

# Evaluation of resistance in new hybrid maize genotypes to Northern leaf blight (*Exserohilum turcicum*)

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**Abstract.** Hidayatullah T, Suliansyah I, Swasti E, Putri NE, Mejaya IMJ. 2025. Evaluation of resistance in new hybrid maize genotypes to Northern leaf blight (*Exserohilum turcicum*). *Biodiversitas* 26: 4967-4975. Northern Leaf Blight Disease (NLB), caused by *Exserohilum turcicum*, is a major foliar disease threatening maize (*Zea mays*) production in Indonesia. This study aimed to evaluate the resistance of 20 new hybrid maize genotypes developed by Universitas Andalas, along with two control varieties, against NLB. Field trials were conducted in West Sumatra using a randomized complete block design to assess disease resistance and agronomic traits. Multivariate analysis was used to assess 20 new hybrid maize genotypes. The collected data were examined across nine parameters: Plant Height (H), Ear Height (EH), Stem Diameter (SD), Dehusked Ear Weight (DEW), Harvest Moisture (HM), Grain Weight per Ear (GWE), 1000-grain Weight (W1000), Yield Potential (YP), and Northern Leaf Blight (NLB). Results showed that most of the hybrids demonstrated Resistance (R) to Moderate Resistance (MR), with genotypes G4-13, G15-16, G18, and G21 exhibiting high levels of resistance and stability. NLB severity showed significant correlations with all parameters except harvest moisture. Principal component analysis identified three components with eigenvalues greater than one, collectively explaining 86.53% of the total variance. The first two components alone accounted for 74.19% of the variance, highlighting their dominant role in discriminating among genotypes. Cluster analysis produced four clusters, such as cluster 1: G7, G12, G3, G14, and G17; Cluster 2: G10, G19, G2, G20; Cluster 3: G21, G6, G22; and the last, Cluster 4: G15, G8, G16, G9, G13, G18, G4, and G5. The findings indicate that G6 is suitable candidates for advancement to preliminary yield trials, combining superior yield potential with notable resistance to NLB. These outcomes provide practical guidance for breeding programs seeking to produce resilient and productive maize hybrids across variable agroclimatic settings.

**Keywords:** *Exserohilum turcicum*, genotype, hybrid maize, leaf blight resistance, plant breeding

**Abbreviations:** CIMMYT: *Improvement Center Centro Internacional de Mejoramiento de Maíz y Trigo*, DAP: Days After Planting, DEW: Dehusked Ear Weight, GWE: Grain Weight per Ear, HM: Harvest Moisture, HS: Highly Susceptible, HR: High Resistance, MR: Moderately Resistance, NLB: Northern Leaf Blight, PCA: Principal Component Analysis, RCBD: Randomized Complete Block Design, R: Resistance, SD: Stem Diameter, W1000: 1000-Grain Weight, YP: Yield Potential

## INTRODUCTION

In Indonesia, it ranks second after rice and plays a crucial role in national food security (Hidayatullah et al. 2023a). According to the Central Statistics Agency (BPS) (GoodStats 2024), Indonesia's national maize production rose by 2.98% in 2024, reaching 15.21 million tons, compared to 14.77 million tons in 2023. The increase occurred across nearly all islands, except in Maluku and Papua. Maize is cultivated throughout Indonesia, including Java and Sumatra in the west, Kalimantan and Nusa Tenggara in the central region, and Sulawesi, Moluccas, and Papua in the east. Domestic demand for maize has steadily increased each year due to its diverse uses, not only as a staple food but also as a primary ingredient in animal feed and a growing component in biofuel production (Choudhary et al. 2020). Despite continuous efforts to enhance production, Indonesia's domestic maize supply

remains insufficient to meet rising market demand, leading to recurring imports.

One of the key obstacles to boosting maize production is its vulnerability to various biotic stresses, especially Northern Leaf Blight (NLB) (Ahangar et al. 2016). NLB is a foliar disease triggered by the fungus *Exserohilum turcicum* (Semangun 2008). *E. turcicum* is among the most damaging pathogens affecting maize, significantly limiting yield potential (Kotze et al. 2019). When the disease establishes before the silking stage, it can cause significant crop losses, especially in grain yield (Nwanosike et al. 2015). NLB disrupts photosynthesis and can reduce grain yield by 28% to as much as 91% (Reddy et al. 2013). The highest levels of disease incidence and severity in Indonesia were observed in Sei Glugur Village, where the average incidence reached 83.64% and the average severity was recorded at 33.15% (Syakira and Safni 2024).

Efforts to improve maize production rely on the use of high-quality grain varieties, such as hybrid maize, which provide higher yields and better resistance to pests and diseases (Wicaksana et al. 2022). Disease control through resistant varieties is considered the most effective and preferred method by farmers (Setyawan et al. 2016a). Therefore, exploring maize genotypes with resistance to NLB stress is essential, as it provides valuable information into both resistant and susceptible varieties (Setyawan et al. 2016b). A characterization study by Hidayatullah et al. (2023b) on five elite inbred lines, followed by a full diallel hybridization design, led to the development of 20 promising hybrid maize candidates. Consequently, it is crucial to evaluate the resistance levels of these 20 F1 hybrid genotypes against major maize diseases, especially NLB. This study employed multivariate analysis approach to evaluate both agronomic traits and resistance-related parameters to NLB in maize. For example, Principal Component Analysis (PCA) is a multivariate statistical technique that simplifies complex data by reducing dimensionality and eliminating inter-correlations among variables. PCA has previously been applied to assess traits linked to fall armyworm resistance in tropical maize genotypes (Wambi et al. 2025). The multivariate analytical approach is robust and applicable to diverse species and trait datasets exhibiting significant inter-trait correlations (Sanches et al. 2019).

This study is part of a broader research initiative aimed at developing new hybrid maize genotypes with higher productivity and better adaptability to the unique agroclimatic conditions of West Sumatra, which differ from those of other provinces (Setyawan et al. 2016a, 2016b; Hidayatullah et al. 2023b). One of the key requirements for releasing a new hybrid maize genotype is its resistance to NLB. Although both parental lines used in these hybrids are known to exhibit resistance to leaf blight, it remains essential to test their hybrids to verify whether this resistance is successfully inherited. Evaluating resistance to major maize diseases, particularly NLB, in these 20 new hybrids aims to identify genotypes with strong resistance that can advance to preliminary yield trials. The main novelty of this study lies in identifying promising new hybrid maize genotypes that will enrich the diversity of maize varieties in Indonesia and potentially serve as grain references for maize cultivation, especially in West Sumatra, Indonesia.

## MATERIALS AND METHODS

### Study area

The field trial was conducted in Pariaman, West Sumatra, Indonesia (0.60° S, 100.13° E; 7 m above sea level). The experimental site is situated in lowland tropical environment. According to Meteorology, Climatology, and Geophysical Agency of Indonesia (BMKG) data from the Jalan Baru rainfall station (July-December 2024), total rainfall reached 3,032.3 mm with 99 rainy days, with the highest monthly precipitation occurring in August (935 mm) and December (606.7 mm). These conditions provided a favorable environment for the natural development of NLB.

### Research material

This study utilized 22 maize genotypes, consisting of 20 promising new single-cross hybrid varieties and two commercial control varieties: P32 (a nationally distributed hybrid maize) and Sukmaraga (a nationally released composite variety). The 20 new hybrid genotypes were developed through a single-cross breeding process and are products of the plant breeding program of Universitas Andalas, which began in 2013. These hybrids were derived from populations such as Arjuna, Bisma, and other widely cultivated hybrids. All field trials were conducted with prior permission from the landowner/farmers in West Sumatra. The research protocol did not involve any endangered or protected germplasm. Institutional approval was obtained from Universitas Andalas for the use of experimental plots and germplasm evaluation.

### Experimental design

The experimental design was a Randomized Complete Block Design (RCBD), non-factorial, with three replications. Each plot measured 3.75 m × 2 m and was prepared using full tillage. Each plot consisted of five rows with a planting distance of 75 cm × 20 cm. Grains were sown at a two grains per hole, targeting 100 plants per plot (20 grains per row × 5 rows). Thinning was conducted before fertilization, resulting in 50 plants per plot (10 plants per row × 5 rows). Maize, known to be susceptible to leaf blight disease, was planted as a spreader row to facilitate natural disease infection. These spreader rows were planted four weeks before the test genotypes. All materials were cultivated and maintained without fungicide protection to allow natural infection. Fertilization was applied twice: the first at 14 Days After Planting (DAP) using NPK and urea at doses of 350 kg/ha and 250 kg/ha, respectively, and the second at 30 DAP using urea at 100 kg/ha (Azrai et al. 2016).

### Field management and inoculation

Disease inoculation was carried out using spreader rows of sweet corn, known for their high susceptibility to NLB. These rows were sown approximately four weeks before the experimental materials to promote early disease development. Infection relied on natural spread, as initial observations confirmed that the research site was an NLB-endemic area, necessitating field-based rather than controlled laboratory experiments. Pest observations were routinely performed, and if pest levels exceeded thresholds, selective non-fungicidal insecticides were applied to avoid interference with NLB infection. Throughout the experiment, no fungicides were applied, weeds were controlled mechanically, nutrient management was kept uniform, and spraying equipment was sanitized to prevent contamination with other pathogens.

### Observed parameters

The observed parameters were categorized into primary and supporting parameters. The primary parameter was the severity of NLB disease. Disease assessment was conducted visually at the end of the flowering stage using a scoring system adapted from Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT 1999; Setyawan et al. 2016a).

Observations were made on all plots, evaluating each of the 50 plants per plot individually. Each plant was assessed for leaf blight symptoms and assigned a score based on the severity of the infection. The plot score was calculated as the average score of all individual plants within the plot. The scoring system followed the standard provided by (CIMMYT 1999).

The NLB scoring criteria are structured as follows: Score 1 (High Resistance/HR) indicates no visible infection on leaves. Score 2 (Resistance/R) is assigned when infection is found on 2-3 leaves below the ear. Score 3 (Moderately Resistance/MR) occurs when infection extends to 2-3 leaves above the ear. Score 4 (Susceptible/S) is given when all leaves, except the top 2-3 leaves, are infected. Score 5 (Highly Susceptible/HS) is assigned when all leaves on the plant show signs of infection.

The supporting parameters in this study included plant growth and yield. Plant growth parameters observed were plant Height (H), measured in centimeter from the base of the stem to the highest panicle at harvest; Stem Diameter (SD), measured in millimeter at 10 cm above the base of the stem; and Ear Height (EH); measured in centimeter from the base of the stem to the highest ear. Yield components observed included Dehusked Ear Weight (DEW), determined in gram by weighing the sample ears; Grain Weight per Ear (GWE), obtained in gram by weighing the shelled grains from each ear; and grains moisture content at Harvest (HM), measured in percentage from the shelled grains to determine moisture levels at the time of harvest using grain moisture tester. The 1,000-grain weight (W1000) was measured in gram by weighing 1000 well-filled, clean grains using a precise analytical balance. This measurement in ton/ha was used to estimate the average grain weight, which reflects grain density and size. Lastly, the Yield Potential (YP) was calculated using the formula below (Efendi 2021):

$$\text{Yield (kg ha}^{-1}\text{)} = \frac{10000}{LP} \times \frac{100 - MC}{100 - 14} \times B \times R$$

Where:

LP : Harvest area (m<sup>2</sup>) equivalent to 10 sampled plants

B : Total weight of dehusked ears (kg)

MC : Grain moisture content (%)

R : Average shelling percentage

The shelling percentage (R) was determined using the following equation:

$$R = \frac{W_{\text{dehusked ears}} - (W_{\text{dehusked ears}} - W_{\text{grain}})}{W_{\text{dehusked ears}}}$$

Where:

$W_{\text{dehusked ears}}$  : weight of ten dehusked ears and  $W_{\text{grain}}$  is the grain weight obtained from those ears.

The harvest area (LP) for ten plants was standardized by converting from the total plot size to the corresponding area occupied by ten plants.

### Data analysis

The quantitative data from each hybrid was first evaluated using an F-test at a 5% significance level. If a significant effect was detected, a follow-up Tukey test (also at 5% significance) was conducted to determine specific differences. The strength of linear relationships between

parameters was assessed using Pearson's correlation coefficient ( $r$ ). A value of  $r = 1$  indicates a perfect positive correlation, while  $r = -1$  represents a perfect negative correlation. All samples were examined and measured based on NLB scoring, plant growth, and yield variables. Principal Component Analysis (PCA) and clustergram-based heatmap modeling were used to analyze these variations. PCA and hierarchical clustering were performed using the R packages FactoMineR for computation, factoextra for visualization, and ggplot2 for advanced graphical customization (Lê et al. 2008; Wickham 2009; Galili 2015; Maechler et al. 2016). Correlation analysis was performed using SRplot (Tang et al. 2023). ANOVA and the Tukey test were assessed using SPSS 27. All analysis scripts used in this study were available from the corresponding author upon reasonable request.

## RESULTS AND DISCUSSION

### Disease severity of NLB among genotypes

Table 1 presents the evaluation of 22 genotypes based on their resistance to Northern leaf blight disease, using a scoring system where lower scores indicate higher resistance. The results showed that most genotypes fall into the Resistant (R) category, with scores ranging from approximately 1.81 to 2.76. Notably, genotypes G4 (2.12±0.14), G5 (2.32±0.32), G6 (2.35±0.02), G7 (2.39±0.14), G8 (1.93±0.03), G9 (1.92±0.06), G10 (1.90±0.07), G11 (1.91±0.06), G12 (2.41±0.45), G13 (1.81±0.14), G15 (1.92±0.13), G16 (2.27±0.09), G18 (1.93±0.08), and G21 (2.33±0.15) exhibited the highest levels of resistance, indicating not only strong but also stable resistance across replications. On the other hand, genotypes such as G1 (2.63±0.22), G2 (2.51±0.20), G3 (2.57±0.28), G14 (2.76±0.16), G17 (2.60±0.14), G19 (2.64±0.40), G20 (2.58±0.09), and G22 (2.51±0.33) were categorized as Moderately Resistant (MR). These results highlight the value of these genotypes for future breeding programs aimed at improving disease resistance traits. Further statistical analysis using ANOVA and Tukey's HSD confirmed significant difference among the 22 genotypes.

### Agronomic performance of hybrid maize genotypes

Table 2 summarizes the ANOVA and Tukey's test results for 22 maize genotypes across eight agronomic traits: H, EH, SD, DEW, HM, GWE, W1000, and YP. Significant variations were observed among genotypes for all traits, indicating substantial genetic diversity that can be exploited in breeding programs. Genotype G21 recorded the tallest H (318.50 cm), the highest EH (156.00 cm), and the greatest YP (10.05 tons/ha), suggesting strong vigor and productivity. Genotype G2 exhibited the largest SD (21.96 mm), while G10 had the highest HM (33.60%). G16 demonstrated favorable performance with W1000 (163.52 g), and G22 showed the highest DEW (233.92 g) and GWE (165.57 g). In contrast, genotype G19 consistently recorded the lowest performance across multiple parameters, such as H (257.50 cm), EH (112.73 cm), GWE (113.79 g), DEW (154.26 g), and W1000 (299.80 g). The analysis revealed statistically significant differences across all tested parameters.

**Table 1.** Northern leaf blight disease severity level

| Genotype | Repetition 1 | Repetition 2 | Repetition 3 | Mean                | Standar deviation | Resistance level |
|----------|--------------|--------------|--------------|---------------------|-------------------|------------------|
| G1       | 2.48         | 2.88         | 2.54         | 2.63 <sup>d-f</sup> | 0.22              | MR               |
| G2       | 2.74         | 2.40         | 2.40         | 2.51 <sup>c-f</sup> | 0.20              | MR               |
| G3       | 2.88         | 2.46         | 2.36         | 2.57 <sup>b</sup>   | 0.28              | MR               |
| G4       | 2.04         | 2.28         | 2.04         | 2.12 <sup>b</sup>   | 0.14              | R                |
| G5       | 2.62         | 2.36         | 1.98         | 2.32 <sup>bc</sup>  | 0.32              | R                |
| G6       | 2.38         | 2.34         | 2.34         | 2.35 <sup>b-e</sup> | 0.02              | R                |
| G7       | 2.28         | 2.34         | 2.54         | 2.39 <sup>b-d</sup> | 0.14              | R                |
| G8       | 1.90         | 1.96         | 1.94         | 1.93 <sup>a</sup>   | 0.03              | R                |
| G9       | 1.92         | 1.98         | 1.86         | 1.92 <sup>a</sup>   | 0.06              | R                |
| G10      | 1.88         | 1.98         | 1.84         | 1.90 <sup>a</sup>   | 0.07              | R                |
| G11      | 1.84         | 1.96         | 1.92         | 1.91 <sup>a</sup>   | 0.06              | R                |
| G12      | 1.92         | 2.52         | 2.80         | 2.41 <sup>b-e</sup> | 0.45              | R                |
| G13      | 1.66         | 1.80         | 1.94         | 1.81 <sup>a</sup>   | 0.14              | R                |
| G14      | 2.68         | 2.66         | 2.94         | 2.76 <sup>f</sup>   | 0.16              | MR               |
| G15      | 1.80         | 1.90         | 2.06         | 1.92 <sup>a</sup>   | 0.13              | R                |
| G16      | 2.36         | 2.18         | 2.28         | 2.27 <sup>bc</sup>  | 0.09              | R                |
| G17      | 2.74         | 2.60         | 2.46         | 2.60 <sup>ef</sup>  | 0.14              | MR               |
| G18      | 1.80         | 1.94         | 1.94         | 1.89 <sup>a</sup>   | 0.08              | R                |
| G19      | 2.84         | 2.90         | 2.18         | 2.64 <sup>d-f</sup> | 0.40              | MR               |
| G20      | 2.68         | 2.54         | 2.52         | 2.58 <sup>c-f</sup> | 0.09              | MR               |
| G21      | 2.30         | 2.20         | 2.50         | 2.33 <sup>b-d</sup> | 0.15              | R                |
| G22      | 2.52         | 2.18         | 2.84         | 2.51 <sup>c-f</sup> | 0.33              | MR               |

Note: HR: High Resistance, R: Resistance, and MR: Moderate Resistance

**Table 2.** Average plant growth characters and yield

| Genotype | H (cm)                | EH (cm)               | SD (mm)              | DEW (g)               | HM (%)               | GWE (g)               | W1000 (g)             | YP (ton/ha)         |
|----------|-----------------------|-----------------------|----------------------|-----------------------|----------------------|-----------------------|-----------------------|---------------------|
| G1       | 272.80 <sup>a-c</sup> | 124.13 <sup>a-d</sup> | 21.22 <sup>b-d</sup> | 180.88 <sup>ab</sup>  | 29.57 <sup>de</sup>  | 131.80 <sup>abc</sup> | 314.56 <sup>a</sup>   | 7.27 <sup>bc</sup>  |
| G2       | 263.73 <sup>ab</sup>  | 115.80 <sup>ab</sup>  | 21.96 <sup>d</sup>   | 176.10 <sup>ab</sup>  | 29.04 <sup>b-e</sup> | 126.71 <sup>ab</sup>  | 338.30 <sup>b</sup>   | 6.96 <sup>b</sup>   |
| G3       | 270.76 <sup>a-c</sup> | 122.66 <sup>a-c</sup> | 20.11 <sup>a-d</sup> | 186.74 <sup>bc</sup>  | 29.73 <sup>de</sup>  | 132.89 <sup>a-c</sup> | 369.30 <sup>g</sup>   | 7.28 <sup>b</sup>   |
| G4       | 275.06 <sup>a-d</sup> | 126.26 <sup>b-d</sup> | 20.93 <sup>b-d</sup> | 212.86 <sup>c-e</sup> | 28.81 <sup>a-d</sup> | 152.35 <sup>c-i</sup> | 345.66 <sup>bcd</sup> | 8.47 <sup>c-g</sup> |
| G5       | 271.86 <sup>a-c</sup> | 125.46 <sup>b-d</sup> | 20.91 <sup>b-d</sup> | 212.75 <sup>c-e</sup> | 29.49 <sup>c-e</sup> | 154.77 <sup>d-j</sup> | 338.66 <sup>b</sup>   | 8.47 <sup>c-g</sup> |
| G6       | 311.50 <sup>d-f</sup> | 140.00 <sup>de</sup>  | 19.95 <sup>cd</sup>  | 232.20 <sup>f</sup>   | 29.70 <sup>a-c</sup> | 162.00 <sup>ij</sup>  | 362.50 <sup>ef</sup>  | 9.62 <sup>hi</sup>  |
| G7       | 279.06 <sup>b-e</sup> | 128.43 <sup>b-d</sup> | 19.92 <sup>a-c</sup> | 203.86 <sup>bcd</sup> | 30.27 <sup>de</sup>  | 143.78 <sup>b-g</sup> | 344.30 <sup>b-d</sup> | 7.82 <sup>b-d</sup> |
| G8       | 287.56 <sup>c-f</sup> | 128.46 <sup>b-d</sup> | 19.58 <sup>ab</sup>  | 234.43 <sup>ef</sup>  | 29.82 <sup>de</sup>  | 160.25 <sup>f-j</sup> | 380.36 <sup>gh</sup>  | 8.75 <sup>fg</sup>  |
| G9       | 276.80 <sup>a-e</sup> | 122.16 <sup>a-c</sup> | 20.78 <sup>a-d</sup> | 211.58 <sup>c-e</sup> | 30.42 <sup>d-f</sup> | 147.04 <sup>b-h</sup> | 347.37 <sup>b-e</sup> | 7.93 <sup>c-f</sup> |
| G10      | 280.50 <sup>b-e</sup> | 128.56 <sup>b-d</sup> | 20.59 <sup>a-d</sup> | 178.88 <sup>ab</sup>  | 33.60 <sup>g</sup>   | 114.81 <sup>a</sup>   | 306.76 <sup>a</sup>   | 5.91 <sup>a</sup>   |
| G11      | 283.03 <sup>b-e</sup> | 127.93 <sup>b-d</sup> | 21.00 <sup>b-d</sup> | 213.54 <sup>c-e</sup> | 32.59 <sup>fg</sup>  | 136.79 <sup>b-e</sup> | 368.10 <sup>fg</sup>  | 7.15 <sup>b</sup>   |
| G12      | 271.26 <sup>a-c</sup> | 124.70 <sup>a-d</sup> | 20.11 <sup>a-d</sup> | 218.79 <sup>d-f</sup> | 30.27 <sup>def</sup> | 150.41 <sup>c-i</sup> | 358.66 <sup>d-f</sup> | 8.14 <sup>d-g</sup> |
| G13      | 283.46 <sup>b-e</sup> | 130.90 <sup>cd</sup>  | 20.39 <sup>a-d</sup> | 213.44 <sup>c-f</sup> | 31.10 <sup>ef</sup>  | 149.61 <sup>b-h</sup> | 358.93 <sup>d-f</sup> | 7.90 <sup>c-e</sup> |
| G14      | 279.16 <sup>b-e</sup> | 122.76 <sup>a-d</sup> | 20.21 <sup>a-d</sup> | 195.91 <sup>b-d</sup> | 29.18 <sup>b-e</sup> | 138.28 <sup>b-f</sup> | 356.16 <sup>c-f</sup> | 7.42 <sup>b-d</sup> |
| G15      | 284.50 <sup>b-e</sup> | 128.50 <sup>b-d</sup> | 21.70 <sup>a-d</sup> | 266.70 <sup>d-f</sup> | 28.45 <sup>de</sup>  | 178.95 <sup>e-j</sup> | 374.80 <sup>g</sup>   | 8.71 <sup>c-g</sup> |
| G16      | 297.36 <sup>c-g</sup> | 132.43 <sup>e</sup>   | 20.94 <sup>c-e</sup> | 234.78 <sup>ef</sup>  | 29.25 <sup>b-e</sup> | 163.52 <sup>g-j</sup> | 388.10 <sup>h</sup>   | 8.96 <sup>gh</sup>  |
| G17      | 276.40 <sup>a-e</sup> | 116.96 <sup>ab</sup>  | 20.53 <sup>a-d</sup> | 195.40 <sup>b-d</sup> | 28.59 <sup>a-d</sup> | 142.04 <sup>b-g</sup> | 359.73 <sup>d-f</sup> | 7.92 <sup>c-f</sup> |
| G18      | 274.00 <sup>c-e</sup> | 128.00 <sup>cd</sup>  | 21.30 <sup>a-d</sup> | 215.20 <sup>c-f</sup> | 27.65 <sup>de</sup>  | 164.65 <sup>e-j</sup> | 322.90 <sup>bc</sup>  | 8.29 <sup>c-g</sup> |
| G19      | 257.50 <sup>a</sup>   | 112.73 <sup>a</sup>   | 19.85 <sup>a-d</sup> | 154.26 <sup>a</sup>   | 30.68 <sup>d-f</sup> | 113.79 <sup>a</sup>   | 299.80 <sup>a</sup>   | 6.12 <sup>a</sup>   |
| G20      | 269.70 <sup>a-c</sup> | 125.46 <sup>a-d</sup> | 20.59 <sup>a-d</sup> | 178.41 <sup>ab</sup>  | 30.68 <sup>def</sup> | 132.75 <sup>a-d</sup> | 335.06 <sup>b</sup>   | 7.13 <sup>b</sup>   |
| G21      | 318.50 <sup>fg</sup>  | 156.00 <sup>b-d</sup> | 18.60 <sup>a</sup>   | 262.55 <sup>ef</sup>  | 26.05 <sup>ab</sup>  | 183.70 <sup>j</sup>   | 352.20 <sup>fg</sup>  | 10.05 <sup>b</sup>  |
| G22      | 312.50 <sup>g</sup>   | 143.05 <sup>c</sup>   | 19.55 <sup>a-c</sup> | 233.92 <sup>ef</sup>  | 28.27 <sup>a</sup>   | 165.57 <sup>h-j</sup> | 376.50 <sup>fg</sup>  | 9.23 <sup>hi</sup>  |

Note: H: Height, EH: Ear Height, SD: Stem Diameter, DEW: Dehusked Ear Weight, HM: Harvest Moisture, GWE: Grain Weight per Ear, W1000: 1000-grain weight, and YP: Yield Potential. Different letters in the Tukey HSD test indicate statistically significant differences between the compared groups

**Correlation analysis between NLB severity and agronomic traits**

Figure 1 shows the correlations between NLB severity and eight agronomic traits. NLB severity exhibited strong positive correlations with H ( $r = 0.92, p < 0.001^{**}$ ), EH ( $r =$

$0.91, p < 0.001^{**}$ ), DEW ( $r = 0.68, p < 0.001^{**}$ ), GWE ( $r = 0.67, p < 0.001^{**}$ ), YP ( $r = 0.71, p < 0.001^{**}$ ), and W1000 ( $r = 0.49, p < 0.05^{*}$ ). A significant negative correlation was found for SD ( $r = -0.52, p < 0.05^{*}$ ), indicating that plants with thicker stems may possess better structural or physiological

resistance. In contrast, HM ( $r = -0.37$ ,  $p > 0.05$ , ns) showed a non-significant correlation, suggesting limited influence on NLB severity. Significance was annotated as  $p < 0.05$  and  $p < 0.01$ .

### Multivariate analysis of key agronomic and resistance parameters

PCA revealed that the first three components (PC1-PC3) explained 86.5% of the total variance with Eigenvalue  $> 1$  (Table 3). PC1 (58.23%) was dominated by yield-related traits, such as YP, DEW, GWE, H, and EH, all with strong positive loadings ( $> 0.85$ ). PC2 (15.96%) was primarily defined by NLB severity, which loaded strongly and negatively ( $-0.86$ ), alongside SD (0.63). This indicates that genotypes positioned on the positive side of PC2 are associated with greater stem thickness but also higher NLB severity, whereas those on the negative side are associated with lower severity. PC3 (12.34%) was influenced by SD and HM, highlighting structural robustness and grain moisture effects.

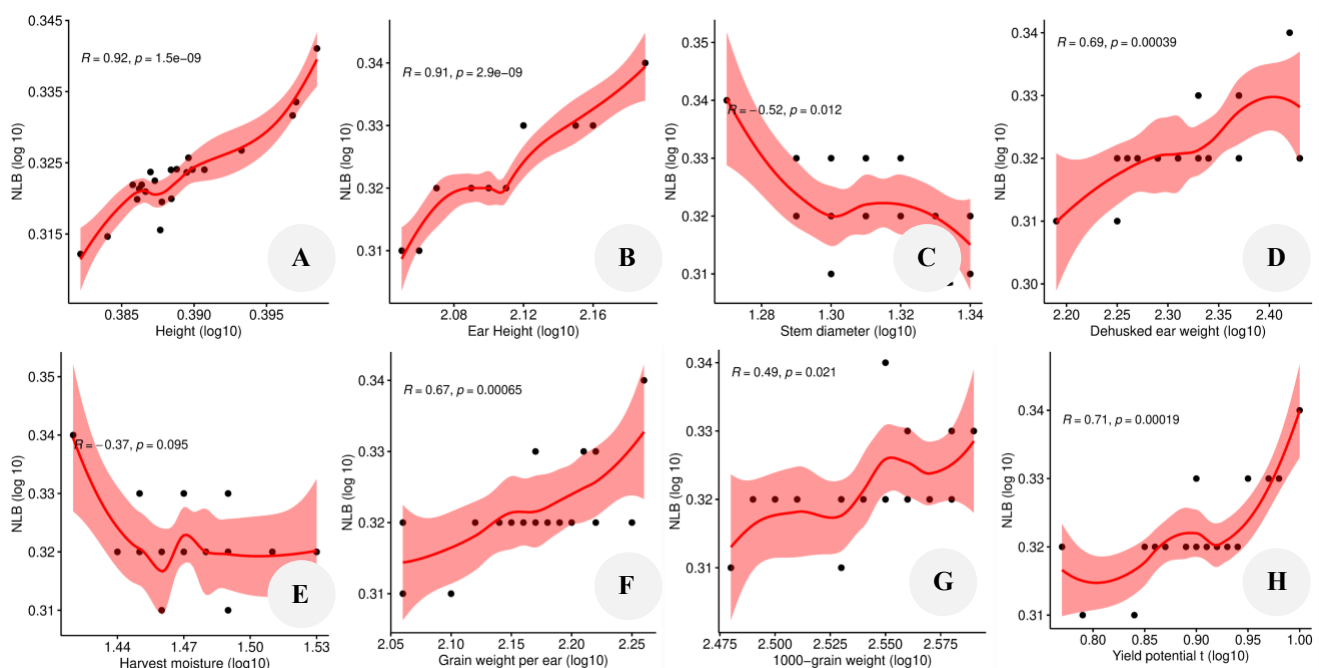
Principal Component Analysis (PCA) identified three components with eigenvalues greater than one, collectively explaining 86.53% of the total variance (Table 3). PC1 accounted for the largest proportion of variance (58.23%), mainly driven by yield-related traits such as YP, DEW, and GWE, along with H and EH. PC2 contributed 15.96% and was strongly associated with NLB severity (negative loading), while PC3 explained 12.34% of the variance, reflecting additional contributions from SD and HM. Together, PC1-PC3 captured nearly 86.5% of the variability among the genotypes.

The scree plot illustrates the relative magnitudes of the eigenvalues, ranking the principal components according to

their importance (Figure 2.A). The first three components, with eigenvalues equal to or greater than 1, were considered significant. Beyond this point, the eigenvalues declined sharply, indicating that the remaining components contribute minimally to the total variance and can be excluded from further analyses. In the PCA biplot (Figure 2.B), genotype G21 was positioned far to the right along PC1, reflecting its superior productivity traits such as the tallest H, highest EH, and greatest YP. Importantly, Table 2 classified G21 as Resistant (R) with a mean severity score of 2.33, which is consistent with its negative PC2 score indicating lower disease severity. In contrast, G22, the control variety Sukmaraga, also occupied the positive side of PC1, highlighting its good yield attributes, but was classified as Moderately Resistant (MR, mean score 2.51). This classification aligns with its relatively positive PC2 position, suggesting higher NLB severity despite favorable agronomic performance. Genotype G6, which clustered near G22, also showed strong yield performance with a yield potential of 9.62 ton/ha. However, unlike G22, G6 had a negative PC2 score, consistent with its Resistant classification (mean score 2.35) and indicating lower NLB severity.

### Cluster analysis of maize genotypes

Based on the dendrogram analysis, four clusters of maize genotypes were successfully identified, each representing groups with similar agronomic characteristics. Figure 3 shows four clusters: Cluster 1: G7, G12, G3, G14, and G17; Cluster 2: G10, G19, G2, G20; Cluster 3: G21, G6, G22; and the last, Cluster 4: G15, G8, G16, G9, G13, G18, G4, and G5. These clusters demonstrate the genetic diversity among the 22 maize genotypes for selection in breeding programs.



**Figure 1.** Correlation between NLB severity level and plant growth and yield parameters

Discussion

In modern plant breeding, species diversity is crucial; greater diversity corresponds to higher potential yield (Syahrudin et al. 2020). The availability of genetic resources needs to be evaluated in terms of the diversity and specific traits to develop superior maize varieties (Swarup et al. 2021). One important aspect to study is plant resistance to diseases, such as NLB (Setyawan et al. 2016a). Furthermore, understanding how resistance traits relate to other agromorphological characteristics is essential (Supriati et al. 2024), as morphological diversity is influenced by the plants' genetic factors and the environmental conditions where they are grown (Yousuf et al. 2021). These considerations drive plant breeders to develop superior varieties using selected germplasm with disease-resistant traits (Setyawan et al. 2016a). Hybrid-based selection plays a key role in producing the most promising varietal lines (Hidayatullah et al. 2023a).

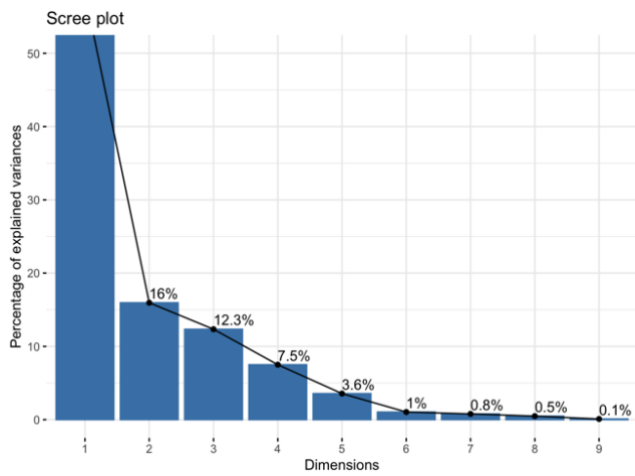
The classification of NLB severity levels among the 22 tested maize genotypes demonstrates that 14 genotypes are resistant, while the remaining eight are moderately resistant. As shown in Table 2, the overall performance of the genotypes under field conditions is favorable. Setyawan et al. (2016a) reported that 8 out of 12 tested varieties were resistant, while others exhibited lower levels of resistance. Similarly, studies on hybrid maize in Northeast China indicate that most tested genotypes possess resistance to NLB (Ma et al. 2022). Environmental factors such as humidity, rainfall, temperature, and wind speed significantly influence the development and severity of NLB (Mirsam et al. 2025). In the early stages of selecting NLB-resistant hybrid maize, the incubation period serves as a critical indicator of resistance response (Permana et al. 2021).

All growth and yield traits showed significant differences among the tested maize varieties. NLB incidence was significantly correlated with most agronomic parameters

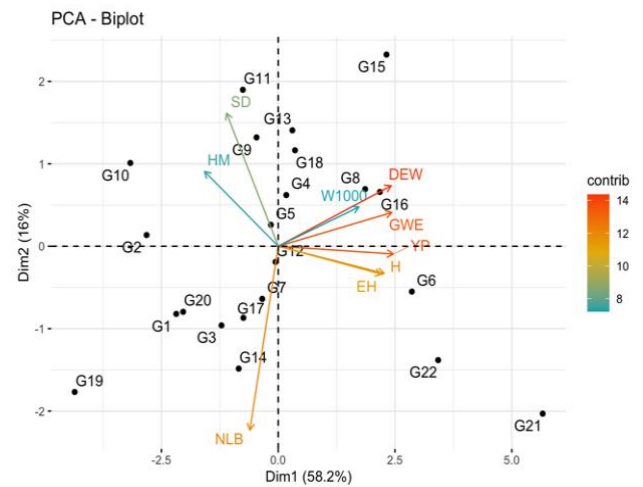
except harvest moisture ( $p>0.05$ ). These correlations suggest that taller, high-yielding plants may be more prone to NLB, whereas plants with thicker stems tend to show greater resistance. This tendency has been reported in earlier studies, where NLB primarily attacked the leaves as the main photosynthetic organs (Setyawan et al. 2016a). *Exserohilum turcicum* is a hemibiotrophic pathogen that can initiate infection within one day after inoculation, leading to tissue damage, xylem blockage, and leaf wilting (Kotze et al. 2019). Damage to the leaves reduces the plant's ability to produce and translocate assimilates to the ears, ultimately decreasing ear weight (Semangun 2008). Thus, increasing NLB severity is generally associated with reduced yield and unfavorable plant characteristics, as also noted by Badu-Apraku et al. (2021).

**Table 3.** Eigenvectors and eigenvalues of the nine Principal Components (PC), only loadings $\geq 0.40$  are shown, blanks indicate loading $<0.40$

| Variables                  | PC 1  | PC 2  | PC 3  |
|----------------------------|-------|-------|-------|
| Variance %                 | 5.24  | 1.43  | 1.11  |
| Cumulative variance %      | 58.23 | 15.96 | 12.34 |
| Eigenvectors               |       |       |       |
| Height (H)                 | 0.88  |       |       |
| Ear Height (EH)            | 0.86  |       |       |
| Stem Diameter (SD)         | -0.43 | 0.63  | 0.53  |
| Dehusked Ear Weight (DEW)  | 0.94  |       |       |
| Harvest Moisture (HM)      | -0.61 |       | -0.63 |
| Grain Weight per Ear (GWE) | 0.94  |       |       |
| 1000-grain Weight (W1000)  | 0.67  |       |       |
| Yield Potential (YP)       | 0.95  |       |       |
| Severity (NLB)             | -     | -0.86 |       |

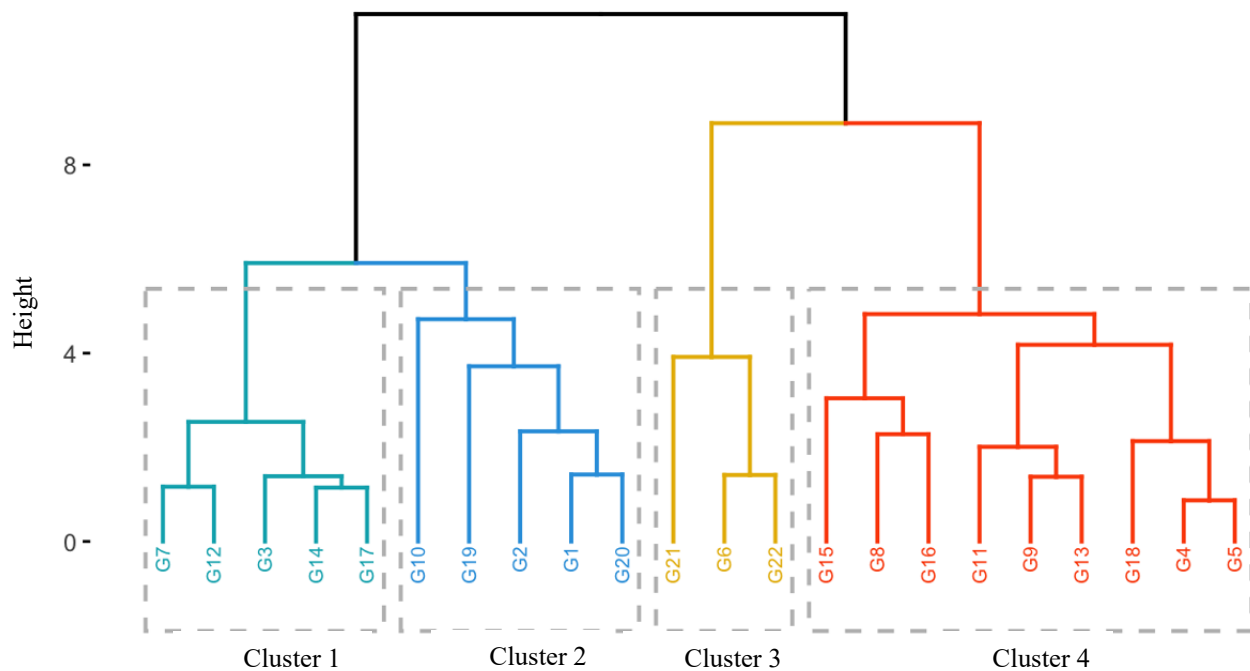


A



B

**Figure 2.** A. Scree plot of Principal Components (PCs), B. PCA biplots of the first two principal components (PC1 and PC2). H: Height, EH: Ear Height, SD: Stem Diameter, DEW: Dehusked Ear Weight, HM: Harvest Moisture, GWE: Grain Weight per Ear, W1000: 1000-grain weight, YP: Yield Potential, and NLB: Northern Leaf Blight



**Figure 3.** Cluster analysis of agronomic characteristics

The multivariate analysis of the nine attributes showed that the first two principal components captured most of the overall variability. According to PCA, nine quantitative traits were identified as key factors explaining most of the variation, making them suitable for maize selection and improvement. PC1 accounted for 58.23% of the variation, considerably more than PC2, which explained 15.96%. Similar results have been observed in previous studies, where PC1 consistently exerted greater influence. For example, study on the selection of superior maize genotypes found that PC1 accounted for 31.06%, compared to PC2 at 21.64% (Carnimeo et al. 2020). This suggests that PC1 is the strongest indicator for selecting traits related to yield improvement (Ilieva et al. 2019).

Height, ear height, dehusked ear weight, harvest moisture, grain weight per ear, 1000-grain weight, and yield potential were tested in this PCA analysis and contributed significantly to PC1. Traits with higher coefficients, usually 0.6 or above, are considered more critical in the study (Afrianto et al. 2017). In general, positive and negative loadings indicate the tendency of positive or negative correlations between components and variables, contributing to diversity and distinguishing clusters (Yeshitila et al. 2023). PCA can be used to examine correlations between traits and identify independent principal components that significantly influence plant attributes, particularly when selecting hybrid maize (Fadhli et al. 2023). This analysis also serves as a dimensionality-reduction method that summarizes many variables into a smaller set containing the most important and highly contributing information (Reddy et al. 2020). Principal components are new variables formed by combining the original ones to capture the most variation in the data (Greenacre et al. 2022).

For a comprehensive interpretation, it is necessary to visualize PCA resulted using a biplot to display the relationships and clustering patterns among plant genotypes (Greenacre et al. 2022). According to Naidoo et al. (2020), a biplot places traits with smaller influences closer to the center of the axis, whereas traits positioned farther from the center have a greater impact. The length of the vector lines and their proximity to the axis generally correspond to the magnitude of vector loadings. Genotypes located close to each other on the biplot suggest similarity in their main characteristics, whereas genotypes such as G21 and G22, which were positioned farther from the center and separated from other groups, may reflect distinct diversity or unique characteristics. Based on the result, Genotype G21 recorded the tallest height (318.50 cm), highest ear height (156.00 cm), and greatest yield potential (10.05 tons/ha). According to Deras and Ketaren (2024), Pioneer P32 is a high-yielding hybrid maize variety with an average productivity of 13.76 tons/ha, net income of Rp 10.01 million/ha, and an OIR of 1.59-1.92, making it economically efficient for farmers. Other advantages include ease of harvesting and bright-colored grains. According to DPKP DIY (2023), genotype G22 or Sukmaraga has a physiological maturity of 105-110 days, plant height 180-220 cm, an average yield of 6.0 tons/ha of dry shelled corn, a potential yield of 8.50 tons/ha of dry shelled corn, a 1000-grain weight of 270 g, grain type pearl, and moderate resistance to downy mildew, leaf spot, and stalk rot. This finding suggests that genotype G6 possesses similarly high yield potential and resistance characteristic.

The results of the cluster analysis further reinforced the PCA findings, as genotypes grouped consistently according to their productivity traits and NLB resistance levels, thereby providing complementary evidence for the multivariate

interpretation. Cluster analysis is another method used to determine the pattern and degree of correlation among genotypes (Hamidah et al. 2024). One of the most effective ways to utilize genetic diversity for yield improvement is clustering genotypes according to their average performance (Farid et al. 2020). The separation of materials from the same origin into different clusters indicates a broad genetic base among genotypes (Anggraeni et al. 2018). Genotypes from the same origin appearing in distinct clusters indicate a broad genetic foundation (Akin-Idowu et al. 2016). A total of 22 maize genotypes were grouped into four clusters based on the average performance of each measured quantitative variable. The cluster analysis indicated that genotype G6 was grouped in the same cluster as genotype G21 and G22 (control).

In conclusion, this study successfully identified several new hybrid maize genotypes with strong resistance to NLB and promising agronomic performance. Among the 22 maize genotypes tested for NLB severity, 14 were classified as resistant, while the other eight showed moderate resistance. NLB severity was positively correlated with H, EH, DEW, GWE, YP ( $p < 0.001$ ; \*\*), and W1000 ( $p < 0.05$ ; \*), negatively correlated with stem diameter ( $p < 0.05$ ; \*). The integration of PCA and clustering analyses provided consistent evidence that genotypes G21 and G22 were distinctly separated from the other genotypes, reflecting their roles as established control varieties with proven superior performance in both yield and stability. Notably, genotype G6 was positioned in close proximity to G21 and G22 in both analyses, suggesting that it shares a similar profile of desirable agronomic attributes, high yield potential, and stable resistance to NLB. This convergence of evidence highlights G6 as a promising candidate for further development and selection, with the potential to serve as a competitive alternative to the control varieties under diverse agroecological conditions. These findings contribute valuable insights for developing resilient and high-yielding maize varieties suitable for diverse agroclimatic conditions, particularly in West Sumatra, Indonesia.

This study offers important insights into NLB resistance and the agronomic performance of hybrid maize genotypes; however, its findings are constrained by evaluation in a single location and season, limited consideration of pathogen diversity, and the absence of molecular or multi-environment analyses needed for thorough validation and broader recommendations.

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