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*Research article*

# Evaluating agronomic traits and selection of low N-tolerant maize hybrids in Indonesia

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**Abstract:** Nitrogen is one of the macro elements that maize needs. Nitrogen deficiency will affect maize's growth and grain yield. This study aimed to determine hybrid maize's growth, grain yield, and tolerance to low N conditions. This research was conducted at the Indonesian Cereal Testing Instrument Standard Institute in Maros, South Sulawesi, Indonesia, from July to November 2022. A nested design was applied with eleven hybrid maize genotypes and three N fertilization levels (N0 = 0 kg N $ha^{-1}$, N1 = 100 kg N $ha^{-1}$, and N2 = 200 kg N $ha^{-1}$) as treatments, replicated three times. Growth and grain yield traits were measured. An analysis of variance was used to determine the effect of fertilization level on growth. Eberhart and Russell stability analysis and the Stress Tolerance Index (STI) were used to determine hybrid maize tolerance and yield stability across the three fertilization levels. The findings indicated that the reduction in nitrogen fertilizer level affected maize agronomic performance and yield reduction. HLN 09 exhibited a mean yield of 7.68 t $ha^{-1}$, surpassing the overall hybrid mean of 7.21 t $ha^{-1}$. HLN 09 also demonstrated moderate stress tolerance at N2-N1, N2-N0, and N1-N0 and was characterized as a stable hybrid with regression coefficient (bi) = 0.99 and deviation from regression ($s^2di$) = −0.22. The HLN 09 maize hybrid was a hybrid maize with good tolerance to low N conditions and high stability and yield.

**Keywords:** low nitrogen; stability; tolerance index; yield; Zea mays L.

## 1. Introduction

Maize is a vital crop for humans. Humans rely on maize for various purposes, including food, feed, industry, and biofuel [1]. In 2022, Indonesian maize production was 16.53 million t, decreasing by around 12.50% in 2023, while maize demand increased at an increasing rate[2,3]. A challenge for increasing maize production is agricultural expansion in areas with low soil nitrogen (N) levels.

Nitrogen (N) is crucial for maize, serving as a vital nutrient for its life cycle [4]. N deficiency can reduce leaf area and photosynthesis rate because more photosynthate is allocated to roots [5]. This deficiency may also decrease plant height, increase the Anthesis-Silking Interval, accelerate senescence [6–8]. Additionally, nitrogen deficiency leads to decreased maize yield during harvest [9–11].Yields can drop by 10–50%, reaching up to 70% under severe stress conditions due to N deficiency [12,13].

The development of maize varieties with low-nitrogen (N) tolerance has addressed the challenge of cultivating crops in areas with insufficient N levels. Globally, breeding maize with low-N tolerance has been a significant focus in maize breeding. Breeding low-N-tolerant maize plants can enhance maize yield in China by 14% [14]. More than 100 inbred lines can be used as parents for breeding with low nitrogen tolerance hybrids that have high stable yield [15,16]. Various hybrid combinations with low N tolerance in maize have also been documented by [17–19]. In the context of Indonesia, the CY 11, G2013631, MR 14, AVLN 118-7, and AVLN 83-2 lines demonstrate good combining ability for yield in low N conditions [20,21]. It is possible to select low-nitrogen-tolerant hybrid maize lines based on secondary characteristics, stress tolerance index, and Simple Sequence Repeat (SSR) markers [22–24]. In Indonesia, there are 15 low-N-tolerant hybrid maize selected based on the Stress Tolerance Index and the Stress Susceptibility Index[25,26].

Low-nitrogen-tolerant hybrid maize is a potential solution for Indonesia's low soil nitrogen (N) problems. However, the current research on this crop is limited and slow. Therefore, more research is required to overcome these challenges. This research aimed to investigate the impact of nitrogen fertilization on the growth and yield of maize hybrids and assess their tolerance to N stress. The results can provide valuable insights for breeding high-yield hybrid maize under low N conditions in Indonesia, improving food security and economic growth.

## 2. Materials and methods

This study was conducted at the Indonesian Cereal Testing Instrument Standard Institute in Maros, South Sulawesi, Indonesia, from July to November 2022. The experiment involved a total of nine promising low-nitrogen-tolerant maize hybrids (HLN 01, HLN 02, HLN 03, HLN 04, HLN 05, HLN 06, HLN 07, HLN 08, and HLN 09) and two control varieties: ADV 777 (hybrid maize that requires high nitrogen) and JH 37 (moderately tolerant to low nitrogen and drought hybrid maize). The genotype arrangement employed a three-replication nested design. The genotypes were organized within the nested structure based on the nitrogen fertilizer levels, i.e., 0 kg N ha$^{-1}$, 100 kg N ha$^{-1}$, and 200 kg N ha$^{-1}$. The 200 kg N ha$^{-1}$ level is the usual nitrogen fertilizer level farmers use for maize in Indonesia. It represents a high fertilizer level. The 100 kg N ha$^{-1}$ level represents half of the usual fertilizer dose and serves as a low fertilizer level. It allows us to observe how maize responds to a reduced fertilizer level. 0 kg N ha$^{-1}$ is the baseline at which no nitrogen is applied. It helps us understand the natural conditions or the minimum nitrogen requirement for maize. The experiment plot was 3 meters by 5 meters, with plants spaced at 75 cm between rows and 20 cm within rows, so there were 100 plants in one plot. This plant spacing corresponded to a population density of 66,666 plants $ha^{-1}$. At 10 days after planting (DAP), the 100 kg N $ha^{-1}$ treatment was applied, while the 200 kg N $ha^{-1}$ treatment was split into two doses: one at 10 DAP and the other at 35 DAP, Phosphorus (P) and potassium (K) fertilizers, each at a rate of 60 kg $ha^{-1}$, were applied ten days after planting (DAP). Optimal plant maintenance practices were implemented, including weeding, watering, and hoarding.

Before the research, a soil test was done (Table 1). The total nitrogen analysis employed the Kjeldahl method [27], while soil organic carbon analysis utilized the Walkley-Black method [28]. The analysis shows that the location has a silty clay texture. The land has a very low level of organic C and low total nitrogen and C/N ratio. That level means the land is suitable for low-N-tolerant maize selection.

**Table 1.** Soil characteristics of the experimental site at the laboratory of the Indonesian Cereal Testing Instrument Standard Institute.

| Parameter | Value | Level |
|---|---|---|
| Texture | | |
| Clay (%) | 39 | |
| Silt (%) | 46 | |
| Sand (%) | 15 | |
| Organic C (%) | 0.89 | Very low |
| Total N (%) | 0.13 | Low |
| C/N | 5.75 | Low |

Source: Soil laboratory of the Indonesian Cereal Testing Instrument Standard Institute.

The observed variables were agronomic traits and yield. The agronomic traits included plant height, ear height, stalk diameter, leaf angle, leaf length, and leaf width. The yield was corrected to t $ha^{-1}$ with 15% moisture, employing the formula

$$\text{Yield } (\text{t ha}^{-1}) = \frac{10^4}{\text{HA}} \text{ x } \frac{\text{100-GM}}{85} \text{ x EHW x SP} \div 1.000 \quad [29] \tag{1}$$

HA = harvested area ($m^2$);
GM = grain moisture (%);
EHW = ear harvested weight (kg);
SP = shelling percentage (%).

An analysis of variance was performed to assess the effects of N fertilizer levels, genotype, and their interaction on the variables observed [30]. If a significant effect was found, a 5% LSD test was conducted to compare the test hybrid with control varieties.

The Stress Tolerance Index (STI) is used to measure maize hybrids' tolerance to low nitrogen (N) conditions. The STI formula is $\frac{Y_s \text{ x } Y_p}{\bar{Y}_p^2}$ [31]. $Y_s$ and $Y_p$ represent the hybrid yield under low and optimum N conditions, respectively, and the average yield of all hybrids under optimum N conditions is $\bar{Y}_p^2$. The tolerance levels of the hybrids are based on their STI values: STI > 1.0 for tolerance, 0.5 < STI ≤ 1.0 for moderate tolerance, and STI ≤ 0.5 for susceptible.

The stability of the hybrid over the three N levels is another factor in determining maize hybrid tolerance. The Eberhart and Russel stability analysis [32] used $b_i = \frac{\sum_j Y_{ij} I_j}{\sum_j I_j^2}$, $S_{di}^2 = \left(\frac{\sum_j \hat{\delta}_{ij}^2}{j-2} - \frac{s_e^2}{r}\right)$, where $b_i$ is the regression coefficient, $S_{di}^2$ is the deviation from regression, i is the genotype number, j is the environment number, r is the replication number, $Y_{ij}$ is the average yield of the i$^{th}$ genotype in the j$^{th}$ environment, $I_j$ is the environmental index = mean index, i.e., the mean yield of the jth environment minus the mean yield of all genotypes, $\sum_j \hat{\delta}_{ij}^2$ = pooled variance, and $\sum_j \hat{\delta}_{ij}^2$ = pooled ANOVA error.

## 3. Results

### *3.1. Nitrogen level effect on maize agronomic traits*

The effect of nitrogen fertilizer, genotype, and their interactions are displayed in Table 2. Table 2 demonstrates that nitrogen fertilizer and genotype significantly affected maize traits and yields. Their interaction was also significant for all variables except leaf width and angle. The variables' coefficients of variation (CVs) varied between 4.70% and 15.20%.

**Table 2.** Nitrogen fertilizer, genotype, and interaction effects on agronomic traits and maize yields.

| Variable | Mean square | | | | | | | | CV (%) |
|---|---|---|---|---|---|---|---|---|---|
| | Nitrogen (N) | | R/N | Hybrid (H) | | H x N | | Error | |
| Plant height | 11777.50 | ** | 66.17 | 571.38 | * | 955.91 | ** | 218.47 | 7.40 |
| Ear height | 3788.14 | ** | 28.78 | 242.23 | ** | 660.90 | ** | 64.87 | 7.60 |
| Stalk diameter | 235.60 | ** | 8.28 | 14.78 | ** | 7.79 | ** | 2.84 | 7.00 |
| Leaf angle | 344.93 | ** | 10.89 | 98.32 | ** | 14.82 | | 14.84 | 15.20 |
| Leaf length | 1689.11 | ** | 34.30 | 152.66 | * | 45.52 | ** | 15.19 | 4.70 |
| Leaf width | 9.12 | ** | 0.49 | 2.19 | ** | 0.33 | | 0.44 | 6.80 |
| Yield | 289.03 | ** | 0.81 | 3.50 | ** | 3.56 | ** | 0.73 | 11.90 |

Note: * = significant at $p < 0.05$, ** = significant at $p < 0.01$, CV = coefficient of variation.

Table 3 illustrates that the agronomic traits of maize vary with each level of fertilizer. For plant height, at 200 kg N ha$^{-1}$, the range is 202.27–249.93 cm. At 100 kg N ha$^{-1}$, it is 187.53–212.00 cm. At 0 kg N ha$^{-1}$, it is 153.6–191.33 cm. HLN 01 and HLN 07 do not differ in plant height across the three fertilizer levels. Only HLN 01 shows no differences across the fertilizer levels for ear height. The ear height had ranges of 94.60–139.67 cm at 200 kg N ha$^{-1}$, 95.47 to 115.00 cm at 100 kg N ha$^{-1}$, and 61.00–111.13 cm at 0 kg N ha$^{-1}$. The stalk diameter at 200 kg N ha$^{-1}$ ranged from 23.80 to 29.58 mm. At 100 kg N ha$^{-1}$, it ranged from 20.78 cm to 26.27 cm. At 0 kg N ha$^{-1}$, it ranged from 18.44 to 24.39 mm. The leaf length was 82.13–95.91 cm at 200 kg N ha$^{-1}$, 76.53–92.20 cm at 100 kg N ha$^{-1}$, and 64.20–85.16 cm at 0 kg N ha$^{-1}$. Only HLN 03 and JH 37 do not show any differences in stalk diameter and leaf length across all levels of fertilizers.

**Table 3.** Plant height, ear height, stalk diameter, and leaf length of hybrid maize at three N levels.

| Hybrid | Plant height (cm) | | | Ear height (cm) | | | Stalk diameter (mm) | | | Leaf length (cm) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N2 | N1 | N0 | N2 | N1 | N0 | N2 | N1 | N0 | N2 | N1 | N0 |
| HLN 01 | 202.27 | 199.33 | 185.40 | 94.60[b] | 111.73 | 111.13 | 26.60 | 22.65 (x) | 20.70 (x) | 88.67 | 87.80[a] | 77.13 (x) |
| HLN 02 | 226.60 | 202.07 (x) | 153.60 (x) | 118.53 | 115.00 | 61.00[ab] (x) | 26.50 | 26.27[ab] | 23.40[a] (x) | 95.91[ab] | 92.20[ab] | 85.16[a] (x) |
| HLN 03 | 249.93 | 202.40 (x) | 177.93 (x) | 139.67 | 111.20 (x) | 99.07 (x) | 27.07 | 25.47[ab] | 24.39[a] | 89.27 | 85.33[a] | 81.13 (x) |
| HLN 04 | 216.27 | 208.53 | 175.07 (x) | 117.00 | 95.47[b] (x) | 100.27 (x) | 29.55 | 24.51[ab] (x) | 22.39 (x) | 82.13 | 76.53 | 66.27 (x) |
| HLN 05 | 210.67 | 188.47[a] | 182.20 (x) | 116.00 | 95.80[b] (x) | 105.00 | 23.80 | 22.78 | 20.86 (x) | 90.13 | 79.67 (x) | 74.53 (x) |
| HLN 06 | 243.47 | 201.20 (x) | 181.60 (x) | 131.60 | 107.20 (x) | 91.80 (x) | 26.56 | 25.13[ab] | 21.24 (x) | 89.40 | 86.27[a] | 64.20 (x) |
| HLN 07 | 205.93 | 187.53[a] | 184.73 | 113.20 | 96.33[b] (x) | 100.80 | 24.61 | 22.49 | 18.44 (x) | 87.33 | 86.33[a] | 69.07 (x) |
| HLN 08 | 242.33 | 206.07 (x) | 191.33 (x) | 128.33 | 114.47 (x) | 96.87 (x) | 27.05 | 26.10[ab] | 21.85 (x) | 91.33[a] | 89.07[a] | 73.13 (x) |
| HLN 09 | 228.33 | 212.00 | 172.93 (x) | 123.93 | 110.67 (x) | 91.27 (x) | 24.60 | 24.34[ab] | 20.20 (x) | 92.20[ab] | 88.60[a] | 74.93 (x) |
| ADV 777 | 214.40 | 196.67 | 176.47 (x) | 122.22 | 100.00 (x) | 89.67 (x) | 29.58 | 20.78 (x) | 20.47 (x) | 84.27 | 77.20 (x) | 78.27 |
| JH 37 | 215.67 | 188.33 (x) | 166.13 (x) | 114.67 | 114.40 (x) | 80.20 | 28.77 | 21.23 (x) | 22.11 (x) | 85.20 | 83.60 | 79.33 |
| Mean | 223.26 | 199.33 | 177.04 | 117.96 | 96.65 | 93.37 | 26.79 | 23.8 | 21.46 | 88.71 | 84.78 | 74.83 |
| LSD 5% | 24.14 | 24.14 | 24.14 | 13.15 | 13.15 | 13.25 | 2.75 | 2.75 | 2.75 | 6.36 | 6.36 | 6.36 |

Note: N0 = 0 kg N $ha^{-1}$, N1 = 100 kg N $ha^{-1}$, N2 = 200 kg N $ha^{-1}$; in a row, (x) = significant difference from 200 kg N $ha^{-1}$ by 5% LSD; in a column, a = better than ADV 777 by 5% LSD, b = better than JH 37 by 5% LSD.

### 3.2. Maize yield at the three nitrogen levels and the tolerance index

Table 4 presents the yields of the hybrids at nitrogen levels of 200 kg N ha$^{-1}$, 100 kg N ha$^{-1}$, and 0 kg N ha$^{-1}$, along with the corresponding yield decreases and the Stress Tolerance Index (STI) levels for each fertilization level. The research study revealed that the yield of hybrid maize varied significantly with different levels of nitrogen fertilization. The yield of maize ranged from 8.42 to 12.57 t ha$^{-1}$ with the application of 200 kg N ha$^{-1}$. With low nitrogen fertilization of 100 kg N ha$^{-1}$, the yield ranged from 4.74 to 8.09 t ha$^{-1}$. However, without any nitrogen fertilizer, the yield decreased significantly. The yield of maize ranged from 4.40 to 5.33 t ha$^{-1}$.

**Table 4.** Yields at 200 kg N ha$^{-1}$, 100 kg N ha$^{-1}$, and 0 kg N ha$^{-1}$, yield reductions and Stress Tolerance Index (STI) values.

| Hybrid | Yield (t ha$^{-1}$) | | | Yield reduction (t ha$^{-1}$) | | | STI | | |
|---|---|---|---|---|---|---|---|---|---|
| | N2 | N1 | N0 | N2-N1 | N2-N0 | N1-N0 | N2-N1 | N2-N0 | N1-N0 |
| HLN 01 | 8.42 | 7.00$^{a}$ | 5.33 | 1.42 | 3.10 | 1.67 | 0.54 (MT) | 0.41 (S) | 0.86 (MT) |
| HLN 02 | 12.05$^{ab}$ | 8.09$^{ab}$ | 4.40 | 3.96 | 7.66 | 3.70 | 0.89 (MT) | 0.49 (S) | 0.82 (MT) |
| HLN 03 | 12.57$^{ab}$ | 5.61 | 4.60 | 6.96 | 7.97 | 1.01 | 0.65 (MT) | 0.53 (MT) | 0.60 (MT) |
| HLN 04 | 8.96 | 6.76$^{a}$ | 4.53 | 2.20 | 4.44 | 2.24 | 0.56 (MT) | 0.37 (S) | 0.71 (MT) |
| HLN 05 | 9.73 | 7.30$^{a}$ | 4.52 | 2.43 | 5.21 | 2.78 | 0.65 (MT) | 0.40 (S) | 0.76 (MT) |
| HLN 06 | 11.02$^{ab}$ | 7.16$^{a}$ | 4.84 | 3.86 | 6.18 | 2.33 | 0.72 (MT) | 0.49 (S) | 0.80 (MT) |
| HLN 07 | 11.45$^{ab}$ | 6.16$^{a}$ | 4.47 | 5.29 | 6.99 | 1.69 | 0.65 (MT) | 0.47 (S) | 0.64 (MT) |
| HLN 08 | 12.42$^{ab}$ | 6.09 | 3.90 | 6.33 | 8.52 | 2.19 | 0.69 (MT) | 0.44 (S) | 0.55 (MT) |
| HLN 09 | 10.83$^{ab}$ | 7.16$^{a}$ | 5.05 | 3.67 | 5.79 | 2.11 | 0.71 (MT) | 0.50 (MT) | 0.84 (MT) |
| ADV 777 | 8.50 | 4.74 | 4.58 | 3.76 | 3.92 | 0.17 | 0.37 (S) | 0.36 (S) | 0.50 (MT) |
| JH 37 | 8.90 | 6.18 | 4.73 | 2.72 | 4.17 | 1.46 | 0.50 (MT) | 0.39 (S) | 0.68 (MT) |
| Mean | 10.44 | 6.57 | 4.63 | 3.87 | 5.81 | 1.94 | | | |
| SE | 0.49 | 0.49 | 0.49 | | | | | | |
| LSD 5% | 1.40 | 1.40 | 1.40 | | | | | | |

Note: N0 = 0 kg N ha$^{-1}$, N1 = 100 kg N ha$^{-1}$, N2 = 200 kg N ha$^{-1}$, a = better than ADV 777 by 5% LSD, b = better than JH 37 by 5% LSD, S = susceptible, MT = moderate tolerance.

As per Table 4, when comparing the yield at a rate of 200 kg N ha$^{-1}$ with that at 100 kg N ha$^{-1}$, the yield reduction ranged from 1.42 to 6.96 t ha$^{-1}$. Similarly, the yield reduction varied from 3.10 to 8.52 t ha$^{-1}$ when comparing the yield at 200 kg N ha$^{-1}$ to that at 0 kg N ha$^{-1}$. The yield reduction from 100 kg N ha$^{-1}$ to 0 kg N ha$^{-1}$ ranged from 0.17 t ha$^{-1}$ to 3.70 t ha$^{-1}$.

The STI values ranged from 0.37 to 0.89 when fertilized with 200 kg N ha$^{-1}$ and 100 kg N ha$^{-1}$. Ten hybrids showed moderate tolerance, while only one hybrid was susceptible. On the other hand, when maize fertilized was with 200 kg N ha$^{-1}$ and 0 kg N ha$^{-1}$, the STI index was between 0.36 and 0.53. Only two hybrids demonstrated moderate tolerance, while the rest were susceptible. At rates of 100 kg N ha$^{-1}$ and 0 kg N ha$^{-1}$, the STI ranged from 0.50 to 0.86, and all hybrids were classified as moderate tolerance (Table 4).

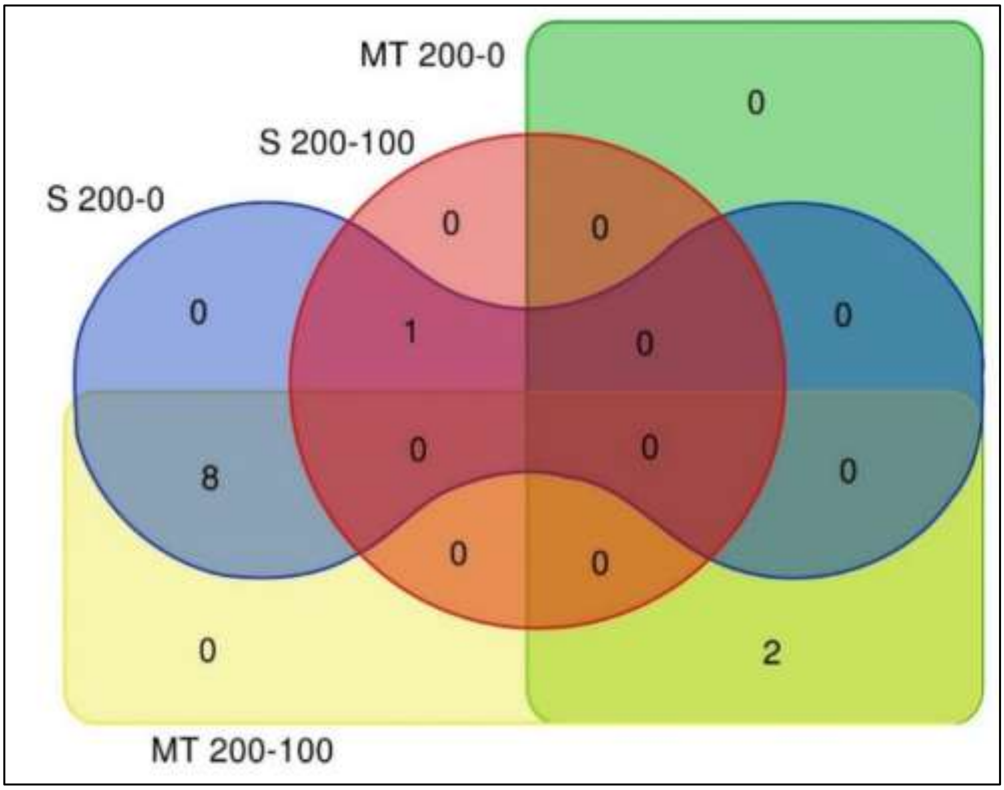


**Figure 1.** Venn diagram of maize hybrids' tolerances based on STI at 200 kg N ha$^{-1}$ and 0 kg N ha$^{-1}$ and 200 kg N ha$^{-1}$ and 100 kg N ha$^{-1}$ fertilization levels. Note: S 200-0 = Susceptible at rate of 200 kg N ha and 0 kg N ha$^{-1}$, S 200-100 = Susceptible at rate of 200 kg N Ha$^{-1}$ and 100 kg N ha$^{-1}$, MT 200-0 = Moderate Tolerance at rate of 200 kg N ha$^{-1}$ and 0 kg N ha$^{-1}$ and MT 200-100 = Moderate Tolerance at rate of 200 kg N ha$^{-1}$ and 100 kg N ha$^{-1}$.

The relationship pattern of tolerance levels of the hybrids based on their STIs of 200 kg N ha$^{-1}$ to 100 kg N ha$^{-1}$, 200 kg N ha$^{-1}$ to 0 kg N ha$^{-1}$, and 100 kg N ha$^{-1}$ to 0 kg N ha$^{-1}$ is displayed in a Venn diagram in Figure 1. Interestingly, the tolerance level at 100 kg N ha$^{-1}$ to 0 kg N ha$^{-1}$ was moderate, the same as for the other dose combination. Therefore, it was not included in the Venn diagram. Only STI values for the other two dose combinations (200 kg N ha$^{-1}$ to 100 kg N ha$^{-1}$ and 200 kg N ha$^{-1}$ to 0 kg N/ha) were shown in the diagram. The diagram shows that one hybrid is susceptible at 200 kg N ha$^{-1}$ to 100 kg N ha$^{-1}$ and 200 kg N ha$^{-1}$ to 0 kg N/ha. Additionally, eight hybrids are moderately tolerant to the first dose combination but susceptible to the second. Two maize genotypes fall into the moderately tolerant category for both dose combinations.

Table 5 shows the average yield, regression coefficient (bi), and regression deviation value ($s^2$di) for eleven maize hybrids at three levels of N fertilizer. The average yield was 7.21 t ha$^{-1}$, ranging from 5.49 t ha$^{-1}$ (ADV 777) to 8.18 t ha$^{-1}$ (HLN 02). Six hybrids (HLN 02, HLN 03, HLN 06, HLN 07, HLN 08, and HLN 09) had above-average yields, while five hybrids (HLN 01, HLN 04, HLN 05, ADV 777, and JH 37) had below-average yields. Most hybrids had bi values close to 1 and $s^2$di values close to zero, except for HLN 01, HLN 03, and HLN 08.

**Table 5.** Mean yield, bi, and $s^2di$ of hybrid maize at the three nitrogen levels.

| Hybrid | Mean yield (t $ha^{-1}$) | bi | $s^2di$ |
|---|---|---|---|
| HLN 01 | 6.92 | 0.51** | 0.02 |
| HLN 02 | 8.18 | 1.28 | 0.59 |
| HLN 03 | 7.60 | 1.43** | 1.50** |
| HLN 04 | 6.75 | 0.74 | 0.12 |
| HLN 05 | 7.18 | 0.86 | 0.45 |
| HLN 06 | 7.67 | 1.05 | −0.20 |
| HLN 07 | 7.36 | 1.23 | 0.02 |
| HLN 08 | 7.47 | 1.49** | 0.03 |
| HLN 09 | 7.68 | 0.99 | −0.22 |
| ADV 777 | 5.94 | 0.72 | 0.60 |
| JH 37 | 6.60 | 0.72 | −0.24 |
| Mean | 7.21 | | |

Note: bi: regression coefficient; $s^2di$: deviation from regression.

## 4. Discussion

### *4.1. Nitrogen level effect on maize agronomic traits*

The data presented in Table 2 shows that both nitrogen fertilizer and the genotype factors significantly affect various traits and the overall yield of maize crops. Specifically, the application of nitrogen fertilizer and the use of hybrid maize varieties were found to have considerable impacts on the traits. The interaction between nitrogen fertilizer and hybrid maize was significant for most of the measured traits. This suggests that combining these two factors can result in different outcomes than expected from each individually. It implies that both factors affect growth and yield and that hybrids respond differently to nitrogen levels. [33]. However, this combined effect did not extend to all traits, as no significant interaction was observed for leaf width and angle. The coefficient of variation (CV) ranged from 4.70% to 15.20% across the variables, indicating the experiment has moderate variance and adequate precision [34].

Generally, the observation variable tends to decline as fertilizer diminishes. Lower nitrogen levels reduced maize growth indicators such as plant height, leaf area, chlorophyll, stalk diameter, ear length, and kernel number [35,36]. Nitrogen (N) is essential for plant growth and development. Maize needs N throughout its life cycle, from the vegetative to the reproductive stage [37]. Maize requires nitrogen to synthesize proteins and chlorophyll and for other metabolic pathways [38]. Chlorophyll, the green pigment for photosynthesis, contains much nitrogen. Without sufficient nitrogen, plant leaves lose their green colour and become pale and yellow due to less chlorophyll [39]. Leaf area index (LAI) and leaf chlorophyll content are crucial in evaluating a plant's photosynthetic capacity, nutrient status, and overall health. LAI is a valuable indicator of the plant's light interception capability for photosynthesis, while leaf chlorophyll content reflects the plant's nutrient status and photosynthetic efficiency [40,41]. The reduced photosynthesis rate affects the plant’s ability to generate energy and biomass, inhibiting plant growth and development. The addition of N fertilizer can enhance the vascular tissue in the stem and the synthesis of enzymes and nucleic acids that regulate protein accumulation and post-translational protein modification [42,43].

Root traits are critical for resource uptake and crop performance under low nitrogen conditions. Maize responds to nitrogen deficiency by enhancing root depth and steepening root growth angles [44,45]. Fine roots exhibit greater nitrogen uptake compared to thicker roots [46]. Root architecture plays a significant role in determining nutrient acquisition efficiency, particularly through root length and density [47,48]. A deeper root system with increased lateral root length increases nitrogen acquisition efficiency [49].

*4.2. Maize yields at the three nitrogen levels and the low-nitrogen tolerance*

The interaction between genotype and environment is beneficial for breeders in plant-stress fields. The interaction causes each genotype to show different responses to different fertilization levels. The response is due to differences in genetic backgrounds. Tolerant genotypes will perform more stable than susceptible ones. Therefore, plant breeders can use these differences to select the desired genotypes according to their purposes [50,51].

The maize yield at each N level is varied. At a 200 kg N ha$^{-1}$ rate, HLN 03 had the maximum yield (12.57 t ha$^{-1}$), while HLN 01 had the minimum (8.42 t ha$^{-1}$). All maize hybrids, except HLN 01, HLN 04, and HLN 05, differed from the control at this level. However, at 100 kg N ha$^{-1}$, HLN 02 was the best, and ADV 777 was the worst. HLN 02 had a significant difference from the controls, achieving a yield of 8.09 t ha$^{-1}$, whereas ADV 777 had the lowest yield at 4.74 t ha$^{-1}$. At a 0 kg ha$^{-1}$ nitrogen rate, HLN 01 exhibited the highest yield at 5.33 t ha$^{-1}$, while HLN 08 had the lowest at 3.90 t ha$^{-1}$ (Table 4). The interaction of genotype and N fertilizer dose led to differences in yield for each genotype at each N fertilization level [52].

The yield reduction between each level of nitrogen fertilization differs depending on the hybrid maize variety. Table 4 shows that the yield at the rate of 200 kg N ha$^{-1}$ instead of 100 kg N ha$^{-1}$ is reduced by 1.42–6.96 t ha$^{-1}$. HLN 03 has the highest yield reduction, and HLN 01 has the lowest. The yield reduction ranges from 3.10 to 8.52 t ha$^{-1}$ when the yield at rate 0 kg N ha$^{-1}$ is compared to that at 200 kg N ha$^{-1}$, with HLN 08 having the most considerable reduction and HLN 01 having the smallest. The yield reduction from 100 kg N ha$^{-1}$ to 0 kg N ha$^{-1}$ ranged from 0.17 t ha$^{-1}$ (ADV 777) to 3.70 t ha$^{-1}$ (HLN 03). The absence of nitrogen in the soil led to a restricted presence of starch metabolizing enzymes and hormone levels in maize, consequently causing a reduction in yield [19].

Table 4 shows the hybrid maize tolerance index values based on STI for different fertilization levels. For 200 kg N ha$^{-1}$ and 100 kg N ha$^{-1}$, the STI values varied from 0.37 (ADV 777) to 0.89 (HLN 02). According to the STI criteria, all hybrid maize corresponds to a moderate-tolerance group, except for ADV 777 (susceptible). For 200 kg N ha$^{-1}$ and 0 kg N ha$^{-1}$, the STI values ranged from 0.36 to 0.53. HLN 03 had the highest STI value, and ADV 777 had the lowest. Only HLN 03 and HLN 09 were encompassed in the moderate-tolerance criteria at this fertilization level, while the rest were susceptible. For 100 kg N ha$^{-1}$ and 0 kg N ha$^{-1}$, the STI values spanned from 0.50 to 0.86. ADV 777 showed the lowest STI value, and HLN 01 showed the highest based on the STI criteria. All hybrid maize belonged to a moderate-tolerance group at this fertilization level.

The STI index can identify maize genotypes with high yields under normal and stressful conditions. The STI index can screen genotypes with high yield potential and tolerance under both normal and stressful conditions [53,54]. Table 4 shows that maize hybrids with above-average yields at three fertilization levels were classified as tolerant or moderately tolerant. A similar pattern in wheat was also found, where genotypes with high yields under heat stress and normal conditions had high STI values, while genotypes with low yields had low STI values [55]. This finding was in line with previous studies by [56–58].

Figure 1 is a Venn diagram that shows the different groups of maize genotypes that can handle 200 kg N ha$^{-1}$ and 100 kg N ha$^{-1}$ of nitrogen fertilization based on their STI values. A Venn diagram is a graphical representation of the relationships among different data sets based on intersections or combinations of several sets [59,60]. Venn diagrams can categorize data by intersections or combinations of sets and are more informative than heat maps and tables for up to five variables in some cases [61,62]. In a Venn diagram, each set is shown as a transparent circle. The overlapping regions indicate the elements that belong to more than one set [63–65]. In Figure 1, the hybrid ADV 777 was classified as susceptible to both fertilizer conditions, meaning it had low yields under both N levels. Eight hybrids (HLN 01, HLN 02, HLN 04, HLN 05, HLN 06, HLN 07, HLN 08, and JH 37) were rated as moderately tolerant at STI 100 kg N ha$^{-1}$ at susceptible to STI 0 kg N ha$^{-1}$. When N levels were normal, their yields were high, but when N levels were stressed, their yields were low. Two maize hybrids (HLN 03 and HLN 09) were classified as moderately tolerant to both fertilizer conditions, meaning they had moderate yields under both N levels.

The bi and $s^2$di values determine the maize hybrid stability. Based on these values, maize hybrids can be classified into four categories [66,67]. The first category consists of hybrids with bi values not significantly different from 1 and $s^2$di values not significantly different from 0. These hybrids are considered stable across environments. The second category comprises hybrids with bi values significantly different from 1 and $s^2$di values not significantly different from 0. These hybrids are adapted to specific environments. The third category includes hybrids with bi values not significantly different from 1 and $s^2$di values significantly different from 0. The fourth category contains hybrids with bi values significantly different from 1 and $s^2$di values significantly different from 0. Hybrids in the third and fourth categories are unstable across environments.

The HLN 03 maize hybrid is unstable due to its bi value of 1.43 (significantly different from 1) and its $s^2$di value of 1.30 (significantly different from 0). These values indicate that HLN 03 has a high level of interaction with the environment. HLN 01 is a genotype-specific hybrid for low-N soil locations. The bi value of 0.51 (significantly lower than 1) and the $s^2$di value of 0.02 (not significantly different from 0) of the HLN 01 maize hybrid indicate that it is suitable for cultivation in marginal environments. The HLN 08 maize hybrid has a bi value of 1.49 (significantly higher than 1) and $s^2$di value of 0.02 (not significantly different from 0), which implies that HLN 08 is a genotype-specific hybrid for optimal environments (high N soil locations). Maize hybrids with bi values close to 1 and $s^2$di values close to 0 have low environmental interaction and are categorized as stable hybrids. Genotypes HLN 02, HLN 04, HLN 05, HLN 06, HLN 07, HLN 09, ADV 777, and JH 37 belong to this category of stable hybrid (Table 5).

The selection that considers the tolerance and stability index in the stress conditions can identify both tolerant and widely adapted genotypes. This method has been employed in various crops, such as rice in saline conditions [68], bread wheat in drought conditions [69], and maize under waterlogging conditions [70]. In the current research, HLN 02, HLN 06, and HLN 07 are stable hybrids with yields higher than average. However, these hybrids exhibit only moderate tolerance at STI 100 kg N $ha^{-1}$ and are susceptible at STI 0 kg N $ha^{-1}$. In contrast, HLN 09 was identified as the most suitable maize hybrid for low-N environments. HLN 09 exhibited a relatively high yield of 7.68 t $ha^{-1}$, surpassing the mean yield of 7.21 t $ha^{-1}$ for all hybrids. The HLN 09 yields at 0 kg N $ha^{-1}$, 100 kg N $ha^{-1}$, and 200 kg N $ha^{-1}$ were 5.05 t $ha^{-1}$, 7.16 t $ha^{-1}$, and 10.83 t $ha^{-1}$, respectively, greater than mean yields at each fertilizer level (4.63 t $ha^{-1}$, 6.57 t $ha^{-1}$, and 10.44 t $ha^{-1}$). The Stress Tolerance Index (STI) values for HLN 09 were 0.71 for N2-N1, 0.50 for N2-N0, and 0.84 for N1-N0. These STI values demonstrate that HLN 09 consistently maintained higher stress tolerance across varying nitrogen levels. Additionally, HLN 09 was characterized as a stable hybrid (bi = 0.99, $s^2di = -0.22$). These facts indicate that HLN 09 has superior performance to other hybrids. As such, HLN 09 represents a stable and promising hybrid for low-N environments.

## 5. Conclusions

A decrease in nitrogen fertilizer dosage for maize significantly affected agronomic traits, followed by a yield decrease among the tested maize hybrid genotypes. This fact indicates that optimal nitrogen levels are essential to optimizing maize yield. Among the tested genotypes, the HLN 09 maize hybrid showed remarkable tolerance to nitrogen-deficient conditions, sustaining both stability and high yield. The hybrid's tolerance to low nitrogen suggests its potential for cultivation in environments with limited nitrogen availability.

## Use of AI tools declaration

The authors declare they have not used artificial intelligence (AI) tools in the creation of this article.

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## Conflict of interest

The authors declare no conflict of interest.

## Authors contributions

Conceptualization: M.A.; data curation: A.M.; formal analysis: R.I. and S.B.P.; investigation: R.I. and N.N.A.; methodology: A.M; project administration: R.E.; resources: S and R.E.; software: S.B.P. and N.N.A.; supervision: M.A.; validation: A.M; visualization: S; writing—original draft: R.E.; writing—review and editing: S.B.P. All authors have read and agreed to the published version of the manuscript.

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