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Genotype-by-environment interaction and tolerance of tropical maize under low nitrogen conditions

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Abstract. *Supriadi D, Bimantara YM, Zendrato YM, Widaryanto E, Kuswanto K, Waluyo B. 2025. Genotype-by-environment interaction and tolerance of tropical maize under low nitrogen conditions. Biodiversitas 26: 3863-3874.* The environment is the main factor affecting the performance of agronomy and the yield of tropical maize. In this context, the development of maize hybrids with low nitrogen (N) tolerance is crucial but challenging since genotype selection is biased toward the existence of Genotypes by Environment Interaction (GEI). Therefore, this research aimed to show the responses as well as determine the tolerance and stability of maize hybrids under low nitrogen environmental conditions in four environments. A total of 10 maize hybrids were analyzed using a randomized complete block design with three replications. The results showed that GEI was significant for most of the observed traits, including grain yield and yield components, as well as physiological traits. The percentage of G × E variance exhibited considerable differences among various traits, with values ranging from 13.68% for stomatal length to 50.00% for the number of ear rows. The low nitrogen environment significantly influenced ear performance and physiological traits, resulting in a lower average grain yield (6.98 t ha^{-1}) compared to normal conditions (10.72 t ha-1), as well as the other traits. G01, G02, and G05 were identified as tolerant hybrids based on the ranking summary of stress tolerance indices. Meanwhile, G01 and G03 were selected as stable hybrids based on stability statistics and Genotype + Genotype × Environment (GGE). G01 (R0211) had the highest yield and remained stable-tolerant in low-nitrogen environments, demonstrating its superiority as a genotype. This study and the above genotypes could be used as genetic material for future maize breeding to develop new varieties that are both high-yielding and stable under low N, especially under tropical regions.

Keywords: Grain yield, maize breeding, nitrogen stress, stability, stress tolerance indices

INTRODUCTION

Maize (*Zea mays* L.) is a popular cereal crop, especially in tropical regions, representing 49% of the global harvested area. Several countries in Southeast Asia, such as Thailand, Indonesia, Vietnam, and the Philippines, are the centers of production for cereal crops with high demand (Khongdee et al. 2022; Von Pinho et al. 2022). Maize cultivation has a significant economic and social impact, which serves a varied and adaptable function in agri-food systems worldwide. This cereal crop plays an important role in ensuring food and nutrition security (Grote et al. 2021; Erestein et al. 2022). In this context, innovation and technology through genetic methods and field management are needed to increase productivity (Cairns et al. 2021; Wang et al. 2023).

The main challenge in maize production is the environmental, which significantly influences the responses of genotypes (Engida et al. 2024; Zendrato et al. 2024a). A significant challenge in enhancing production is the low levels of macronutrients, such as nitrogen (N) (Grandy et al. 2022). Some regions, especially those with tropical climates, are characterized by low nitrogen inputs, such as Sub-Saharan Africa (Jacobs et al. 2024; Masso et al. 2024). Nitrogen plays a role in photosynthesis, protein synthesis, and chlorophyll formation (Shah et al. 2024). Low-N conditions inhibit the vegetative and reproductive growth of maize, leading to lower biomass accumulation and smaller ear size, directly reducing yield potential (Adu et al. 2018; Amegbor et al. 2022; Efendi et al. 2024), ranging from 26% to 65% (Ertiro et al. 2022), which directly impacts economic outcomes for farmers, as well as leading to food insecurity and price increases (Wang et al. 2022). The deficiency also affects root development, leading to dehydration and nutrient deficiencies during reproductive stages when water and nutrient demand are highest (Maywald et al. 2022; Lopez et al. 2023).

In this context, maize breeding efforts are directed toward developing resilient cultivars that show tolerance and stability to abiotic stress (Azrai et al. 2022; Herawati et al. 2023). This condition faces the complex challenges inherent in genotype selection. Several studies suggested methods for selecting the tolerant genotype using secondary traits (Bhandari et al. 2024; Menkir et al. 2024; Zendrato et al. 2024b); however, some of the selected genotypes had lower grain yield than the average mean. In addition, various soil and low-N conditions led to the existence of Genotype by Environment Interaction (GEI), resulting a complex analysis due to the different responses (Matongera et al. 2023; Yue et al. 2025). GEI under low-N affects yield of maize, indicated by a hybrid that performs well in normal environment and may fail in low-N conditions (Lemaire and Ciampitti 2020; Ciampitti et al. 2021; Sun et al. 2025). The studies showed that this conditions not only affects the grain yield but also the quality of grain, such as protein and provitamin A, as well as photosynthesis and chlorophyll (Ortiz-Covarrubias et al. 2019; Wu et al. 2019; Dosho et al. 2022), making it a significant challenge for farmers and breeders for selecting the best genotype (Ciampitti et al. 2021; Zendrato et al. 2025). It is crucial to use stability analysis methods to evaluate maize performance to show the sensitivity and stability of the genotypes (Abdillah et al. 2023; Anshori et al. 2024).

Understanding the GEI and performance of maize hybrids, especially under low-N, is essential to provide valuable insights for selecting the superior tolerant genotype for maize breeding (Gela et al. 2022; Costa et al. 2024; Kumar et al. 2025). The integration of tolerance indices and stability analysis methods under low-N would be interesting to investigate further for maize breeding program. Each method, including tolerance indices and stability statistics, has its strengths and weaknesses in addressing tolerant and stable genotypes (Supriadi et al. 2024). It will provide a more comprehensive understanding of the GEI and stress tolerance based on many method perspectives, as well as help breeders in assigning breeding strategies for nitrogen deficiency resilience (Azrai et al. 2022; Shojaei et al. 2022). Therefore, this study aimed to evaluate the performance, tolerance, and stability of tropical maize hybrids under low-N environments using tolerance indices and stability statistics.

MATERIALS AND METHODS

Description of the study area

The experiment was conducted from April to October 2023 at Plosoklaten-Kediri and Bandarkedungmulyo-Jombang in East Java, Indonesia, during the cropping season. Each location was designed to have two different environmental conditions, under optimum and low nitrogen treatment. Furthermore, the experimental sites had different agroecology and agro-climatic conditions based on measurements performed. Rainfall and climate data were gathered from meteorogical stations near the experimental sites. The soil in Jombang (E01 and E02) had an alluvial type with a clay loam texture at an altitude of 179 m above sea level (masl), with minimum and maximum temperatures of 23.48°C and 31.23°C, respectively. Meanwhile, the environment in Kediri (E03 and E04) had an andosol soil type with a sandy loam texture and a height of 110 masl, with minimum and maximum temperatures of 22.16°C and 32.81°C, respectively. The distinct agroclimatic and agroecological conditions emphasize the critical role of environmental factors in assessing the performance of maize hybrids, as these factors significantly impact plant growth and development. Figure 1 shows the monthly differences in rainfall at locations, and Table 1 shows the details of the research area.

Plant materials, experimental design, and field management

A total of 10 promising low nitrogen-tolerant maize hybrids were used in the field experiments consisting of eight test hybrids developed by PT Restu Agropro Jayamas, Indonesia, namely R0211 (G01), R0J020 (G02), R0654 (G03), R0105 (G04), R0118 (G05), R0498 (G06), R0641 (G07), and R0J016 (G08), with RSA002 (G09) and NK7328 (G10) commercial hybrids as checks. G01 to G08 represent potential for a new candidate superior varieties selected from PT Restu Agropro Jayamas maize breeding program, characterized by high yield screening under low nitrogen. These maize hybrids were developed from selected inbred lines developed from crosses between commercial varieties in Indonesia, such as ADV 777, BISI 18, NK212, NK7328, DK95, DK979 and etc, which use ear-to-row and recurrent for selection.

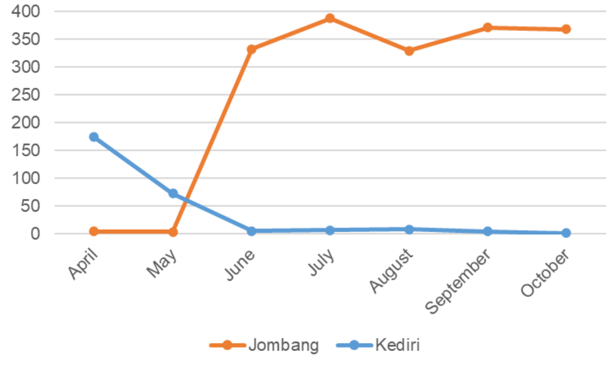


Figure 1. The monthly rainfall in two locations of the experiment

Table 1. Description of four environmental conditions used for N-treatment of maize hybrids evaluation

Environmental code	Location	Soil type	Soil texture	Altitude	Average annual rainfall (mm)	Min temperature	Max temperature	Condition
E01	Bandarkedungmulyo, Jombang	Alluvial	Clay loam	179	1390	23.48	31.23	Optimal N
E02	Bandarkedungmulyo, Jombang	Alluvial	Clay loam	179	1390	23.48	31.23	Low N
E03	Plosoklaten, Kediri	Andosol	Sandy loam	110	1330	22.16	32.81	Optimal N
E04	Plosoklaten, Kediri	Andosol	Sandy loam	110	1330	22.16	32.81	Low N

The genotypes were randomly assigned to each plot and the experiments were arranged in a Randomized Complete Block Design (RCBD) with three replications. The experimental plot size was 2.8×5 m (14 m^2) with a planting distance of 70 cm and 20 cm between and within rows, respectively. Border rows were used in each field to minimize edge effects and full tillage was performed before planting. Subsequently, two seeds were sown per hole, and the seedlings were thinned to one plant per hole 10 Days After Planting (DAP). This research included two varied experimental conditions, with the first and second trials using optimum/normal nitrogen (N) and low nitrogen treatment, respectively. These two experiments were carried out at both locations in Jombang and Kediri. Fertilization was also carried out twice, at 10 and 30 DAP. The low nitrogen growing condition experiment used a dose of 100 kg N ha^{-1} (50 kg N ha^{-1}for first fertilization + 50 kg N ha^{-1} for second fertilization), while 300 kg N ha^{-1} (150 kg N ha^{-1} for first fertilization + 150 kg N ha^{-1} for second fertilization) was adopted for normal conditions. The 300 kg N ha^{-1} level is the usual nitrogen fertilizer level farmers use for maize in Indonesia and represents the optimum fertilizer level. The 100 kg N ha^{-1} level is one-third of the usual fertilizer dose, represents low fertilizer, and can be used for the selection of maize tolerant to low nitrogen conditions (Farid et al. 2020). All crop management practices, including weeding, irrigation, ridging, and other practices, were followed according to the Indonesian Ministry of Agriculture's technical guidelines at each site. The weeding process entailed the removal of weeds surrounding the plants, whereas mulching was performed by elevating the mounds and loosening the soil to enhance aeration. This practice also mitigated the risk of root and stem bending in the maize hybrids. Irrigation was applied during the vegetative and flowering stages, extending into the initial phase of seed formation, depending on soil moisture levels when rainfall was inadequate. Additional agricultural practices, including ridging, pest control, and disease management were implemented in accordance with established technical protocols. Harvesting was performed at physiological maturity, as indicated by the appearance of a black grain layer.

Data collection

A total of 10 sample ears were randomly selected from each hybrid to become the source of the yield component data. The observed traits consisted of ear length (cm), ear diameter (mm), number of ear rows, number of kernels per row, grain weight (kg), weight of 1000 kernels (g), ear weight (kg), moisture content (%), shelling percentage (5), grain yield (t ha-1), and several physiological traits, including Chlorophyll A, B, and total number of stomatal, stomatal length, and stomatal width. In addition, the grain yield was estimated using the following equation (Azrai et al. 2022).

$$Grain\ yield\ (t\ ha^{-1}) = \frac{10000}{PS} \times \frac{100 - MC}{100 - 15} \times \frac{EW}{1000} \times SP$$

Where :
MC : Actual grain moisture content at harvest
PS : Harvested plot size (14 m^2)
EW : Ear Weight per plot (kg)
SP : Shelling Percentage

Chlorophyll was observed by weighing 2 g of the leaf sample and grinding with a mortar and pestle. The leaves were taken 8 weeks after planting by taking the second leaf from the tip of the plant. Sampling was carried out at 10:00 am until 11:00 a.m. with three maize leaf samples taken from each genotype. The leaf paste was placed into a film wattle (30 mL), mixed with 10 mL acetone PA (Pro Analyst), and closed. The solution was left in a refrigerator for 24 hours and filtered through the Whatman 42 paper. 1-mL of the filter was pipetted and placed into a test tube for dilution, which was achieved by adding 9 mL of acetone and homogenizing. The extract solution obtained was placed into a cuvette, and the absorbance was measured in a spectrophotometer at wavelengths of 645 nm and 663 nm.

Stomatal samples were taken from plants at 60 DAP by collecting the second leaf at the tip of the plant. Leaves were taken at 10:00 a.m. with three leaf samples taken from each genotype. Stomatal observations were conducted using an Olympus BX51-type light microscope equipped with an Olympus DP24 digital camera. The preparation was placed on a glass object, and observations were made using an objective lens at 40× magnification. The view of the microscope with the configuration has dimensions of 513.9 µm × 689.9 µm, leading to a field of 354,539.6 µm^2. This area was used to count the number of stomatal contained in each specific area unit for quantitative analysis of stomatal density.

Statistical analysis

The data were visualized in boxplots and scatter plots before being analyzed to determine the outlier data of each observation trait and to ensure the suitability of the data. Additionally, missing data patterns were examined to determine if they were random, and appropriate imputation methods were applied where necessary. A combined Analysis of Variance (ANOVA) was carried out to analyze the effect of nitrogen treatment (condition), environment, genotype, and GEI on the observed traits, using the following linear model.

$$Y_{ijkl} = \mu + N_i + E_{j(i)} + B_{k(ji)} + G_l + (GE)_{lj(i)} + (GN)_{li} + \varepsilon_{ljkl}Y_{ijkl}$$

Where :
$Y_{ijkl}Y_{ijkl}$: Trait response of nitrogen treatment-i, environment-j, replication-k, and genotype-l
$\mu\mu$: Grand mean
N_iN_i : Effect of nitrogen treatment
$E_{j(i)}E_{j(i)}$: Effect of the environment trial nested in nitrogen treatment
$B_{k(ji)}B_{k(ji)}$: Effect of block nested in location and nitrogen treatment
$GE_{lj(i)}GE_{lj(i)}$: Interaction effect of genotype and environment
$GN_{li}GN_{li}$: Interaction effect of genotype and nitrogen treatment
$\varepsilon_{ljkl}\varepsilon_{ljkl}$: Experimental error

Pearson correlation analysis was used to determine the relationship between grain yield and other traits, as well as stress tolerance indices. Several stress tolerance indices are used to determine the response and ranking of each genotype, including the Tolerance Index (TOL) (Rosielle and Hamblin 1981), Harmonic Mean (HM) (Schneider et al. 1997), Stress Susceptibility Index (SSI), Mean Productivity (MP), Geometric Mean Productivity (GMP), and Stress Tolerance Index (STI) (Fernandez 1992), Yield Index (YI) (Gavuzzi et al. 1997), Yield Stability Index (YSI) (Bouslama and Schapaugh 1984), and Modified Stress Tolerance Index (MSTI) (Farshadfar and Sutka 2002). Each tolerance index has unique criteria for selecting tolerant genotypes, such as TOL, which identifies tolerance based on the difference in grain yield under two environmental conditions; HM, MP, GMP, and STI, which identify tolerant genotypes that have above-average grain yield; SSI is used to assess the severity of genotypes under stressful environments and reflects the susceptibility of genotypes to yield loss; YSI describes consistent genotypes across environments; YI calculates yield under stressful conditons relative to the average stress yield, whereas MSTI modifies STI in assessing tolerant genotypes based on the two environments used. The use of multiple tolerance indices can provide comprehensive information for assessing tolerant genotypes (Bhandari et al. 2024). The estimation was carried out according to the following formula.

TOL = $Y_p - Y_s Y_p - Y_s$

MP = $\frac{(Y_p + Y_s)}{2} \frac{(Y_p + Y_s)}{2}$

HM = $\frac{2(Y_p \times Y_s)}{(Y_p + Y_s)} \frac{2(Y_p \times Y_s)}{(Y_p + Y_s)}$

SSI = $\frac{1 - \frac{Y_s}{Y_p}}{1 - \frac{\bar{Y}_s}{\bar{Y}_p}} \frac{1 - \frac{Y_s}{Y_p}}{1 - \frac{\bar{Y}_s}{\bar{Y}_p}}$

GMP = $(Y_p \times Y_s)^{0.5} (Y_p \times Y_s)^{0.5}$

STI = $\frac{Y_p \times Y_s}{(\bar{Y}_p)^2} \frac{Y_p \times Y_s}{(\bar{Y}_p)^2}$

YI = $\frac{Y_s}{\bar{Y}_s} \frac{Y_s}{\bar{Y}_s}$

YSI = $\frac{Y_s}{Y_p} \frac{Y_s}{Y_p}$

MSTI = k_iSTI, where k1 = $\frac{(Y_p)^2}{(\bar{Y}_p)^2} \frac{(Y_p)^2}{(\bar{Y}_p)^2}$ and k2 = $\frac{(Y_s)^2}{(\bar{Y}_s)^2} \frac{(Y_s)^2}{(\bar{Y}_s)^2}$

Where :
$Y_p Y_p$: Mean yield of each hybrid under normal conditions
$Y_s Y_s$: Mean yield of each genotype under nitrogen stress conditions
$\bar{Y}_p \bar{Y}_p$: Grand mean of yield under normal conditions
$\bar{Y}_s \bar{Y}_s$: Grand mean of yield under nitrogen stress conditions

Rank summary of genotypes from all tolerance indices using the ranking equation mean + standard deviation of ranking.

The genotypic stability was quantified under four environment using mixed models. Stability analyses were performed when GEI significantly affected the grain using several methods including yield and stability index (Kang 1993), coefficient of variations (Francis and Kannenberg 1978), regression of genotype means yield on the environmental index (Finlay and Wilkinson 1963; Eberhart and Russel 1966), ecovalence (Wricke 1962), and Weighted Average of Absolute Scores (WAASB) (Olivoto et al. 2019). In addition, Genotype + Genotype × Environment (GGE) biplot analysis (Yan et al. 2000) was carried out to assess hybrid stability and adaptation to specific environments using discriminativeness *vs*. representativeness, mean *vs*. stability, genotype ranking, and which-won-where methods. The analyses used SAS OnDemand for Academics (welcome.oda.sas.com), RStudio (R version 4.1.2), and Microsoft Excel. SAS OnDemand for Academics and Microsoft Excel were adopted for ANOVA, and calculation of tolerance indices, respectively. Meanwhile, RStudio was adopted for stability statistics and 3D scatter plots using the "scatterplot3d" R package as well as analyses of correlations and stability statistics through "metan" R package.

RESULTS AND DISCUSSION

Combined analysis of variance and mean performance

Table 2 shows the combined ANOVA across the four environmental conditions for the observed traits. Low nitrogen significantly affected grain yield and components due to the differences in the response of maize hybrids to the two treatment conditions. In addition, the low nitrogen treatment had a significant effect on all physiological traits, such as chlorophyll and stomatal traits except stomatal length. The genotype effect was significant ($p<0.05$) for all observed traits, except moisture content and the number of stomatal. Therefore, the hybrids evaluated were phenotypically diverse in yield, component, and physiological traits. Grain yield was significantly influenced by the interaction between genotype and low nitrogen treatment (G × N) as well as genotype and location (G × L/N). Several yield components, such as ear length, number of ear rows, grain weight, ear weight, moisture content, shelling percentage, and chlorophyll b, were also significantly influenced by the two sources of interaction. Chlorophyll a, chlorophyll total, number of stomatal, and stomatal width showed significance in G × N, while ear diameter, weight of 1000 kernels, and stomatal length were significant in G × L/N. The number of kernels per row did not show significance; hence, there are differences in the responses and performances of maize hybrids under different environmental conditions. The percentage of G × E variance varied significantly for each trait, ranging from 13.68% (stomatal length) to 50.00% (number of ear rows), as well as the percentage of genetic and environmental variance, indicating differences in heritability between traits.

Table 3 shows a comparison of the performance of maize hybrids under low nitrogen treatment and normal conditions. Maize hybrids under low nitrogen conditions had lower average means than normal in terms of yield and components, except for moisture content. Meanwhile, grain yield, grain and ear weight, and the weight of 1000 kernels reported considerable reductions in genotypic means. The chlorophyll content and stomatal traits showed a significant difference under low nitrogen and normal conditions, indicating the mean performance depending on the existing environmental conditions. These results suggested that low nitrogen stress could significantly inhibit the ear development and physiology of maize. The performance mean of each genotypes of maize across environments was shown in Table 4. Figure 2 shows the difference in the hybrid grain yield response across the environments, where G01 is the highest, followed by G04 and G05, while G09 and G10 have the lowest grain yield in all environments. The performance grain yield of maize under four environments was shown in Table 5.

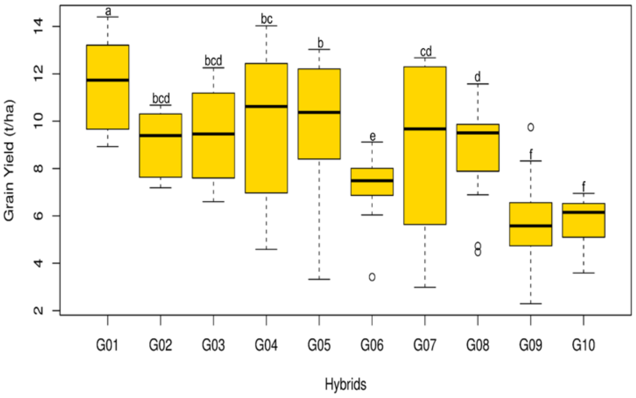


Figure 2. The distribution of maize hybrids on grain yield (t ha^{-1}) across the environments. Note: a to f represents the LSD test for each genotype across environments

Table 2. Combined analysis of variance on all observed traits of maize hybrids under normal and low nitrogen treatment

Source	df	EL	ED	ER	KR	GW	W1000	EW	GY
Low nitrogen (N)	1	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Location (L)/N	2	0.003	0.840	0.290	0.926	0.063	0.019	0.040	0.036
Replication/L N	8	0.851	0.173	0.333	0.256	0.154	0.586	0.640	0.541
Genotype (G)	9	<.0001	<.0001	<.0001	0.006	<.0001	<.0001	<.0001	<.0001
G x N	9	0.001	0.274	<.0001	0.637	0.016	0.429	<.0001	<.0001
G x L/N	18	0.026	<.0001	<.0001	0.643	<.0001	0.007	0.006	0.004
cv, %		5.83	3.31	3.77	7.24	14.73	9.18	14.01	13.84
σ_g^2		18.13%	3.49%	8.70%	27.69%	55.80%	26.84%	52.72%	53.45%
σ_{ge}^2		26.37%	43.01%	50.00%	16.75%	17.63%	16.56%	20.32%	21.42%
σ_e^2		55.49%	53.49%	41.3%	55.56%	26.57%	56.61%	26.96%	25.13%
Source	**df**	**MC**	**SP**	**ChlorA**	**ChlorB**	**ChlorT**	**NSt**	**StL**	**StW**
Low nitrogen (N)	1	<.0001	<.0001	<.0001	<.0001	<.0001	0.020	0.302	<.0001
Location (L)/N	2	0.430	0.248	<.0001	<.0001	<.0001	0.297	0.015	0.017
Replication/L N	8	0.002	0.946	0.704	0.876	0.880	0.661	0.586	0.520
Genotype (G)	9	0.100	<.0001	<.0001	<.0001	<.0001	0.360	0.001	<.0001
G x N	9	0.025	0.018	0.002	<.0001	<.0001	0.002	0.688	<.0001
G x L/N	18	0.002	<.0001	0.138	0.047	0.138	0.178	0.023	0.384
cv, %		5.59	2.73	7.53	25.18	11.52	10.9	7.05	8.97
σ_g^2		-4.24%	15.38%	14.36%	9.17%	12.19%	-6.12%	12.05%	10.92%
σ_{ge}^2		36.75%	29.31%	22.66%	30.87%	29.32%	27.21%	13.68%	33.55%
σ_e^2		67.49%	55.31%	62.98%	59.96%	58.50%	78.91%	74.27%	55.54%

Note: df: Degree of freedom, cv: Coefficient of variation, EL: Ear Length (cm), ED: Ear Diameter (mm), ER: Number of Ear Rows, KR: Number of Kernels per Row, GW: Grain Weight (kg), W1000: Weight of 1000 kernels (g), EW: Ear Weight (kg), GY: Grain Yield (t ha^{-1}), MC: Moisture Content (%), SP: Shelling Percentage, ChlorA: Chlorophyll A, ChlorB: Chlorophyll B, ChlorT: Chlorophyll total, NSt: Number of Stomatal, StL: Stomatal Length, StW: Stomatal Width

Table 3. Performance means of yield components and physiological traits in maize under normal and low nitrogen conditions

Condition	EL	ED	ER	KR	GW	W1000	EW	GY
P-value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Low nitrogen	16.67±0.21	46.42±0.36	15.91±0.16	33.83±0.56	5.69±0.28	281.25±5.56	6.92±0.26	6.98±0.28
Normal	17.79±0.12	48.09±0.14	16.83±0.07	36.65±0.26	8.53±0.23	323.64±3.72	9.62±0.30	10.27±0.32
Condition	**MC**	**SP**	**ChlorA**	**ChlorB**	**ChlorT**	**NSt**	**StL**	**StW**
P-value	<.0001	<.0001	<.0001	<.0001	<.0001	0.020	0.302	<.0001
Low nitrogen	26.43±0.25	81.27±0.41	115.71±1.39	50.96±1.94	166.67±2.84	9.66±0.14	103.43±1.13	50.50±0.94
Normal	23±0.20	82.54±0.34	135.99±1.80	63.83±3.24	199.82±4.73	10.13±0.17	104.82±1.08	53.67±0.64

Note: EL: Ear Length (cm), ED: Ear Diameter (mm), ER: Number of Ear Rows, KR: Number of Kernels per Row, GW: Grain Weight (kg), W1000: Weight of 1000 kernels (g), EW: Ear Weight (kg), GY: Grain Yield (t ha^{-1}), MC: Moisture Content (%), SP: Shelling Percentage, ChlorA: Chlorophyll A, ChlorB: Chlorophyll B, ChlorT: Chlorophyll total, NSt: Number of Stomatal, StL: Stomatal Length, StW: Stomatal Width

Table 4. The performance traits of maize genotypes across four environments

Genotype	EL	ED	ER	KR	GW	EW	GY	MC	SP	ChlorA	ChlorB	ChlorT	NSt	StL	StW
G01	18.51	48.12	16.62	37.63	9.24	10.97	11.57	24.50	83.00	135.31	70.17	205.48	10.19	108.36	55.03
G02	17.81	45.81	16.38	37.14	8.10	8.70	9.06	24.05	81.40	122.25	47.91	170.16	9.33	107.19	48.58
G03	17.27	46.98	16.27	36.29	7.77	9.15	9.43	24.44	80.62	129.56	52.44	182.00	10.25	99.69	53.91
G04	17.73	48.35	16.53	35.11	7.68	9.45	9.80	24.27	80.65	130.94	57.84	188.77	10.35	108.85	55.59
G05	17.21	47.88	16.83	38.43	8.12	9.39	9.94	25.35	83.95	126.93	64.17	191.10	9.62	103.55	54.55
G06	17.24	47.80	15.87	34.05	7.05	6.86	7.21	24.38	82.91	120.15	47.61	167.76	10.10	102.16	49.73
G07	16.94	46.94	16.47	34.88	7.10	8.62	8.95	25.77	82.45	132.27	66.73	199.00	9.75	106.23	53.26
G08	17.31	47.97	17.18	33.56	7.45	8.27	8.72	24.70	82.81	120.75	49.82	170.57	9.94	105.34	53.87
G09	15.93	45.77	15.95	32.58	4.45	5.53	5.79	25.18	82.62	117.29	50.90	168.19	9.60	96.25	47.43
G10	16.34	46.92	15.57	32.73	4.15	5.76	5.77	24.48	78.63	123.03	66.34	189.37	9.77	103.62	48.89
Mean	17.23	47.25	16.37	35.24	7.11	8.27	8.62	24.71	81.90	125.85	57.39	183.24	9.89	104.12	52.08
LSD 0.05	0.68	1.06	0.42	1.73	0.71	0.79	0.81	0.94	1.52	6.45	9.83	14.36	0.73	5.00	3.18

Note: EL: ear length (cm), ED: ear diameter (mm), ER: number of ear rows, KR: number of kernels per row, GW: grain weight (kg), W1000: weight of 1000 kernels (g), EW: ear weight (kg), GY: grain yield (t ha^{-1}), MC: moisture content (%), SP: shelling percentage, ChlorA: chlorophyll A, ChlorB: chlorophyll B, ChlorT: Chlorophyll total, NSt: number of stomata, StL: stomata length, StW: stomata width

Table 5. Hybrid performance and stress tolerance indices on grain yield for each hybrid were evaluated under normal and low nitrogen conditions

Genotype	Stress tolerance indices											
	Yp	Ys	TOL	MP	HM	GMP	SSI	STI	YI	YSI	k1STI	k2STI
G01	13.25	9.90	3.35	11.57	11.33	11.45	0.79	1.24	1.42	0.75	2.07	2.50
G02	9.66	8.46	1.20	9.06	9.02	9.04	0.39	0.77	1.21	0.88	0.68	1.14
G03	11.27	7.59	3.69	9.43	9.07	9.25	1.02	0.81	1.09	0.67	0.98	0.96
G04	12.51	7.10	5.41	9.80	9.06	9.42	1.35	0.84	1.02	0.57	1.25	0.87
G05	12.18	7.71	4.48	9.94	9.44	9.69	1.15	0.89	1.10	0.63	1.25	1.08
G06	8.00	6.43	1.57	7.21	7.13	7.17	0.61	0.49	0.92	0.80	0.30	0.41
G07	12.24	5.67	6.57	8.95	7.75	8.33	1.67	0.66	0.81	0.46	0.93	0.43
G08	10.11	7.33	2.78	8.72	8.50	8.61	0.86	0.70	1.05	0.73	0.68	0.78
G09	7.07	4.51	2.56	5.79	5.50	5.64	1.13	0.30	0.65	0.64	0.14	0.13
G10	6.44	5.10	1.34	5.77	5.69	5.73	0.65	0.31	0.73	0.79	0.12	0.17
Tolerant hybrids	G01, G04, G07	G01, G02, G05	G01, G10, G06	G01. G05, G04	G01, G05, G03	G01, G05, G04	G01, G06, G10	G01, G05, G04	G01, G02, G05	G02, G06, G03	G01, G05, G04	G01, G02, G05
Ranking summary						G01, G02, G05						

Note: Yp: Grain yield under normal conditions (t ha^{-1}), Ys: Grain yield under low nitrogen conditions (t ha^{-1}), TOL: Tolerance Index, MP: Mean Productivity, HM: Harmonic Mean, GMP: Geometric Mean Productivity, SSI: Stress Susceptibility Index, STI: Stress Tolerance Index, YI: Yield Index, YSI: Yield Stability Index, k1STI, and k2STI: Modified stress tolerance index

Low nitrogen stress tolerance indices

Several stress tolerance indices were used to elucidate the response of maize hybrids under low nitrogen treatment conditions (Table 6), and three hybrids were selected based on each parameter. G01, G04, and G07 were the hybrids with the highest yields under normal conditions, while G01, G02, and G05 were under low nitrogen treatment. The TOL and SSI selected G01, G10, and G06 as tolerant hybrids based on the smallest to largest values. Meanwhile, other parameters were used to select tolerant hybrids based on the largest to smallest values. G01, G05, and G05 were selected based on four STI parameters, namely Mean Productivity (MP), Geometric Mean Productivity (GMP), STI, and Modified Stress Tolerance Index-1 (k1STI). Harmonic Mean (HM) selected G01, G05, and G03 as tolerant hybrids, while Yield Index (YI) selected G01. In addition, the Yield Stability Index (YSI) selected hybrids G02, G06, and G03. The Yield Index (YI) and Modified Stress Tolerance Index-2 (k2STI) selected G01, G02, and G05. Based on the summary ranking, G01, G02, and G05 were the best and most tolerant hybrids from all stress tolerance indices.

Figure 3.A shows the correlation between each stress tolerance parameter. Yield under normal conditions (Yp) was significantly correlated with all stress tolerance indices except SSI and YSI, while yield under low nitrogen conditions (Ys) was not correlated with TOL, SSI, and YSI. TOL did not show a significant correlation with the tolerance index parameters. However, there was a strong positive and negative correlation with SSI and YSI, respectively. The same is shown by the SSI against YSI with a strong negative correlation value. This shows that the higher the TOL and SSI values, the lower the YSI value. Strong positive and significant correlations were shown between GMP, MP, HM, STI, and k2STI, as proven by the same tolerant hybrids selected for the parameters.

The Modified Stress Tolerance Indices (k1STI and k2STI) showed a tendency for correlation with other stress tolerance parameters.

Figure 3.B presents a three-dimensional scatterplot to visualize the average of each hybrid under normal and stress conditions as well as the grain yield tolerance value for low nitrogen stress conditions using TOL. Additionally, G09 and G10 have low grain yield in both environmental conditions. Hybrids G02, G10, and G06 showed a small reduction under low nitrogen treatment conditions, while G07 and G04 experienced a large reduction. The grain yield of G01 also decreased but remained the highest-yielding hybrid under both environmental conditions.

Stability analysis on grain yield of hybrids

The average grain yield (Y_i) of ten maize hybrids reached 8.62 t ha^{-1}, with G01 and G10 having the highest and lowest grain yield of 11.57 t ha^{-1} and 5.77 t ha^{-1}, respectively (Table 7). Several stability statistics were used to identify the performance of hybrids, including the Yield and Stability index (YS_i), Coefficient of Variation (CV_i), regression and environmental indices (b_i and s^2_{di}), ecovalence (W^2) to the latest stability index, and Weighted Average of Absolute Scores (WAASB). Meanwhile, G01, G02, G03, G04, and G05 were identified as the best genotypes based on YS_i. This is because the hybrids had a value greater than the average YS_i and the yield tended to be greater. Therefore, the hybrids are relatively high-yielding and stable across the test environments. Only G01, G02, and G03 had low Coefficients of Variation (CV) of 16.85%, 7.81%, and 22.59%, which were smaller than the average CV of 24.63%. Based on b_i and s^2_{di}, genotypes that were not significant at 1.00 and 0.00 were stable hybrids G01, G03, and G05. These three hybrids were selected as stable using W^2 and WAASB based on the ranking of all test hybrids. Among the stability statistics, G01 and G03 were consistently selected as stable hybrids in the test environment.

Genotype + Genotype × Environment (GGE) biplot analysis (Figure 4) was used to evaluate the stability and adaptation level of hybrids in specific environments to explain 90.65% of the G+GE variability, with the first two Principal Components (PC1 and PC2) of 78.63% and 12.02%, respectively. This multivariate analysis provides several tools through graphical plotting to visualize the response of genotypes in different environments, which in this study used four graphical plots. Figure 4.A shows the environmental vector view, which shows that the environment with the longest vector line is an environment with high diversity. E04 had the longest vector lines, followed by E02, indicating the varying grain yields of the hybrids in the two environments. Figures 4.B and 4.C show the ranking of each hybrid and the stability of the test locations. The genotype closest to the center of the symmetrical circle had the highest grain yield, while the genotype with the vertical length closest to the Average Environmental Coordinate (AEC) axis was the most stable genotype. G01 is a hybrid with the highest and most stable grain yield. Several other hybrids, such as G03, G05, and G08 tended to be stable, as determined by the length of vertical distances from the AEC axis. In this context, GEI effects had less influence on the hybrids. G09 also showed stability but had a below-average grain yield. G07 possessed the largest vertical distance, indicating that the hybrid was the most unstable under the existing environmental conditions. In Figure 4.D, there are five hybrid sectors and environments in the same sector. This “which-won-where” view illustrates the relationship between genotypes and environment, where the genotypes that are in the same sector as the environment indicate that the genotype is adaptive to that environment. G01 showed the best performance and was adaptive in all environments, followed by G03 and G05. Meanwhile, none of the other hybrids reported specific adaptability to the environment.

Table 6. The grain yield mean of maize genotypes under four environments

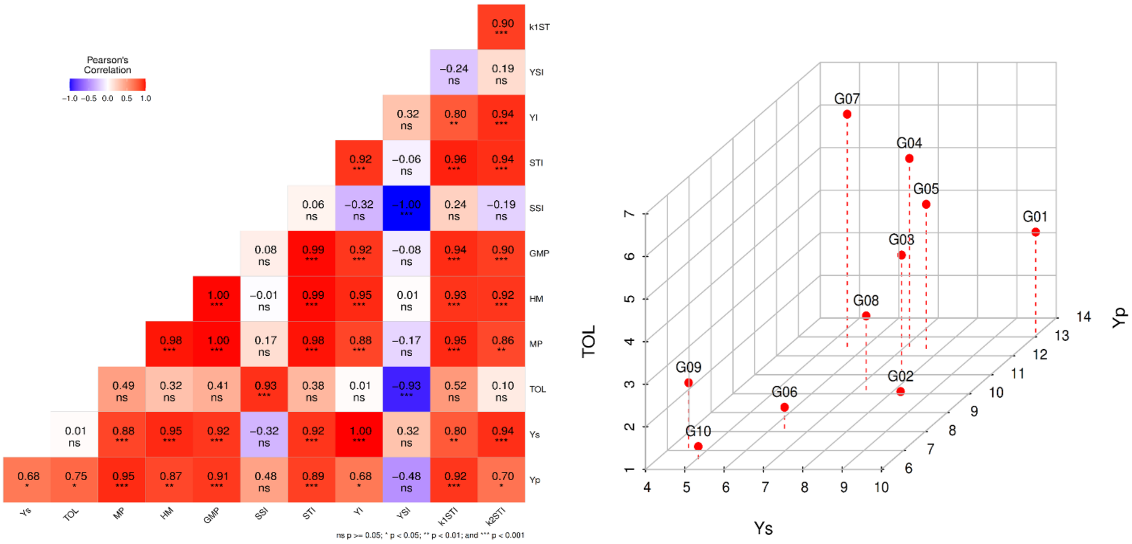
Genotype	E01	E02	E03	E04	Mean
G01	9.60	13.27	10.19	13.23	11.57
G02	8.45	9.83	8.47	9.49	9.06
G03	7.65	11.31	7.52	11.24	9.43
G04	7.66	13.19	6.53	11.82	9.80
G05	8.45	11.66	6.96	12.70	9.94
G06	7.46	8.46	5.39	7.54	7.21
G07	5.38	12.21	5.95	12.26	8.95
G08	9.30	10.63	5.36	9.59	8.72
G09	5.42	8.36	3.59	5.78	5.79
G10	4.20	6.40	6.00	6.48	5.77
Mean	7.36	10.53	6.60	10.01	8.62
LSD 0.05	1.71	1.18	2.57	0.71	0.81
CV (%)	16.45	7.92	27.49	4.99	13.84

Note: E01: Bandarkedungmulyo, Jombang (low N), E02: Bandarkedungmulyo, Jombang (normal), E03: Plosoklaten, Kediri (low N), E04: Plosoklaten, Kediri (normal)

Table 7. Stability statistics on grain yield (t ha^{-1}) for each hybrid across environments

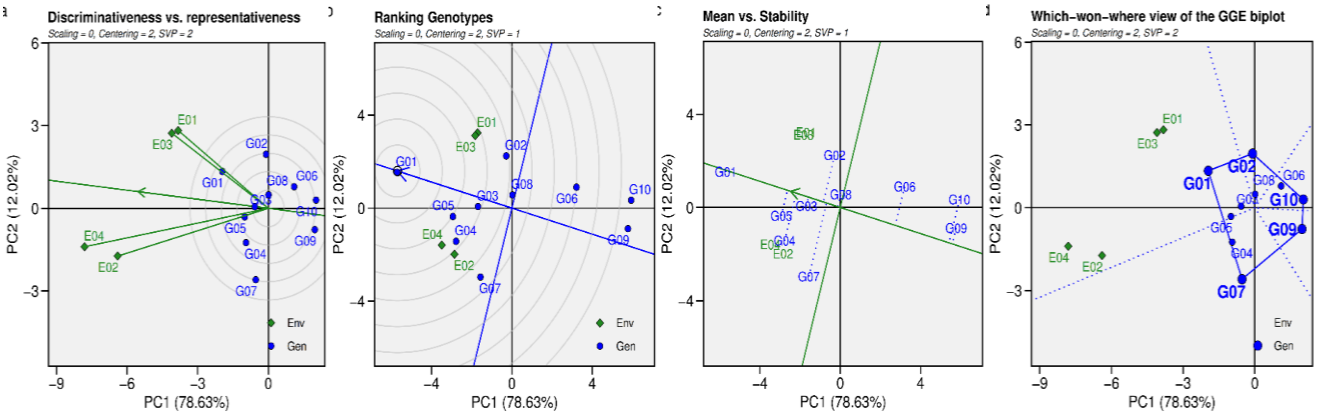
Genotype	Y_i	YS_i	CV_i	b_i	s^2_{di}	W^2	WAASB
G01	11.57a	13+	16.85	0.96ns	0.03ns	1.03	0.20
G02	9.06bcd	3+	7.81	0.36**	-0.45ns	4.70	0.57
G03	9.43bcd	8+	22.59	1.08ns	-0.29ns	0.45	0.18
G04	9.80bc	6+	32.70	1.65**	-0.4ns	4.90	0.58
G05	9.94b	11+	27.05	1.33ns	0.34ns	2.89	0.38
G06	7.21e	-3	17.95	0.55*	0.33ns	3.90	0.53
G07	8.95cd	-2	42.45	1.9***	0.85ns	11.76	0.87
G08	8.72d	-3	26.48	0.97ns	2.24**	5.44	0.46
G09	5.79f	-3	33.92	0.87ns	1.04*	3.23	0.43
G10	5.77f	-10	18.53	0.33**	0.63ns	7.28	0.70
Average	8.62	2.00	24.63	1.00	0.43	4.56	0.49

Note: Y_i: Average yield, a to f: LSD test for each grain yield of genotype across environments, YS_i: Yield and Stability Index (+: greater than the average), CV_i: Coefficient of Variations, b_i: The regression coefficient (*: Significantly different from b_i = 0.0 at $p<0.05$, **: at $p<0.01$, ns: Not significant), s^2_{di}: Deviation from regression (*: Significantly different from s^2_{di} = 0.0 at $p<0.05$, **: at $p<0.01$, ns: Not significant), W^2: The ecovalence of Wricke, WAASB: Weighted Average of Absolute Scores



A B

Figure 3. A. The Pearson correlation among grain yield under both environments and stress tolerance indices, B. The average grain yield of each hybrid under normal (Yp) and low nitrogen (Ys) conditions and the tolerance (TOL) values. TOL: Tolerance Index, MP: Mean Productivity, HM: Harmonic Mean, GMP: Geometric Mean Productivity, SSI: Stress Susceptibility Index, STI: Stress Tolerance Index, YI: Yield Index, YSI: Yield Stability Index, k1STI, and k2STI: Modified Stress Tolerance Index



A B C D

Figure 4. Genotype + Genotype × Environment (GGE) biplot. A. Discriminativeness vs. representativeness, B. Ranking genotypes, C. Mean vs. stability, D. Which-won-where view of GGE biplot of hybrids across the environment

Discussion

The environment is a significant factor in plant breeding, affecting various aspects of performance, including agronomic traits, yields, components, and physiology (Alam et al. 2022; Mbe et al. 2024). This study conducted to examine the response and performance of ear traits, grain yield, and chlorophyll as well as stomatal content of tropical maize hybrids under normal and low-N conditions. The results showed that low nitrogen significantly affected the components of ear traits and most physiological traits. Similarly, the genotype effect influenced grain yield and all observed traits, except for moisture content and the number of stomatal. The difference between environmental factors and the genotype used led to GEI. This caused the failure of the genotype to be stable under varied environments (Azrai et al. 2022). GEI occurs during plant breeding and significantly influences quantitative traits, which can be a significant challenge in genotype selection (Singamsetti et al. 2021). The phenomenon results from the differences in the relative performance of genotypes due to low nitrogen stress (Aga et al. 2024). Table 2 shows the significance of GEI effects on most observed traits, including grain yield, chlorophyll content, and stomatal traits. However, GEI does not affect ear diameter, number of kernel rows, moisture content, and stomatal length. The emphasis on GEI shows the importance of using various stability analysis methods, especially on grain yield. These methods are crucial for understanding the interactions, assessing the yield potential and stability of tested hybrids in different environments, and identifying hybrids with good adaptation to specific environmental conditions (Supriadi et al. 2024).

Nitrogen deficiency in maize can lead to significant yield losses, making the management a critical component of the production. This condition reduced vegetative and reproductive growth, leading to lower biomass accumulation and smaller ear size (Aga et al. 2024). Table 3 shows a comparison of the ear performance and grain yield of maize under normal and low-N conditions. Maize hybrids under nitrogen-deficient conditions have poorer ear performance than under normal conditions, affecting grain yield performance. All ear components, such as ear length and diameter, number of kernels, the weight of kernel, and shelling percentage, show a lower average mean, except the moisture under low-N. The average grain yield of hybrids under normal conditions reached 10.27 t ha^{-1}. Meanwhile, the grain yield was 6.98 t ha$^{-1,}$ or reduced by 32.04% under low-N conditions. Adu et al. (2018) showed that the grain yield of maize experienced a 39 % reduction at low-N compared to high-N environment conditions. A lower nitrogen environment can reduce maize growth, such as plant height, leaf length, ear length and diameter, kernel number, chlorophyll, and stomatal, as well as the quality of grain such as protein and provitamin A (Biswas and Ma 2016; Ortiz-Covarrubias et al. 2019; Dosho et al. 2022; Efendi et al. 2024). This research showed that chlorophyll and stomatal were lower under low-N than normal conditions. In this context, plants experience a loss of green leaf color and become pale yellow due to a lack of chlorophyll under low nitrogen conditions, affecting the photosynthesis rate (Wu et al. 2019). Subsequently, energy and biomass generation are affected as well as plant growth and development (Asibi et al. 2019; Ren et al. 2021).

Developing maize hybrids that are resilient to suboptimal conditions is crucial for sustainable agriculture, particularly in nitrogen-deficient soils (Kamara et al. 2024). Achieving high yield and nutrient use efficiency concurrently may be possible through the utilization of novel cultivars capable of efficiently absorbing and utilizing nutrient resources, thereby producing a high grain yield with reasonable fertilizer input (Chen et al. 2021). In this study, we compared the performance of maize under both low and normal conditions, aiming to determine the optimal tolerance of maize hybrids across different environments by examining the grain yield trait. G01 had the best grain yield under normal conditions followed by G04 and G05, while G09 and G10 had the lowest yield across the environments.

The development of maize hybrids with high grain yields needs to be analyzed by determining tolerance to environmental changes (Shojaei et al. 2022). The evaluation of the tolerance capacity used several STI methods to select hybrids. Table 6 shows the best of selected hybrids under normal and low-N environmental conditions, as well as the tolerant hybrids based on the stress tolerance indices. G01, G04, and G07 had the highest yield under optimum environment, while G01, G02, and G05 were under low-N conditions. Furthermore, G01 was the best-tolerant hybrid selected by all indices, except the YSI. Several stress tolerance indices, such as MP, GMP, STI, and k1STI selected G01, G05, and G04 as the tolerant hybrids under low-N. TOL and SSI selected G01, G10, and G06, while YI and k2STI selected G01, G02, and G05. Several studies showed that the use of many tolerance indices was considered more effective in determining tolerant genotypes than relying on only one parameter. However, assessing and selecting truly tolerant genotypes is challenging based on all the parameters used (Ararisa et al. 2024; Bhandari et al. 2024; Kumar et al. 2024). This research adopted a ranking summary with the mean rank and standard deviation to select G01, G02, and G05 as the best hybrids.

Stress tolerance indices, such as MP, HM, GMP, STI, YI, k1STI, and k2STI, showed a highly significant positive correlation with the yield of hybrids under normal and low-N conditions. Several studies showed that these indices were positively associated with grain yield under abiotic stress conditions (Bennani et al. 2016; Lamba et al. 2023; Al-Ashkar 2024). Stress tolerance indices in line with grain yield under normal and abiotic stresses can be used as selection criteria for breeders and researchers to determine the tolerance level of genotypes, such as STI (Masuda et al. 2021; Azrai et al. 2022). However, Bhandari et al. (2024) reported that the strength of the correlation for certain indices decreased with increased stress levels, leading to a grain yield loss of 50%. Based on the description, understanding the predictive efficacy of various stress tolerance indices is important concerning stress levels. This research showed that G02 possessed the best yield retention in low-N, while G01 and G05 showed the most adaptive and highest yield based on a three-dimensional scatterplot.

Diverse environmental conditions due to abiotic stress, such as low nitrogen have a major effect on the presence of GEI, becoming a challenge in evaluating the performance of hybrids (Rezende et al. 2020). The effects of GEI on grain yield were reported across environments and locations using several stability statistics, including parametric, non-parametric, and multivariate analyses using a GGE biplot to sort stable hybrids. G01, G02, G03, G04, and G05 showed high and positive yield as well as stability indices, as reported by Kang (1993). However, the Coefficient of Variation (CV) method (Finlay and Kannenberg 1963) showed that G01, G02, and G03 had CV$_s$ lower than average estimates and grain yield higher than the average mean. In this context, only these hybrids are favorable and stable across different environmental conditions. Regression coefficient (b_i), deviation from regression ($s^2_d i$), ecovalence of wricke, and WAASB methods showed that G01, G03, and G05 performed well in terms of stability and broad adaptation (Wricke 1962; Finlay and Wilkinson 1963; Eberhart and Russel 1966; Olivoto et al. 2019). Each stability statistic is defined by a distinct criteria and model for evaluating genotype stability, with the parameter offering specific advantages and disadvantages. The use of multiple stability statistics is more accurate and efficient than a single measure to identify stable hybrids (Supriadi et al. 2024). Several studies used stability statistics to select maize varieties that are stable and high-yielding across diverse environmental conditions (Azrai et al. 2022; Wicaksana et al. 2022; Engida et al. 2024). In this research, G01 and G03 were identified as the most stable hybrids in all stability statistics.

The GGE biplot is a graphical tool for explaining the effect of GEI two-way data and identifying the ideal environments, the best genotypes, and the stable genotypes, as well as the wide and specific adaptability (Mossie et al. 2024; Mullualem et al. 2024). Figure 4 reports four types of GGE biplots that explain the 90.56% accuracy of the data analysis performed. According to the “discriminativeness vs. representativeness” view, E04 and E02 had a stronger ability to differentiate between genotypes. These two environments were the low nitrogen treatment conditions, which allowed variation between the response of growth and grain yield of hybrids. Similar results to other stability statistics, G01 and G03 were the most stable grain yields across different environmental conditions, indicating the stability statistics complemented by the GGE biplot can be applied for simultaneous selection in genotype evaluation. G01, G05, and G04 performed and adapted well under normal and low nitrogen conditions based on the “which-won-where” view, while the other hybrids did not specifically adapt to the environment used. The sensitivity and adaptability of genotypes to certain environments are crucial information for assessing superior genotypes to changes (Abdillah et al. 2023; Supriadi et al. 2024). G01 and G03 were selected using stability statistical methods, and G01, G02, and G05 were selected using tolerance index methods based on their grain yield performance. In addition to high yields, these genotypes exhibited superior physiological traits across all environments compared to other genotypes, particularly in terms of chlorophyll content and stomatal performance. Chlorophyll plays a crucial role in maintaining leaf greenness, which is strongly correlated with grain formation (Wu et al. 2019; Chibane et al. 2021; Linders et al. 2024), whereas stomatal traits promote good nitrogen and water uptake (Kunrath et al. 2020). Understanding GEI of grain yield and performance of maize is crucial for developing the resilient varieties that perform well across different nitrogen levels. The adaptability of these selected genotypes allows them to perform optimally under low-N stress conditions, resulting in maximum yield.

Variations in environmental conditions, defined by differences in agroclimate and agroecology, are pivotal in influencing the agronomic outcomes of maize cultivation in the state. Even when agroclimatic and agroecological conditions are relatively same within a specific location, variations in low-N treatments, as observed at E02 and E04, can result in notable differences in grain yield and other phenotypic traits for each genotype when compared to normal nitrogen treatments (E01 and E03). The interactions between genotype and environment, shaped by factors such as soil type and texture, temperature, rainfall, and treatment, significantly affect the yield stability and tolerance levels of each genotype.

In conclusion, the integration of tolerance and stability analyses in evaluating genotype performance, particularly under abiotic stress conditions, presents an effective strategy for breeders in the selection of genotypes. This analysis facilitates a more comprehensive comparison of genotypes characterized by high yield, stress tolerance, and stability across diverse environments. Assessing the adaptability, stability, and tolerance of hybrids is a crucial part of selecting superior cultivars in the realm of plant genetic enhancement. Breeding strategies should focus on selecting and identifying genotypes that perform well in nitrogen-limited environment. G01 was the most superior maize hybrid, with tolerance and stability under low nitrogen conditions, and had the highest grain yield. The wide adaptability of this genotype implies that it consistently produces stable yields despite fluctuations in environmental conditions, particularly in agronomic settings where nitrogen is limited. This hybrid was reported as a superior candidate for release genotype from PT Restu Agropro Jayamas, named “R0211”. Some hybrids also possessed the potential to be superior hybrids, such as G02, G03, and G05 that can be used for low nitrogen input trials. Therefore, future research could be carried out to analyze these genetic materials for maize breeding programs (e.g., more multi-location testing and farmer trials) in tropical regions.

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