Plant Archives Vol. 26, No. 1, 2026 pp. 93-102 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org

DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2026.v26.no.1.012

TRAIT ASSOCIATION BETWEEN TURCICUM LEAF BLIGHT-RELATED DISEASE TRAITS AND GRAIN YIELD IN MAIZE UNDER ARTIFICIAL EPIPHYTOTIC CONDITIONS

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(Date of Receiving-07-11-2025; Date of Revision-12-12-2025; Date of Acceptance-26-01-2026)

ABSTRACT

Maize (*Zea mays* L.) productivity is frequently constrained by foliar diseases, among which Turcicum leaf blight (TLB), caused by *Exserohilum turcicum*, represents a major yield-limiting factor in many maize-growing regions. The present study evaluated nine parental lines and 36 hybrids under artificial TLB infection across two locations to elucidate genetic variation and disease–yield relationships. Combined ANOVA revealed highly significant genetic variation for all disease component traits *viz.*, disease score, lesion length, number of infected leaves per plant, and lesions per plant, as well as grain yield, with largely non-significant genotype × environment interactions for disease components. Best Linear Unbiased Predictors (BLUPs) were therefore employed to obtain environment-adjusted genotypic estimates and to ensure robust assessment of genetic relationships. BLUP-based correlation and path analyses demonstrated strong interrelationships among disease component traits in both parental lines and hybrids. Associations between disease traits and grain yield were comparatively weaker in parents, whereas in hybrids, lesion length and number of infected leaves exhibited relatively stronger direct and total negative effects on grain yield, despite residual effects of similar magnitude. Disease score and lesion number contributed mainly indirect or smaller effects. These results demonstrate that yield loss in hybrids is primarily driven by lesion expansion and canopy-level infection, emphasizing lesion length and infected leaves as priority traits for selection, whereas disease expression in parental lines has limited predictive value for hybrid performance. Incorporating these traits into multi-trait selection indices could provide a practical strategy to enhance TLB resistance without compromising grain yield, thereby supporting more effective maize breeding under foliar disease stress.

***Key words*: BLUPs, Correlation, Disease resistance, Grain yield, Genotype × environment interaction, Selection**

Introduction

Maize (*Zea mays* L.) is a cereal crop of global significance; however, its productivity is frequently constrained by Turcicum Leaf Blight (TLB), caused by *Exserohilum turcicum*. The disease adversely affects photosynthetic efficiency and plant growth, leading to considerable reduction in grain yield, particularly under conducive environmental conditions (Rijal *et al.*, 2016; Jakhar *et al.*, 2021). Breeding for TLB resistance while maintaining high yield potential necessitates a clear understanding of the relationships between disease-related traits and grain yield. In this context, correlation and path coefficient analyses are widely employed quantitative tools in crop genetics and breeding, as they enable systematic dissection of complex inter-trait relationships. While correlation analysis measures the degree and direction of association between traits, path coefficient analysis further partitions these correlations into direct and indirect effects, thereby identifying traits that exert the greatest influence on a target trait such as grain yield (Dewey & Lu, 1959; Singh & Chaudhary, 1985). Such analytical partitioning is particularly valuable under biotic stress conditions like TLB, where yield is governed by multiple interacting disease components.

Nevertheless, the reliability of correlation and path coefficient analyses critically depends on accurate estimation of genotypic values, particularly under multi-environment testing where genotype × environment (G×E) interactions can obscure true genetic relationships. Failure to account for these interactions may lead to biased selection decisions and inconsistent genotype performance. Therefore, multi-environment evaluation is essential for reliable assessment of genetic potential. Best Linear Unbiased Predictions (BLUPs), derived from mixed-model approaches, provide a robust statistical framework for estimating genotypic effects while accounting for environmental variation, experimental design structure, and residual error (Piepho *et al.*, 2008; Rincent *et al.*, 2012). By integrating information across environments and exploiting variance–covariance structures, BLUPs generate more precise and stable genotypic estimates than simple means, thereby strengthening inference in correlation and path coefficient analyses.

The present study was therefore undertaken to examine the interrelationships between TLB-related disease traits and grain yield in maize under artificial epiphytotic conditions. Nine maize parental lines and their 36 hybrids, generated through a half-diallel mating design, were evaluated under artificially induced TLB infection across two locations to capture environmental variation. The inclusion of both parents and hybrids enabled a comprehensive assessment of trait associations, encompassing both additive and non-additive genetic effects. Associations between disease-related traits and grain yield were analyzed using BLUP-based correlation and path coefficient analyses to identify key disease components exerting significant influence on yield performance under TLB pressure. Given the complex and environmentally modulated nature of grain yield under disease stress, the findings provide a robust basis for indirect selection of TLB-resistant and high-yielding genotypes through component disease traits in maize breeding programmes.

Material and Methods

Plant material, experimental design, and disease evaluation

The experimental material comprised nine maize parental lines, namely MIL-2-27P (P_1), EC-0758084 (P_2), HKI-163 (P_3), IC-212886 (P_4), SKV-50 (P_5), IC-212893 (P_6), HKI-161 (P_7), CM-600 (P_8), and UMI-1200 (P_9). These parental lines were crossed in a 9 × 9 half-diallel mating design during the *rabi* season of 2023-24 at the Winter Nursery Centre, ICAR-Indian Institute of Maize Research (IIMR), Hyderabad, to generate hybrid combinations. During the subsequent *kharif* season of 2024, a total of 45 entries, consisting of the nine parents and their 36 hybrids, were evaluated in a randomized block design (RBD) with two replications at two locations in Karnataka: All India Coordinated Research Project (AICRP) on Maize, Zonal Agricultural Research Station (ZARS), Mandya (12°342 8.973 N, 76°482 47.863 E), and AICRP on Maize, Main Agricultural Research Station (MARS), University of Agricultural Sciences (UAS), Dharwad (15°272 2.633 N, 75°02 11.523 E). Each entry was planted in a single 4-m-long row, accommodating 15 plants per replication, following recommended agronomic practices.

To ensure uniform and adequate Turcicum leaf blight (TLB) pressure, all entries at both locations were artificially inoculated with *Exserohilum turcicum* culture using standard inoculation procedures. This ensured consistent disease development across genotypes and environments, allowing reliable assessment of genetic responses under controlled disease pressure. TLB disease severity, expressed as disease score (DS), was recorded using a 1-9 visual rating scale based on the percentage of leaf area infected as suggested by Hooda *et al.* (2018). Disease scoring was carried out on ten randomly selected plants per row at 75-90 days after sowing, coinciding with the dough stage of crop development, when disease expression was maximal. For the assessment of other disease-related traits *viz.*, lesion length (LL recorded in cm), number of infected leaves per plant (IL), and lesions per plant (LPP), data was recorded from five randomly selected plants within each row. Grain yield (GY), however, was recorded on a plot basis by harvesting all ears from each plot. Harvested ears were dehusked and weighed to record ear weight, and shelling percentage was determined. GY was calculated as the product of ear weight and shelling percentage and subsequently adjusted for moisture content using the following equations:

$$GY_0 = \frac{GY\ (100 - Moisture\ \%)}{100}$$

$$GY_{15} = \frac{GY_0 \times\ 100}{85}$$

Where

GY_{15} and GY_0 represent grain yield standardized to 0% and 15% moisture content, respectively. Final grain yield values were thus adjusted to 15% moisture content and converted to tonnes per hectare (t ha^{-1}) for statistical analysis.

Statistical analysis

A combined analysis of variance (ANOVA) was initially performed to assess the effects of genotype, environment (location), and genotype × environment (G×E) interaction for disease traits and grain yield across locations. In this preliminary analysis, genotypes were treated as fixed effects and locations as random effects to test the significance of G×E interaction and to characterize the overall phenotypic response of disease component traits across environments, following standard procedures in multi-environment crop trials (Singh & Chaudhary, 1985; Hallauer *et al.*, 2010). Although pooled ANOVA indicated non-significant G×E interaction for disease traits, subsequent genetic evaluation was conducted using mixed linear models to obtain Best Linear Unbiased Predictors (BLUPs) for all traits. In the mixed-model framework, genotypes were treated as random effects, while locations and replications nested within locations were considered fixed effects. This model specification is consistent with plant breeding practice, where genotypes represent a random sample from a breeding population and BLUPs provide unbiased predictions of genetic merit across environments (Piepho *et al.*, 2008; Bernardo, 2020; Bernado *et al.*, 2020). BLUPs were estimated using restricted maximum likelihood (REML), which enables simultaneous estimation of variance components and shrinkage-adjusted predictions of genotypic performance by accounting for environmental heterogeneity, experimental design effects, and residual variation (Piepho *et al.*, 2008). The use of BLUPs avoids potential bias associated with pooled phenotypic means, even when G×E interaction is weak or statistically non-significant, and improves the precision and reliability of genetic parameter estimation across environments (Piepho *et al.*, 2008).

BLUP estimates were used consistently for all downstream analyses to ensure that trait relationships reflected underlying genetic associations rather than environment-specific phenotypic variation. Pairwise genetic associations among disease traits and grain yield were quantified using Pearson's correlation coefficients computed from BLUP values. To further dissect these associations, path coefficient analysis was performed using BLUP-based correlation matrices, partitioning total correlations into direct and indirect effects of individual disease components on grain yield, following the classical methodology of Dewey and Lu (1959) and Singh and Chaudhary (1985).

All statistical analyses were carried out using R software (R Core Team). Combined ANOVA and mixed-model analyses were performed using the *lme4* and *lmer Test* packages. BLUP extraction and model diagnostics were conducted using *emmeans* and *performance*. Correlation analyses were implemented using *Hmisc* and *psych*, while path coefficient analysis was conducted using *lavaan* with graphical support from *semPlot*. Data visualization and heatmaps were generated using *ggplot2* and *pheatmap* packages in R.

Results and Discussion

Combined ANOVA across locations for disease traits and yield

The combined ANOVA revealed highly significant genetic differences among parental lines for all evaluated traits, including disease score (DS), lesions per plant (LPP), lesion length (LL), number of infected leaves (IL), and grain yield (GY) (p < 0.01; Table 1), indicating substantial genetic variability for both disease response and productivity. The non-significant genotype ×

Table 1: Combined analysis of variance for disease traits and grain yield of parents.

Source of variation	df	DS	LPP	LL	IL	GY
Line	8	31.14**	12.60**	45.20**	7.80**	10.59**
Location	1	1.59	180.20	2.80	0.90	180.20
Line × Location	8	0.64	124.20**	3.50**	0.70	3.55**
Residuals	16	1.06	19.60	0.90	0.50	0.28

*Mean squares are presented. Df-degrees of freedom; * Significant at p < 0.05; ** Significant at p < 0.01. Location effects were considered random and thus were not tested for significance. Traits measured: disease score (DS), lesions per plant (LPP), lesion length (LL), number of infected leaves per plant (IL), and grain yield (GY).*

Table 2: Combined analysis of variance for disease traits and grain yield of hybrids.

Source of variation	df	DS	LPP	LL	IL	GY
Line	35	3.79**	49.11**	57.50**	17.90**	10.60**
Location	1	5.31	341.42	191.29	17.71	180.25
Line × Location	35	0.14	2.51	1.68	0.88	3.55**
Residuals	70	0.26	4.41	6.58	1.19	0.28

*Mean squares are presented. Df-degrees of freedom; * Significant at $p < 0.05$; ** Significant at $p < 0.01$. Location effects were considered random and thus were not tested for significance. Traits measured: disease score (DS), lesions per plant (LPP), lesion length (LL), number of infected leaves per plant (IL), and grain yield (GY).*

environment (G × E) interaction for DS and IL suggested that these traits, which primarily reflect overall disease severity and host reaction, were relatively stable across environments and likely represent inherent resistance or susceptibility governed by genetic factors (Hallauer *et al.,* 2010; Bernardo, 2020). In contrast, the highly significant G × E interaction observed for LPP, LL, and GY indicated that these traits were more environment-sensitive. Lesion number and length were influenced by temperature, humidity, and disease pressure, which affect pathogen growth and host-pathogen interactions (Agrios, 2005; Madden *et al.,* 2007). Grain yield was environment-sensitive because environmental conditions affected both plant growth and the severity of disease lesions, which in turn influenced kernel development and total yield. Low residual mean squares indicated minimal experimental error and good model fit.

Similarly, in hybrids evaluated across two locations, genotypic effects were highly significant ($p < 0.01$) for all traits, demonstrating substantial genetic variability among the 36 single crosses (Table 2). The G × E interaction was significant only for GY, suggesting differential yield performance across environments, whereas disease-related traits (DS, LPP, LL, and IL) showed non-significant interactions. This indicated that resistance traits in hybrids were relatively stable, while yield expression reflected the combined effects of genotype, environment, and disease development. Overall, these results suggested that DS is a reliable indicator of genetic resistance or susceptibility, whereas lesion components and grain yield are more responsive to environmental variation and their interaction with genotype (Agrios, 2005; Madden *et al.,* 2007; Hallauer *et al.,* 2010; Bernardo, 2020).

Although the G × E interaction was largely non-significant for disease traits, pooled phenotypic means were not used for downstream analyses. Instead, best linear unbiased predictions (BLUPs) were applied for correlation and path analyses because they provide environment-adjusted estimates of genotypic performance, effectively accounting for residual variation due to location, replication, and experimental error. This approach allowed trait associations to reflect true genetic relationships among genotypes, rather than being confounded by environmental heterogeneity or experimental noise (Smith *et al.,* 2005; Piepho *et al.,* 2008). By using BLUPs, the analysis more accurately captured the genetic architecture of disease resistance and its relationship with grain yield, even when environmental effects were minimal.

Supplementary Table 1:
BLUP estimates of parents for disease severity components and grain yield

Parents	DS	LPP	LL	IL	GY
P_1	-2.37	-3.74	-10.23	-2.97	-0.94
P_2	-2.09	-4.25	-11.24	-2.92	0.31
P_3	-1.81	-0.19	-7.48	-1.24	0.45
P_4	2.22	1.70	11.19	2.37	0.08
P_5	-1.02	0.14	1.80	-2.15	1.05
P_6	1.73	7.79	6.26	1.31	-0.28
P_7	1.98	0.35	4.84	2.38	0.02
P_8	1.38	-0.05	7.48	4.98	-0.09
P_9	-0.02	-1.74	-2.63	-1.75	-0.61

Traits measured: disease score (DS), lesions per plant (LPP), lesion length (LL), number of infected leaves per plant (IL), and grain yield (GY).

Estimation of BLUPs for disease traits and grain yield

BLUP estimates of parental lines revealed substantial genetic variation for all disease components (DS, LPP, LL, and IL) and grain yield (Supplementary Table 1), indicating strong genetic contrasts among parents. Parents P_1, P_2, and P_3 consistently exhibited negative BLUPs for disease traits, reflecting enhanced genetic resistance, whereas P_4, P_6, P_7, and P_8 showed positive BLUPs, indicating higher susceptibility; grain yield BLUPs also varied widely, with P_5, P_3, and P_2 expressing favorable genetic effects under disease pressure. These contrasting BLUP patterns generate measurable genetic covariation between disease traits and yield, enabling robust estimation of genetic associations rather than environmentally driven relationships (Holland, 2007; Piepho *et al.,* 2008; Bernardo, 2020). Similarly, hybrids displayed broad genetic variability for all disease traits and grain yield (Supplementary Table 2), with marked differences in BLUP magnitude and direction across combinations. Hybrids with negative BLUPs for DS, LL,

Supplementary Table 2: BLUP estimates of hybrids for disease severity components and grain yield

Hybrid	DS	LPP	LL	IL	GY
$P_1 \times P_2$	-2.16	-3.86	-5.36	-3.56	1.06
$P_1 \times P_3$	-0.79	2.84	-3.51	-2.08	0.39
$P_1 \times P_4$	0.04	-0.22	-2.18	-1.19	0.81
$P_1 \times P_5$	-1.64	-1.74	-5.66	-4.66	2.43
$P_1 \times P_6$	0.41	-1.64	-3.43	0.21	-1.37
$P_1 \times P_7$	-0.29	-3.23	-6.37	-1.10	-0.37
$P_1 \times P_8$	0.05	1.49	1.71	0.00	-0.81
$P_1 \times P_9$	-1.12	-2.23	-3.05	-1.33	-0.37
$P_2 \times P_3$	-1.19	-0.80	-3.05	-2.97	1.42
$P_2 \times P_4$	-0.28	-3.31	-1.11	-0.29	0.03
$P_2 \times P_5$	-1.24	-1.59	-2.43	-2.78	2.02
$P_2 \times P_6$	0.19	1.37	0.27	-0.12	0.27
$P_2 \times P_7$	-0.11	-2.22	-1.66	-0.73	2.53
$P_2 \times P_8$	0.19	-0.21	1.39	0.03	-0.06
$P_2 \times P_9$	-0.58	-2.69	-1.52	-0.47	-0.73
$P_3 \times P_4$	0.75	9.39	0.47	0.49	-0.27
$P_3 \times P_5$	-0.76	3.02	0.52	-1.39	3.83
$P_3 \times P_6$	-0.52	-1.26	2.81	-0.72	-0.56
$P_3 \times P_7$	-0.29	-0.30	-2.73	-0.54	1.45
$P_3 \times P_8$	0.00	-1.57	3.68	-0.35	-0.28
$P_3 \times P_9$	-0.92	-1.52	0.98	-1.05	-1.15
$P_4 \times P_5$	0.85	1.94	2.57	1.17	-0.59
$P_4 \times P_6$	1.31	-1.86	5.05	2.32	-0.82
$P_4 \times P_7$	1.70	1.69	5.10	2.79	-1.70
$P_4 \times P_8$	0.90	-0.83	4.59	2.60	-1.75
$P_4 \times P_9$	0.67	-2.88	-2.78	0.49	-0.68
$P_5 \times P_6$	0.00	-5.13	-0.36	-0.26	0.99
$P_5 \times P_7$	0.09	-0.03	-3.28	0.66	2.45
$P_5 \times P_8$	-0.02	-1.13	-1.56	0.58	0.57
$P_5 \times P_9$	-0.61	-4.56	-1.14	-1.19	-0.32
$P_6 \times P_7$	1.06	0.40	-0.81	2.65	-1.54
$P_6 \times P_8$	1.16	5.37	8.28	3.45	-2.02
$P_6 \times P_9$	-0.11	3.10	4.09	0.47	-2.02
$P_7 \times P_8$	1.82	6.31	3.22	3.63	-0.62
$P_7 \times P_9$	1.13	1.75	4.46	3.77	-1.01
$P_8 \times P_9$	0.30	6.16	2.81	1.48	-1.24

and IL expressed superior genetic resistance, whereas positive values indicated increased susceptibility, while grain yield BLUPs spanned a wide range, confirming strong genotypic control under disease stress. Hybrids combining reduced disease expression with favorable yield effects represent genetically superior combinations, notably $P_1 \times P_5$, which showed consistently negative BLUPs for all disease traits and a strong positive yield BLUP under TLB stress; $P_3 \times P_5$ recorded the highest positive yield BLUP, while $P_2 \times P_5$ and $P_5 \times P_7$ also combined improved resistance with enhanced yield, whereas $P_6 \times P_8$ exhibited high susceptibility coupled with poor yield performance. The continuous distribution and diversity of BLUP profiles observed among parents and hybrids reflected substantial quantitative variation in disease resistance, consistent with earlier reports on maize foliar diseases (Mueller *et al.*, 2020; Zhu *et al.*, 2023; Gong *et al.*, 2025), and provided the basis for subsequent correlation analysis of genetic relationships among disease traits and grain yield.

Table 3: BLUP-based correlation matrix among disease traits and grain yield in parents.

Trait	DS	LPP	LL	IL	GY
DS	1.00	0.68*	0.93**	0.86**	-0.08
LPP	0.68*	1.00	0.70*	0.52	0.08
LL	0.93**	0.70*	1.00	0.84**	0.12
IL	0.86**	0.52	0.84**	1.00	-0.03
GY	-0.08	0.08	0.12	-0.03	1.00

** Significant at $p < 0.05$; ** Significant at $p < 0.01$.*

Correlation structure among disease traits and grain yield

BLUP-based correlation analysis among parental lines revealed strong and highly significant positive associations among disease traits, indicating tightly coordinated genetic control of disease expression (Table 3). Specifically, disease score was very strongly correlated with LL and IL, suggesting that variation in disease severity among parents is primarily driven by lesion expansion and canopy infection. The association between DS and LPP further indicated that lesion number contributes to overall disease expression, although its influence is secondary to lesion enlargement and leaf infection. Lesion length also showed strong positive correlations with IL and LPP, confirming that lesion enlargement and infection spread are genetically linked processes in parental germplasm These strong interrelationships among disease components are consistent with the polygenic and integrative genetic control of quantitative disease resistance in cereal crops, wherein multiple disease processes jointly determine resistance expression (Holland, 2007; Piepho *et al.*, 2008; Jakhar *et al.*, 2021).

Grain yield, however, exhibited weak and non-significant correlations with all disease traits (r = -0.08 to 0.12), indicating that, at the parental level, genetic variation for yield is largely independent of disease expression. This aligns with established evidence that yield performance in inbred parents is often a poor predictor of hybrid productivity and tends to be weakly associated with stress-response traits due to reduced vigor and absence of heterosis (Hallauer *et al.*, 2010; Bernardo, 2020). Therefore, parental BLUPs primarily inform the

Table 4: BLUP-based correlation matrix among disease traits and grain yield in hybrids.

Trait	DS	LPP	LL	IL	GY
DS	1.00	0.47**	0.67**	0.94**	-0.56**
LPP	0.47**	1.00	0.48**	0.45**	-0.22
LL	0.67**	0.48**	1.00	0.75**	-0.56**
IL	0.94**	0.45**	0.75**	1.00	-0.63**
GY	-0.56**	-0.22	-0.56**	-0.63**	1.00

** Significant at p < 0.05; ** Significant at p < 0.01.*

genetic architecture and interdependence of disease resistance traits rather than yield performance, justifying their inclusion for dissecting trait correlations and identifying key components of TLB resistance, while yield impacts are more appropriately assessed at the hybrid level.

BLUP-based correlation analysis among maize hybrids revealed strong and highly significant positive associations among disease components, indicating tight genetic relationships among traits describing disease development under TLB pressure (Table 4). Disease score exhibited a very strong positive correlation with IL, demonstrating that disease expression in hybrids is largely governed by the extent of canopy infection rather than isolated lesion occurrence. Significant positive correlations were also observed between DS and LL, as well as between LL and IL, indicating that lesion expansion and infection spread act in a coordinated manner to determine overall disease expression. Such strong interrelationships among disease components reflect the quantitative and polygenic nature of resistance to northern corn leaf blight, in which multiple, genetically correlated traits collectively define the host response (Welz & Geiger, 2000; Rashid *et al.*, 2020; Li *et al.*, 2025).

Consistent with this integrated disease response, grain yield in hybrids showed negative associations with all

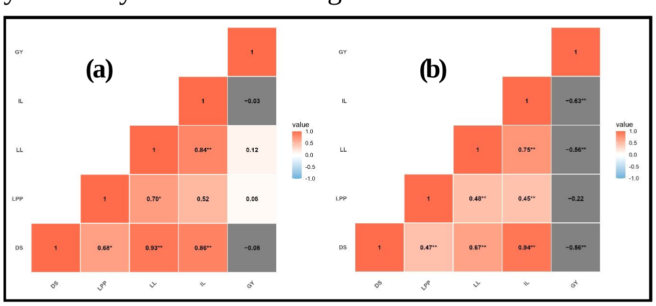

Fig. 1: BLUP-based correlation heatmaps of disease and yield traits in **(a)** parents and **(b)** hybrids. [*Color intensity reflects the magnitude and direction of associations, with positive correlations shown in red shades and negative correlations in grey to bluish shades. Values within cells represent correlation coefficients, and asterisks denote levels of statistical significance*].

disease traits, confirming an antagonistic relationship between disease development and productivity under TLB stress. his pattern contrasted with parental lines and reflected the expression of heterosis in hybrids, where fully realized yield potential is more sensitive to disease pressure. The strongest negative correlations were observed between GY and IL, as well as with DS and lesion length LL, indicating that yield reduction is more closely linked to canopy-level infection and lesion expansion than to lesion number alone. Accordingly, the weak and non-significant association between LPP and GY suggested that lesion number has limited predictive value for yield loss compared with the severity and spatial spread of infection within the canopy.

Previous studies have established that resistance to foliar diseases is mediated by molecular defense mechanisms, including the activation of pathogenesis-related proteins, reactive oxygen species scavenging systems, and cell wall strengthening enzymes, which collectively limit lesion expansion and help preserve photosynthetically active leaf area (Naz *et al.*, 2021; Hamidi *et al.*, 2024; Jiao *et al.*, 2025; Xu *et al.*, 2025). In line with these mechanisms, hybrids that effectively restrict lesion expansion and canopy damage are better able to maintain photosynthetic capacity and consequently experience smaller yield penalties under TLB stress. Similar relationships between yield loss and disease severity have been widely reported in maize, where reductions in yield are primarily driven by loss of functional leaf area rather than lesion frequency *per se* (Perkins & Pedersen, 1987; Byrnes *et al.*, 1989; Batchelor *et al.*, 2020; Saquee *et al.*, 2023; Garoma *et al.*, 2024).

Together, these findings indicate that traits capturing canopy damage and lesion expansion are more informative selection criteria for minimizing yield loss under TLB stress than lesion number alone. This supports the concept that maintenance of green leaf area duration is a critical

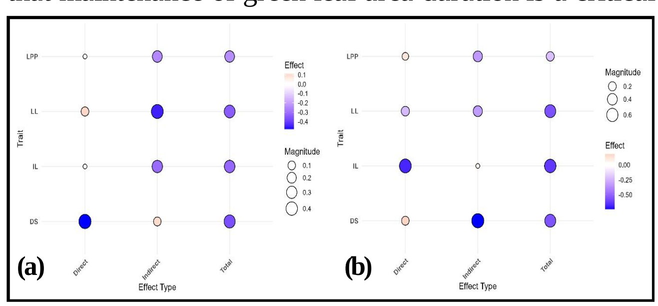

Fig. 2: BLUP-based path effects of disease traits on grain yield in **(a)** parents and **(b)** hybrids. [*Circle color indicates the direction and sign of the path coefficient (blue = negative, light/neutral = weak to near-zero effects), while circle size represents the magnitude (absolute value) of the effect*].

Table 5: Path coefficient analysis showing direct and indirect effects of disease traits on grain yield in parents (BLUP-based).

Trait	Direct effect	Indirect *via* DS	Indirect *via* LPP	Indirect *via* LL	Indirect *via* IL	Total indirect effect	Total effect on GY
DS	-0.48	0.00	0.01	0.10	-0.01	0.10	-0.37
LPP	0.01	-0.32	0.00	0.08	0.00	-0.25	-0.24
LL	0.11	-0.45	0.01	0.00	-0.01	-0.45	-0.34
IL	-0.01	-0.41	0.01	0.09	0.00	-0.31	-0.32
Residual effect							**0.83**

determinant of yield under foliar disease pressure in cereals (Bancal *et al.,* 2007; Iseghohi *et al.,* 2023). Given the strong intercorrelations among disease traits and their differential associations with grain yield, path coefficient analysis was therefore employed to disentangle direct and indirect effects of individual disease components and to identify traits with the greatest causal relevance for selection. The overall correlation structure was further summarized using heatmaps (Fig. 1), illustrating the strength and direction of trait associations.

Path analysis and partitioning of effects

Path coefficient analysis based on BLUPs partitioned relationships among disease traits and grain yield into direct and indirect effects, providing clearer causal insight than simple correlations (Table 5). In parental lines, disease score exhibited a moderate negative direct effect on GY, whereas LPP and LL showed small positive direct effects, and IL had a negligible direct effect. Despite these relatively weak direct effects, all disease components exerted substantial negative indirect effects on GY *via* their interrelationships, particularly through severity and infection spread, resulting in negative total effects on yield. This discrepancy between simple correlations and total effects reflects the pervasive issue of multicollinearity among interrelated disease traits under stress conditions, a recognized challenge in path coefficient models (Olivoto *et al.,* 2017). In parents, the high residual effect of the path model ~83% of the variation in grain yield was unexplained by the assessed disease traits. This substantial residual variance reflects that yield in inbred lines is largely governed by genetic background, inherent vigor, and non-disease factors rather than disease expression alone (Holland, 2007; Bernardo, 2010).

In hybrids, path coefficient analysis resolved correlations among disease components and grain yield (GY) into direct and indirect effects, revealing that disease score and lesions per plant had small positive direct effects on GY but substantial negative indirect effects mediated through lesion length and infected leaves, resulting in overall negative total effects (Table 6). Lesion length and infected leaves exerted strong negative direct effects, with infected leaves contributing the largest total reduction in yield, underscoring canopy-level infection spread as a key determinant of yield loss. A moderate residual effect (0.75) indicated that ~25% of GY variation was explained by disease traits, reflecting a stronger association between disease expression and grain yield in in hybrids and supporting evidence that loss of photosynthetically active leaf area, rather than isolated lesion metrics, drives yield decline under foliar disease stress (Perkins & Pedersen, 1987; Byrnes *et al.,* 1989; Bernardo, 2010). Fig. 2 presents heatmaps summarizing the magnitude and direction of direct, indirect, and total effects in parental lines and hybrids.

Path analysis revealed clear differences between parents and hybrids. In parents, disease traits showed weak direct effects on grain yield, and negative total effects were mainly indirect, with a high residual indicating that yield was largely independent of disease expression. In contrast, hybrids showed strong disease-yield relationships, with lesion length and infected leaves exerting clear negative direct effects and explaining a larger proportion of yield variation. Overall, hybrid performance was markedly more sensitive to disease-induced canopy damage than parental performance (Vivek *et al.,* 2010; Ohunakin *et al.,* 2023).

Table 6: Path coefficient analysis showing direct and indirect effects of disease traits on grain yield in hybrids (BLUP-based).

Trait	Direct effect	Indirect *via* DS	Indirect *via* LPP	Indirect *via* LL	Indirect *via* IL	Total indirect effect	Total effect on GY
DS	0.18	0.00	0.06	-0.16	-0.64	-0.74	-0.56
LPP	0.12	0.09	0.00	-0.11	-0.31	-0.34	-0.22
LL	-0.23	0.12	0.06	0.00	-0.51	-0.33	-0.56
IL	-0.68	0.17	0.05	-0.17	0.00	0.05	-0.63
Residual effect							**0.75**

Conclusion

Combined ANOVA across locations revealed highly significant genetic variation for all disease traits and grain yield in both parental lines and hybrids. Disease score and number of infected leaves exhibited largely non-significant genotype × environment interactions, indicating stable expression across environments and their suitability as reliable indicators of inherent resistance, whereas lesion-related traits and grain yield showed greater environmental sensitivity. To account for these differences and to obtain environment-adjusted genotypic estimates, BLUPs were employed, ensuring that subsequent correlation and path analyses captured underlying genetic relationships rather than environmentally induced variation. Together, ANOVA established the presence and stability of genetic variation under TLB stress, while BLUP-based analyses revealed broad and continuous genetic variation across parents and hybrids, supporting a quantitative, polygenic basis of TLB resistance in maize.

BLUP-based correlation analyses revealed that disease expression was primarily governed by lesion expansion and canopy-level infection, as evidenced by strong positive associations of disease score with lesion length and number of infected leaves, whereas lesion number *per se* was comparatively less informative. In hybrids, BLUP-derived grain yield showed consistent negative associations with disease component traits, underscoring the impact of disease-induced loss of functional leaf area on productivity; in contrast, yield in parental lines exhibited weak correlations with disease traits, reflecting the limited predictive value of inbred performance for hybrid yield. BLUP-based path coefficient analysis further partitioned these relationships, demonstrating that in parental lines disease components exerted only minor direct effects on yield, with most influences expressed indirectly through interrelated traits. Conversely, in hybrids, lesion length and number of infected leaves showed strong negative direct effects on grain yield, confirming that canopy-level infection and lesion expansion constitute the principal determinants of yield loss under TLB stress, while other disease traits contributed mainly through indirect pathways.

Collectively, these results indicated that lesion length and number of infected leaves could be prioritized in selection strategies, whereas disease score and lesions per plant remain useful for phenotyping but are less predictive of yield loss. Incorporating these key traits into multi-trait selection indices offers a practical approach to enhance resistance without compromising grain yield, thereby providing clear guidance for maize breeding under TLB stress.

Authors contribution

Conceptualization of research (KRY, CGK, SC, VH); Designing of the experiments (CGK, KRY, SC, VH); Contribution and maintenance of experimental materials (CGK, KRY, RKD, OK, SN, SP, JK, SRJ, BS, SP); Execution of field/lab experiments and data collection (SC, MN, PGU, KRY, CGK); Analysis of data and interpretation (SC, CGK, KRY); Preparation of the manuscript (SC, KRY, CGK).

Acknowledgements

The authors acknowledge the Indian Council of Agricultural Research (ICAR) - Indian Institute of Maize Research, Ludhiana; All India Co-ordinated Research Project (AICRP) on Maize, Zonal Agricultural Research Station, Mandya and AICRP on Maize, Main Agricultural Research Station, UAS, Dharwad AICRP for support in carrying out the research work.

Funding : This research work is funded by ICAR under Consortium Research Platform on Agrobiodiversity (CRPAB).

Data availability: All data supporting the findings of this study are available within the paper.

Conflict of interest: The authors declare no competing interests.

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