in mind, the present study was conducted to evaluate rice genotypes against leaffolder under various environmental condition to identify stable resistant genotypes for utilization in crop improvement programs.

Materials and methods

Plant materials

The study included 94 unique landraces of various regions of Odisha, along with a standard resistant check (TKM6) and a susceptible check (TN1). The seed material was sourced from the gene bank at the ICAR-National Rice Research Institute, Cuttack, India.

Insect culture

The leaffolder culture was maintained in accordance with the method outlined by Waldbauer and Marciano (1979), with a constant temperature of 25 ± 5 °C and a relative humidity maintained at $60 \pm 10\%$. Adult leaffolders, sourced from the field, were introduced into ant-proof wooden oviposition cages measuring 50 cm \times 50 cm \times 75 cm and containing 30-day-old TN1 potted plants. To sustain the adults, a honey solution (20%) on a cotton ball was provided as food. Upon egg hatching, 25–30 first-instar larvae were carefully transferred to a new TN1 plant of the same age to facilitate continued

development. After completing the larval stage, pupae were collected and housed inside an adult emergence cage. The adult moths emerging from this cage were utilized in the subsequent phases of the study.

Field evaluation of rice landraces

The field experiment took place at the experimental plots of ICAR-NRRI in Cuttack, Odisha, India. The response of landraces and standard checks was evaluated using a rapid screening method (Padmavati et al. 2017) conducted over three consecutive seasons for two years (*Kharif*, 2021, *Rabi*, 2021–2022 and *Kharif*, 2022). The landraces were cultivated in a randomized block design with two replications, transplanted in rows of 20 hills. Standard checks (TN1 and TKM6) were interspersed after every 10 rows of test lines. Planting was done at a spacing of 20 cm \times 15 cm, with two rows of the susceptible check (TN1) serving as a border. Increased nitrogen doses were applied to enhance leaffolder populations in the border susceptible lines. All recommended agronomic practices were followed, excluding plant protection measures. Thirty days after transplanting, all the genotypes were covered with a nylon net. Paired adults moths of the leaf folder were released inside the net twice (at 40 and 60 days after transplanting), each time with 100 adults. Observations were recorded 20 days after each release on 10 randomly selected plants in both replications. The percentage of damage in each genotype was determined by counting damaged leaves, converted to an adjusted damaged leaves rating (ADLR) and scored from 0 to 9 using the standard evaluation score (SES) for rice (IRRI 2013) (Table 1). The test was deemed valid only if there was at least 50% damage in susceptible checks.

Damaged leaves (%)

$$= \frac{\text{Number of damaged leaves in a hill/plant}}{\text{Total Number of observed in a hill/plant}} \times 100$$

Adjusted damaged leaves rating (ADLE)

$$= \frac{\% \text{ damaged in the test entry}}{\% \text{ damaged in the susceptible check}} \times 100$$

Table 1 Standard evaluation system for rice International Rice Research Institute, Philippines (IRRI 2013)

Scale	ADLR	Reaction
0	No damage	R*
1	1–20%	
3	21–40%	
5	41–60%	MR
7	61–80%	S
9	81–100%	

R* Resistance, MR moderately resistance, S susceptible

Statistical analysis

The performance of 94 landraces and the standard checks was assessed across all three seasons using a Randomized Block Design (RBD). Descriptive statistics, including mean, standard error (SE), range, and coefficient of variation (CV%), were calculated for each season. The Analysis of Variance (ANOVA) was conducted using the Grapes statistical software version 1.0.0.

AMMI and GGE biplot analysis

The adjusted damaged leaves rating (ADLR) data obtained from the test environments were adapted to fit the additive main effects and multiplicative interaction (AMMI) model, employing the statistical model and computational methods as outlined by Gauch (2013). Analysis of variance (ANOVA) was utilized to evaluate the variation in resistance among testing environments (E) and genotypes (G) within the population, including the genotype \times environment ($G \times E$) interactions among the 94 landraces. Additionally, the resistance reactions between entries and standard checks in different testing environments were compared. To visually assess the stability of genotypes across the three test environments, Genotype and Genotype by Environment (GGE) biplot analysis was performed, following the methods outlined by Mukherjee et al. (2013) and Gauch (2013). All analyses, including ANOVA, AMMI biplot, and GGE analysis, were carried out using the Plant Breeding Tools (PB Tools 2014) software version 1.4, developed by the International Rice Research Institute (IRRI) in the Philippines.

Results

Phenotypic variation of rice genotypes

The genotypes underwent phenotypic evaluation for leaf-folter resistance over three consecutive seasons: *Kharif*, 2021 (E1), *Rabi*, 2021–2022 (E2) and *Kharif*, 2022 (E3). Histograms depicting the adjusted damaged leaves rating (ADLR) and damage scores across these seasons exhibited a normal frequency distribution (Fig. 1). During *Kharif*, 2021 (E1), 14 genotypes demonstrated an ADLR of less than 20%, while 30 genotypes fell within the ADLR range of 21–40%. Additionally, 34 genotypes showed an ADLR range of 41–60%, 8 genotypes ranged from 61 to 80%, and 10 genotypes fell within the 80–110% ADLR range. During *Rabi*, 2021–2022 (E2) minor phenotypic variation was displayed with comparatively lower mean ADLR%. During this period, 19 genotypes had an ADLR of less than 20%, 29 genotypes ranged from 21 to 40%, 33 genotypes fell within the 41–60% ADLR range, 8 genotypes ranged from 61 to

80%, and 7 genotypes showed an ADLR between 80 and 110%. Moving on to the *Kharif*, 2022 (E3), 14 genotypes had an ADLR below 20%, 30 genotypes ranged from 21 to 40%, 32 genotypes fell within the 41–60% ADLR range, 12 genotypes ranged from 61 to 80% and 8 genotypes exhibited an ADLR between 81 and 120%. During *Kharif*, 2021, a total of 44 genotypes were classified as resistant (damage score between 1 and 3), 34 were moderately resistant (damage score between 3.1 and 5) and 18 were susceptible (damage score between 5.1 and 9). Similarly, during *Rabi*, 2021–2022, 48 genotypes were identified as resistant, 33 as moderately resistant and 15 as susceptible and in the *Kharif*, 2022, 44 genotypes were recorded as resistant, 32 were moderately resistant and 20 were susceptible based on their damage scores.

Performance of the rice genotypes under varying environments

Field screening was executed to evaluate the performance of both the test genotypes and standard checks across all three environments. ANOVA was employed to discern variations in resistance among genotypes (G) and in different environments (E). Additionally, the $G \times E$ interactions of 94 landraces and the standard checks were thoroughly assessed. Notably, during *Rabi*, 2021–22, a higher number of genotypes exhibited resistance compared to *Kharif*, 2021 and 2022.

The overall mean, representing the resistant reaction of rice genotypes under diverse climatic conditions, is detailed in Table 2. A marginal variation in the mean adjusted damaged leaves rating percentage (ADLR%) is evident during the *Rabi*, 2021–2022 (41.44%) compared to *Kharif*, 2021 (42.18%) and *Kharif*, 2022 (44.12%). The highest mean ADLR% was observed during *Kharif*, 2022. Skewness and kurtosis values in Table 2 depict a platykurtic distribution (kurtosis < 3) across all three environments.

AMMI analysis of variance

The AMMI analysis of variance conducted on 94 landraces and two standard checks, testing them against leaf-folter resistance over three environments, revealed that 89.93% of the total sum of square (SS) of damaged area was attributed to the genotype (G) effect, 5.22% to the environment (E) effect, and 4.24% to genotype by environment ($G \times E$) interaction effects (Table 3). Genotypes or environments appearing nearly on perpendicular lines to the axis exhibited similar mean performance. Moreover, landraces or environments on the right side of the perpendicular lines through the origin demonstrated higher leaf-folter damage scores than those on the left side (Fig. 2A).

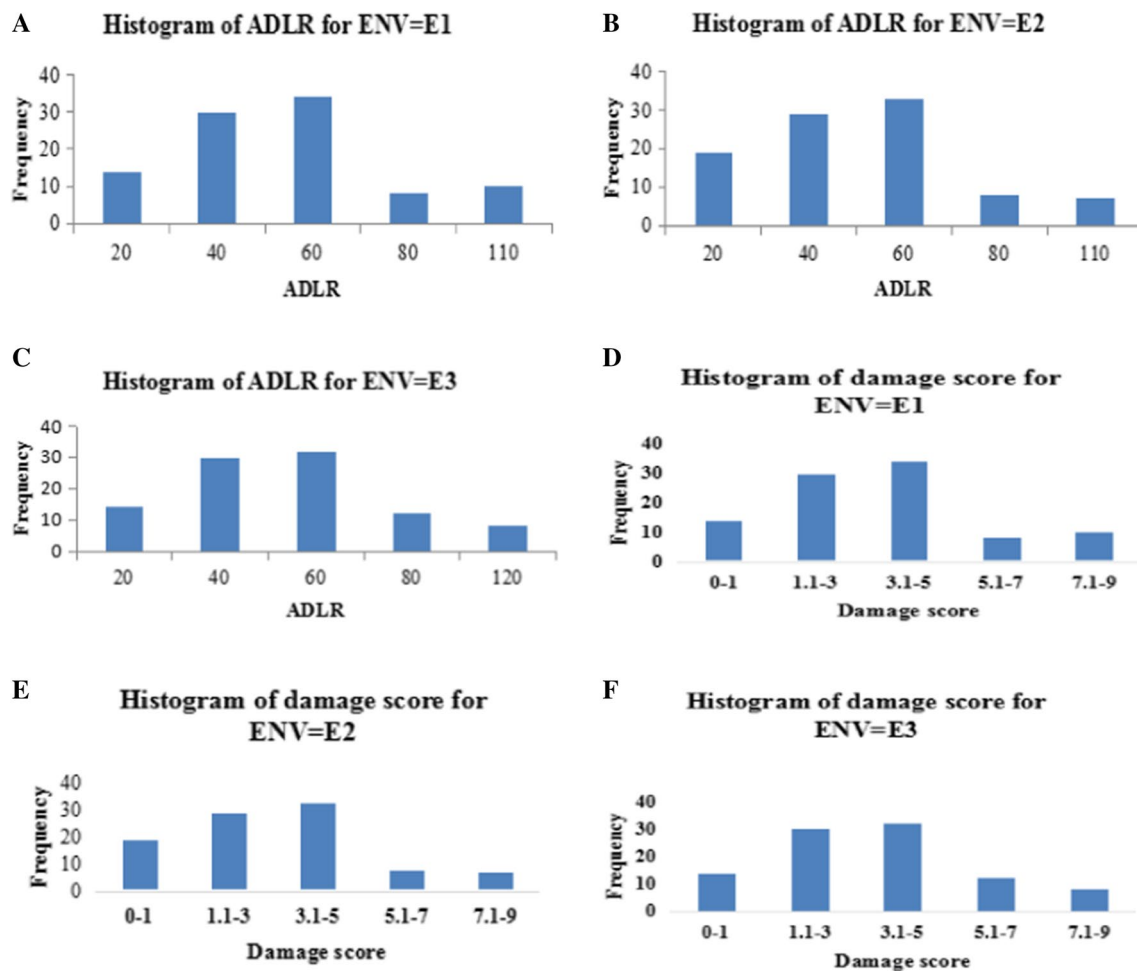


Fig. 1 Frequency distribution of leaffolder resistance during different environmental condition. **A–C** showing histograms of Adjusted damaged leaves rating (ADLR) and **D–F** showing histograms of damage scores

Table 2 Descriptive statistics and mean performance of rice genotypes under different environmental situations

Environment	E1	E2	E3
Mean	42.18	41.44	44.12
Minimum	7.45	6.84	7.67
Maximum	109.5	100.00	115.55
Range	102.05	93.16	107.88
Skewness	0.94	0.74	0.88
Kurtosis	0.58	0.27	0.53
Standard error	2.36	2.20	2.35
Variance	515.80	502.69	510.58
Standard deviation	23.19	21.43	23.12

E1 = *Kharif*, 2021; E2 = *Rabi*, 2021–2022; E3 = *Kharif*, 2022

AMMI identified two significant principal components, PC1 and PC2, explaining 84.6% and 15.4% of the sum of squares, respectively (Table 3). The AMMI biplot, with

ADLR on the X-axis and PC1 scores on the Y-axis (Fig. 2B), showcases that genotypes located farthest on the X-axis have higher mean ADLR% values. On the Y-axis, genotypes with PC1 scores closer to zero are more stable, exhibiting lower mean ADLR%.

Among the evaluated genotypes, 44 numbers of genotype had lower mean ADLR% (1–40%) with damage scores ranging from 1 to 3, including the resistant check G95 (TKM6), exhibited stable resistant reactions across all the test environments. The AMMI biplot for ADLR (Fig. 2B and supplementary table S1) revealed stable resistant genotypes, such as G1 (Manepuri), G2 (Mahasuri), G3 (Jangalijata), G4 (Pahadiabanki), G6 (Kalajeera-B), G7 (Baiganamanji), G9 (Basumatibanki), G10 (OR258), G12 (Ankul), G13 (Benababar), G14 (Baikani-D), G15 (Bhutia), G16 (Biradiabankoi), G17 (Chamarmani), G18 (Balibhuta), G19 (Basudha), G20 (Bayabhanda), G21 (Bhalunki), G22 (Bhatta), G23 (Champaneuli), G24 (Champasola), G31 (Gelhei), G37 (Kadalikenda), G40 (Kalakusuma), G42 (Kalamulia),

Table 3 AMMI ANOVA for genotype \times environment interaction

Source of variation	Df	Sum Sq.	Mean Sq.	F value	Pr(>F)	SS%	Sig.
Environment (E)	2	6704.903	3352.452	116.914	0	5.22	***
Genotypes (G)	95	115,440.968	1215.168	42.378	0	89.93	**
Replication	6	124.468	20.745	19.345	0	0.10	**
G \times E	190	5448.156	28.675	25.331	0	4.24	**
PC1	96	7275.890	75.79	3.67×10^{17}	0	84.60	***
PC2	94	1322.441	14.07	6.81×10^{16}	0	15.40	***
PC3	92	0.00	0.00	0.00	0	0	NS
Residuals	570	645.247	1.132	NA	NA	0.50	

ADLR adjusted damaged leaves rating, Df degree of freedom, Sig. significance

Significance level: ** $P < 0.05$ and *** $P < 0.01$

G43 (Kaliasaru), G44 (Kanakchampa), G45 (Kanhav), G46 (Kansapuri majhalijhuli), G47 (Karpuramoti), G51 (Langudi), G62 (Makadhan), G63 (Makarkand), G64 (Malata), G65 (Mayurkantha-k), G66 (Menaka), G67 (Mogra), G70 (Nagara), G73 (Padmakesari), G75 (Pandukalyan), G79 (Saraswati), G81 (Radhajugal), and the standard resistant check G95 (TKM6). Similarly, 15 number of genotypes such as G72 (Nimei), G78 (Ruksal), G80 (N.Umerchudi), G82 (Sarian), G83 (Ujadanga), G84 (Ratnakanta), G85 (Safari), G86 (Samudrabali), G87 (Tumba), G88 (Tikimasuri), G89 (Ramakrushna bilasha), G91 (Bhajana), G93 (Dhosara), G94 (Laxmibilasa), and the susceptible check G96 (TN1) were identified as stable susceptible genotypes with higher mean ADLR% ($> 60\%$, damage score between 7 to 9). All three environments, namely E1 (Kharif, 2021), E2 (Rabi, 2021–2022) and E3 (Kharif, 2022), were positioned far from the origin, indicating their distinctiveness and favorability for leaffolder infestation (Fig. 2B).

GGEbiplot analysis

The GGE biplot analysis elucidated 97.1% (PC1) of the total variation from both environmental and genotype perspectives (Table 4; Fig. 2C–F). The positive correlation among all three environments (E1, E2, and E3) indicated that they provided similar information about the genotypes. GGE biplot environment view (Fig. 2C, D) showed that although the locations were the same for all three environments, E1 (Kharif, 2021) and E3 (Kharif, 2022) exhibited a more congruent plot compared to E2 (Rabi, 2021–2022). E1 (Kharif, 2021) was found to be the closest to the ADLR value, making it the most effective for leaffolder screening, followed by E3 (Kharif, 2022) and E2 (Rabi, 2021–2022).

What-won-where biplot

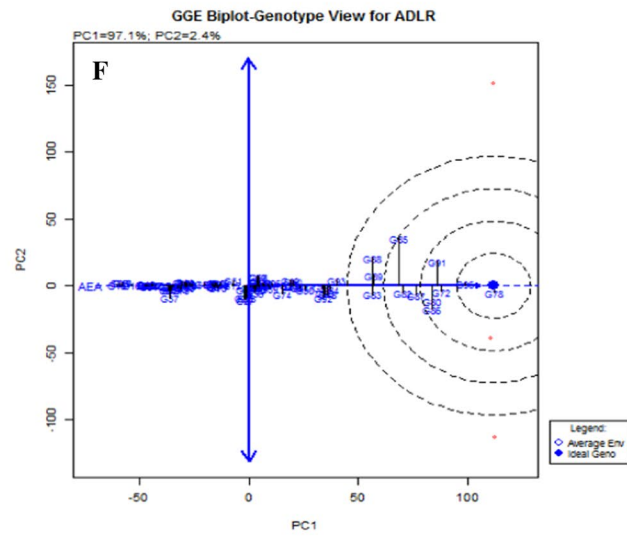
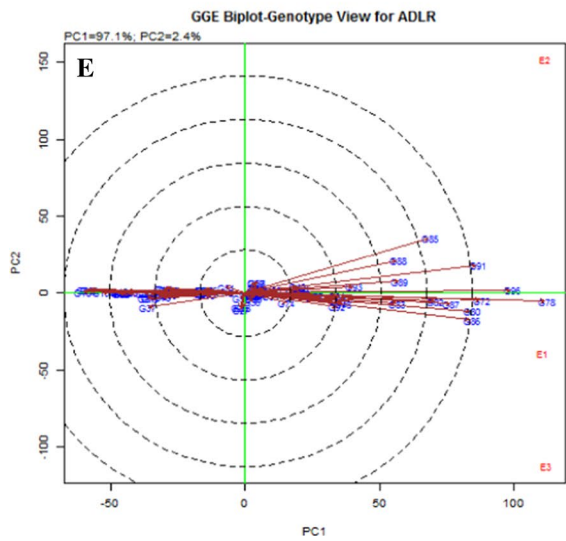
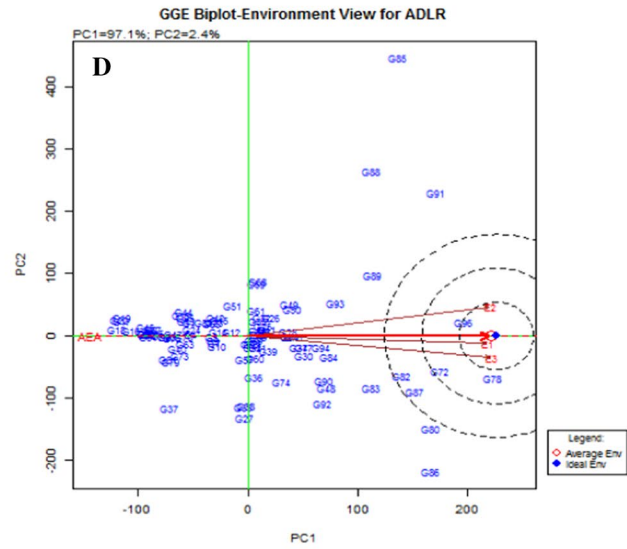
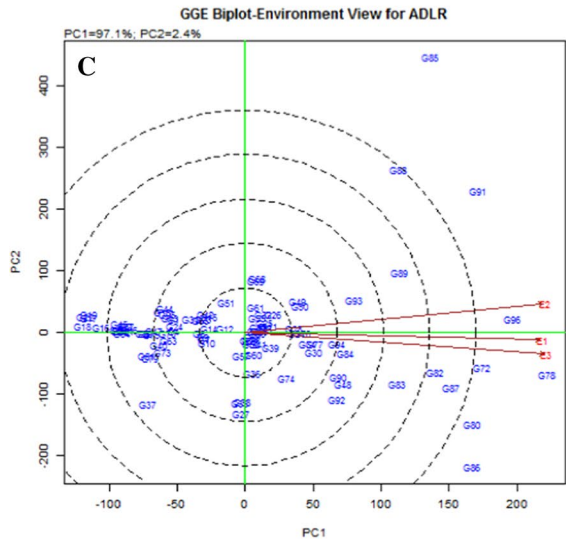
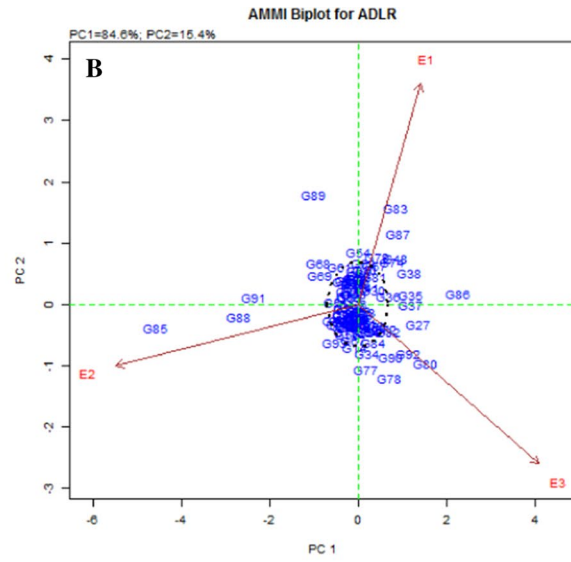
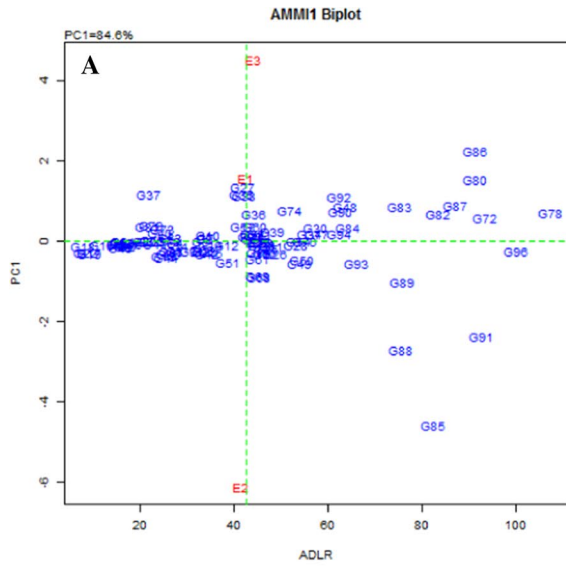
The what-won-where plot revealed a distinct pattern, indicating that the target environments could be grouped into two major sectors. E1 and E3 formed one sector, while E2

occupied another. In the mega-environment of E1 and E3, G86 (Samudrabali), G78 (Ruksal), G96 (TN1), G72 (Nimei), G82 (Sarian), G83 (Ujadanga), G80 (N.umerchudi), G87 (Tumba), G89 (Ramakrushna Bilasha), and G93 (Dhosara) consistently exhibited stable leaffolder susceptibility. On the other hand, in E2, genotypes like G88 (Tikimasuri), G85 (Safari) and G91 (Bhajana) were identified as winners, displaying stable susceptibility (Fig. 3).

In contrast, certain genotypes such as G19 (Basudha), G37 (Kadalikenda), G18 (Balibhuta), G44 (Kanakchampa), G14 (Baikani-D), G17 (Chamarmani) demonstrated stable resistance by appearing in the extreme opposite direction of the winners, indicating lower ADLR scores, which is preferable in resistance studies. The what-won-where plot highlighted genotypes like G85 (Safari), G78 (Ruksal), G86 (Samudrabali), and G96 (TN1) with higher mean ADLR%, as well as G19 (Basudha), G37 (Kadalikenda) with lower mean ADLR%, appearing at extreme vertices on either side of the axis going through the origin. This precise demarcation by GGE biplots showcased the susceptibility and resistance of genotypes. Genotypes clustering toward the origin consistently exhibited a stable resistant status with lower mean ADLR% (1–40%). These entries are very crucial with stable resistance, making them noteworthy for further studies and breeding programs.

Discussion

The rice leaffolder stands as a significant biotic constraint, inflicting considerable damage on rice crops. Farmers commonly resort to chemical pesticides to control this pest, which is usually associated with environmental and soil pollution (Chintalapati et al. 2016). Growing resistant rice varieties in leaffolder-prone areas emerges as an effective pest management strategy, offering advantages such as labor, time, and cost savings, coupled with increased yields. Consequently, identifying stable sources of resistance becomes a primary objective. This study evaluated 94 landraces under



◀**Fig. 2** AMMI and GGE biplot for primary component (PC1) and mean effect of landraces in different seasons. **A** AMMI biplot **B** AMMI Biplot for ADLR **C** GGE Biplot-Environmental view for ADLR **D** GGE Biplot-Environmental view for ADLR **E** GGE Biplot-Genotype view for ADLR **F** GGE Biplot-Genotype view for ADLR

diverse environmental conditions to assess their resistance stability against leaffolder. Stable resistant lines prove valuable tools from the perspectives of entomologists and plant breeders. Crossing stable resistant and susceptible lines can facilitate genetic analysis of various traits and the dissection of leaffolder resistance mechanisms (Chintalapati et al. 2019).

The frequency distribution (Fig. 1) suggested a normal platykurtic distribution, indicating that resistance or

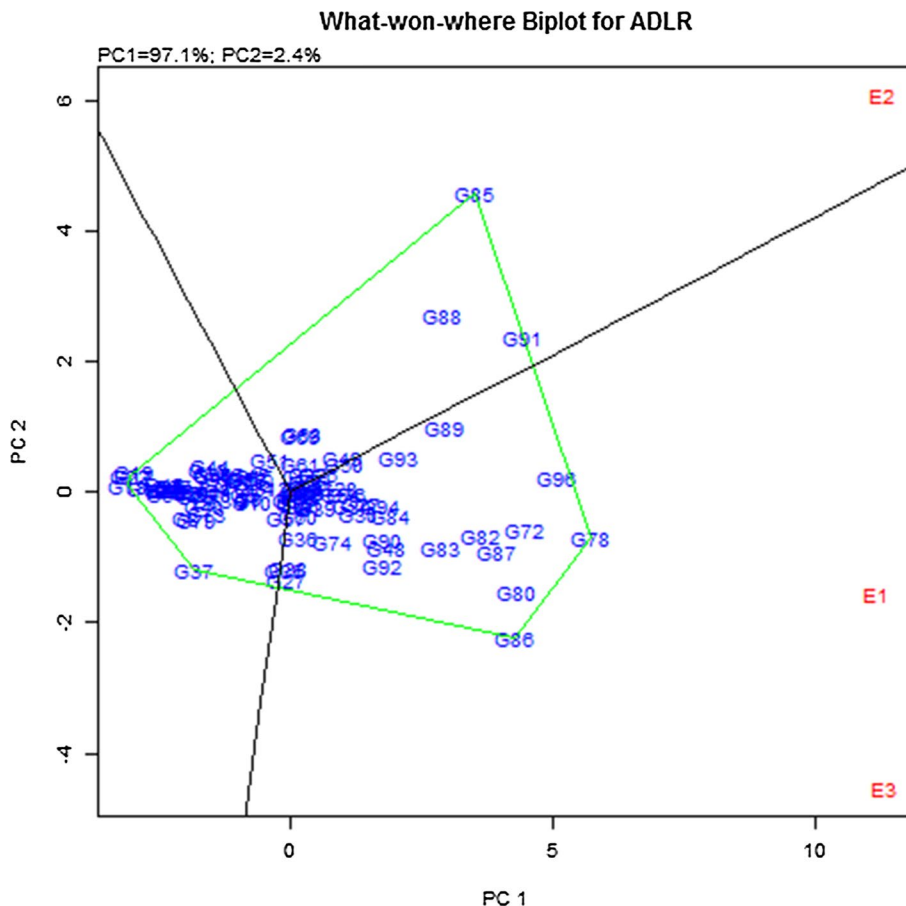
susceptibility is under polygenic control (Sahu et al. 2023). Out of the 96 genotypes assessed, 44 were identified as stably resistant in E1 (Kharif, 2021) and E3 (Kharif, 2022), while 48 were resistant in E2 (Rabi, 2021–2022). Additionally, 18 and 20 genotypes were categorized as susceptible in E1 and E3, respectively, with 15 genotypes marked as susceptible in E2. Among the three environments considered, similar ADLR% was recorded in E1 and E3, ranging between 7.45–109.50 and 7.67–115.55, respectively, while in Rabi 2021–2022, the ADLR% was lower at 6.84–100%. This difference suggests some sort of environmental impact on leaffolder population buildup, leading to reduced leaf damage. Leaffolder incidence tends to be higher in Kharif seasons (Gangwar 2015) as compared to the dry seasons and is negatively correlated with high temperatures while

Table 4 Genotype and genotype × environment analysis

	Percent	Acum	Df	Sum Sq.	Mean Sq.	F value	Pr(>F)	SS%	Sig.
PC1	97.1	97.1	96	292,314.54	3044.94	1.47 × 10 ¹⁹	0	97.14	***
PC2	2.4	99.5	94	7275.68	77.40	3.75 × 10 ¹⁷	0	2.42	**
PC3	0.4	100	92	1313.63	14.28	6.92 × 10 ¹⁶	0	0.44	***

Df degree of freedom, Sig. significance
Significance level: **P < 0.05 and ***P < 0.01

Fig. 3 What-won-where Biplot for ADLR



positively correlated with rainfall and relative humidity (Zainab et al. 2017). However, the AMMI ANOVA results in the current investigation highlighted a higher genotype effect (89.93%), with the environment effect being minimal (5.22%) and $G \times E$ interaction contributing 4.24%. This suggests a stable set of genotypes less influenced by changes in environmental conditions. These findings align with those of Chintalapati et al. (2019), where the genotype response was 69.08%, and the environmental response was 21.71% in the response variable for damage area against leafhopper. The responses of E and $G \times E$ indicate that resistance is primarily due to genotype effects rather than environmental factors.

Discriminating environments should be employed as test environments, as they offer more information on the phenotypic expression of genotypes (Yan and Tinker 2006). The GGE biplot analysis revealed that all three environments were representative of ADLR with minor deviations from the average environment axis. *Kharif*, 2021 (E1) appeared closest to the ideal test environment for ADLR followed by *Kharif*, 2022 and were deemed the most suitable for leafhopper phenotyping. E1 and E3 were grouped in the same mega-environment, while E2 occupied a separate mega-environment, indicating minor variations in ADLR values. This observed variability is primarily attributed to seasonal changes, including weather parameters.

Conclusions

The field screening conducted across three test environments demonstrated that among the 96 genotypes assessed, 44 genotypes exhibited stable resistance across all three seasons. The data recorded in both *Kharif* seasons showed notable similarities, while minor variations were observed in the *Rabi* season due to changes in weather parameters. In the AMMI ANOVA, the $G \times E$ interaction indicated that the role of the environment in influencing resistance reactions was minimal, while the genotype factor significantly contributed to the observed resistance reactions, emphasizing the importance of the genotype's main effect. The current study successfully identified several genotypes with stable resistance and susceptibility, offering valuable candidates for genotyping to further explore the mechanisms underlying resistance against leafhopper.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s42976-023-00484-1>.

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Declarations

Conflict of interest The authors have no financial or non-financial interests to declare that are directly or indirectly relevant to the content of this article.

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