



Assessment of Stress Indices in Elite Single-Cross Maize (*Zea mays* L.) Hybrids Under Low and Optimal Nitrogen Conditions using the iPASTIC Toolkit

ADESIKE OLADOYIN OLAYINKA^{1*}, MOSES ADEOLU ADEBAYO², SOLOMON ADEYEMI OYEKALE³ and IBRAHIM AKINTUNDE RAJI¹

¹Department of Crop Production and Soil Science, Ladoko Akintola University of Technology, Ogbomoso, Nigeria.

²Department of Crop and Animal Sciences, Ajayi Crowther University, Oyo, Nigeria.

³Department of Agriculture, Landmark University, Omu-Aran, Kwara State, Nigeria.

Abstract

Low soil nitrogen is a significant stress factor, often leading to reduced maize (*Zea mays* L.) grain yield. This study assessed the impact of low nitrogen on grain yield and agronomic traits of 10 single-cross extra-early provitamin A quality protein maize hybrids developed via Griffing's diallel method IV. A split-plot trial with four replications set up in a randomized complete block design was conducted under two nitrogen conditions (30 and 90 kg N ha⁻¹) as main plots, with the hybrids and a local check as sub-plots. Grain yield and related traits were measured under different nitrogen levels, and stress indices were calculated. Significant yield differences among hybrids were observed across nitrogen environments. Hybrids TZEEIORQ 63 × TZEEIORQ 53 and TZEEIORQ 63 × TZEEIORQ 52 performed best under both conditions, with TZEEIORQ 63 × TZEEIORQ 53 yielding 62.4% higher than the average of other hybrids and comparable to the local check. Stress indices, including mean productivity, geometric mean productivity, harmonic mean, stress tolerance index, and yield index, showed strong positive correlations with grain yield, effectively predicting low nitrogen tolerance. Multivariate biplot analysis linked TZEEIORQ 63 × TZEEIORQ 53 closely with these indices, while rank sum analysis consistently identified TZEEIORQ 53 × TZEEIORQ 52, TZEEIORQ 63 × TZEEIORQ 52, and TZEEIORQ 63 × TZEEIORQ 53 as tolerant to low nitrogen. Upon further testing, these hybrids may be suitable for cultivation in low soil nitrogen areas where farmers cannot afford optimal fertilizer application.



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CONTACT Adesike Oladoyin Olayinka ✉ aokolawole@lautaech.edu.ng 📍 Department of Crop Production and Soil Science, Ladoko Akintola University of Technology, Ogbomoso, Nigeria.



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Introduction

Maize (*Zea mays* L.) is an economically significant staple cereal crop that is consumed in a variety of forms throughout Africa.¹ It is grown virtually in all agroecologies in Nigeria due to its critical importance as a food, feed, and industrial crop. The North Central region of Nigeria is the leading maize-producing region, recording a mean grain yield of 1.8 t ha⁻¹ on the farmer's field and a marginal annual increase of 2 - 3%. The productivity is due mainly to poor management practices, unimproved maize varieties, and the devastating impact of biotic and abiotic stresses.² Low soil nitrogen (N) is one of the important abiotic stresses that reduces maize yield depending on its severity, plant growth stage, and genotype susceptibility level. It has been reported that maize is a heavy feeder on N with a high response to phosphorus (P); however, the most lacking nutrient in the soil is N, frequently limiting maize yield.³ The intensive cropping system being practiced across Nigerian agroecological zones necessitates the continuous application of organic and inorganic fertilizers for soil fertility sustainability and optimal plant growth. Nitrogen fertilization is critical to the physiological and metabolic functions of the maize plant, resulting in increased vegetative growth, kernel filling, and ultimately, enhanced productivity. Reduction in optimal N fertilization reduces photosynthesis, directly impacting the maize grain yield by diminishing the size and weight of the ears.⁴ Recently, farmers report abysmal grain yields as a result of little or no fertilizer application due to the exorbitant price increases year after year and the inability to obtain loans or credit facilities to finance their farm operations.⁵ As a result, maize hybrids that have been developed with genes for high productivity in low N conditions are highly desirable.

Weather variations are currently a source of concern in the derived Guinea Savanna agroecology. When rains are delayed or erratic, extra-early maturing maize hybrids with high levels of provitamin A, tryptophan, and lysine contents that mature in 85 to 90 days are suitable for planting. Farmers who planted maize varieties that belong to this maturity class benefited from early market access before the main season sales, filling a large gap after the dry season. Thus, breeding biofortified extra-early maturing maize hybrids that are tolerant to low soil N stress is a cost-effective, sustainable, and economically viable strategy for ensuring food

security. Developing maize hybrids from biofortified inbred lines and testing them in both low and optimal N research conditions will improve the identification of superior hybrids with high grain yield potential in both soil environments.

Estimating stress tolerance in newly developed genetic materials will significantly improve genetic gain in a breeding programme.⁶ There are differential reactions of elite maize genotypes to diverse production stresses. High-yield performance is the primary indicator of stress tolerance in most crops, and genotypes with high yields in both stressed and non-stressed environments are considered tolerant.⁷ However, high-yielding genotypes may not necessarily be stress-tolerant and vice versa; hence, the use of stress-tolerant indices is a more effective technique than mere selection through high grain yield and associated traits. Previous studies by Badu-Apraku *et al.*,⁸ Obeng-Bio *et al.*,⁹ and Kolawole *et al.*¹⁰ assessed N stress tolerance in maize by manually estimating multiple trait-based indices under low N research conditions. This approach is time-consuming and may be prone to human error. Hence, there is a need to employ the recently created user-friendly abiotic stress calculator, which performs a huge variety of computations conveniently and rapidly. The Plant Abiotic Stress Index Calculator (iPASTIC), developed Pour-Aboughadareh *et al.* is a web-based application utilizing JavaScript and R to compute prevalent stress tolerance and susceptibility indices for diverse crop characteristics. These indices consist of two groups. The first group consist of geometric mean productivity (GMP), harmonic mean (HM), stress tolerance index (STI), and mean productivity (MP), which have the potential to identify stress-tolerant genotypes. The second group comprises stress susceptibility index (SSI) and yield stability index (YSI), which are useful for discriminating yield and stability performance of newly developed crop varieties. The other indices that iPASTIC software computes include: tolerance index (TOL), yield index (YI), and relative stress index (RSI).

These indices measure not only the yield but also the stability and adaptability, helping breeders select genotypes with both yield potential and resilience to stress. Using stress-tolerant indices allows breeders to account for complex physiological and genetic interactions influencing tolerance, beyond what yield

alone can reveal. They also offer practical tools for screening large numbers of genotypes efficiently, correlating agronomic performance to stress tolerance. Thus, incorporating stress-tolerance indices in breeding programmes is scientifically justified to improve crop resilience and productivity under changing environmental conditions.

The suitability and effectiveness of this online toolkit (iPASTIC) have been documented in diverse crop improvement programmes that encompass the screening of drought-tolerant genotypes (good performance under stress and non-stress conditions) in various crops such as wheat,¹¹⁻¹² bush bean,¹³ maize,¹⁴⁻¹⁵ and sorghum.¹⁶ Notwithstanding the recognised application of these indices in agronomy and plant breeding programmes, limited studies have compared, identified, and selected promising N stress screening indices for maize grain yield. The objectives of this study were to (i) compare the grain yields of single-cross maize hybrids grown in optimum and low N environments, (ii) identify N stress tolerant maize hybrids using the stress indices, and (iii) assess the relationship among the stress indices and their effectiveness for identifying N stress-tolerant maize hybrids.

Materials and Methods

Genetic Materials

The inbred lines used to generate the hybrids evaluated in this study were selected from the tropical *Zea* extra-early provitamin A (PVA) quality protein maize (QPM) inbred lines (TZEEIORQ) developed by the maize improvement programme of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria.¹⁷ The selected inbred lines were evaluated, and five inbreds with high grain yield potential, which possess genes for PVA, quality protein (tryptophan and lysine), as reported by Kolawole *et al.*,¹⁸ were selected and crossed using Griffing's diallel method IV to generate 10 single-cross extra-early maize hybrids without reciprocals.

Experimental Site, Design, and Cultural Practices

In the 2022 and 2023 main growing seasons, the 10 single-cross maize hybrids generated, viz. TZEEIORQ 53 × TZEEIORQ 52, TZEEIORQ 55 × TZEEIORQ 52, TZEEIORQ 57 × TZEEIORQ 52, TZEEIORQ 63 × TZEEIORQ 52, TZEEIORQ 55 × TZEEIORQ 53, TZEEIORQ 57 × TZEEIORQ 53, TZEEIORQ 63 × TZEEIORQ 53, TZEEIORQ 57 ×

TZEEIORQ 55, TZEEIORQ 63 × TZEEIORQ 55, TZEEIORQ 63 × TZEEIORQ 57 and a commercial maize hybrid planted by farmers used as local check were evaluated at the Ladoke Akintola University of Technology, Ogbomoso, Nigeria, Teaching and Research (LAUTECH T&R) Farm, (8° 10'N, 4° 10'E and altitude 341 m above sea level). The study site experiences an annual rainfall between 1,000 and 1,200 mm, with daily temperatures fluctuating from 28 to 30°C. The soils at the experimental location are classified as Alfisol,¹⁹ which are typically characterized by low nitrogen (N) content. Historically, the site has been continuously cultivated with maize over several years, with minimal or no nitrogen fertilizer application. Additionally, after each maize harvest, all plant residues were removed from the field to prepare for subsequent planting seasons. This practice has likely contributed to the depletion of soil nitrogen levels. Before establishing the trial, soil samples were collected and analyzed at the Department of Agronomy's soil laboratory, University of Ibadan, Ibadan, Nigeria, to assess nutrient status. Land preparation involved mechanical tillage using a tractor-mounted plough, followed by the division of the field into two blocks representing distinct nitrogen application regimes: 30 kg N ha⁻¹ and 90 kg N ha⁻¹. These nitrogen environments were separated by a 3-meter alley, with a constructed gutter implemented to inhibit lateral nitrogen movement in the soil.

A split-plot arrangement in a randomized complete block design with four replications served as the experimental setup. The main plots consisted of the two nitrogen environments, while sub-plots comprised different maize hybrids. Each experimental unit was a single-row plot measuring 5 meters in length, with rows spaced 0.75 meters apart and intra-row spacing of 0.50 meters between hills. Three seeds were sown per hill, and thinning was conducted two weeks after planting to maintain two plants per stand, resulting in a final plant density of 53,333 plants per hectare.

Phosphorus and potassium were applied as basal fertilizers at rates of 60 kg ha⁻¹ each, using single superphosphate (SSP) and muriate of potash (MOP), respectively, within the 30 kg N ha⁻¹ treatments. For the 90 kg N ha⁻¹ treatments, nitrogen was supplied in two split applications: the first dose at two weeks post-sowing and the second dose two weeks thereafter. Weed control involved the application of a mixture of Gramoxone and Primextra herbicides at

a rate of 5.0 liters per hectare at planting, followed by manual weeding to maintain weed-free plots. Fall armyworm (*Spodoptera frugiperda*) infestations were managed using Caterpillar Force, containing Emamectin benzoate at 10 g per 15 liters of water, applied at two-week intervals.

Data Collection

Agronomic data were recorded at the plot level. Anthesis date was defined as the number of days from planting until 50% of plants shed pollen. Silking date was the number of days from sowing until 50% of the plants extruded silks. The anthesis-silking interval (ASI) was calculated as the difference between silking and anthesis dates. Plant height was measured from the base to the first tassel branch, while ear height was measured from the base to the ear-bearing node (or uppermost ear in prolific hybrids). Plant aspect was scored on a 1-9 scale assessing overall phenotypic appeal, where 1 indicated excellent and 9 poor appearance. Husk cover was rated from 1 (very tight husk extending beyond the ear tip) to 9 (exposed ear tip). Ear aspect was rated on a 1-9 scale, where 1 indicated clean, uniform, large, and well-filled ears, and 9 corresponded to rotten, poorly filled, and small ears. The number of ears per plant was calculated as the ratio of harvested ears to plants at harvest. Stay-green under low nitrogen conditions was evaluated 70 days after planting on a 1–9 scale, where 1 corresponded to <10% dead leaf area and 9 = >80% dead leaf area. Grain yield was estimated from ear weight.¹ The ear weight (kg) was adjusted to 15% moisture content (MC) and an 80% shelling percentage for conversion to kilograms per hectare (kg ha⁻¹) as follows:

$$GY \text{ (kg ha}^{-1}\text{)} = GWT \text{ (kg plot}^{-1}\text{)} \times \frac{100 - MC}{100 - 15} \times \frac{10,000 \text{ m}^2}{\text{plot size m}^2} \times 0.80$$

Where: GWT = grain weight of harvested plot, MC = moisture content of maize grain at harvest, moisture content for maize grain storage = 15 %, 1 hectare = 10,000 m².

Statistical Analysis

The N research conditions (low and optimal) by year combinations constituted environments. All agronomic data collected under each N research

condition and across environments were subjected to Analysis of variance (ANOVA) using the General Linear Model procedure (PROC GLM) in Statistical Analysis System (SAS) software release 9.4.²⁰ The model was fitted with environments, replicates within environments, and hybrid × environment interaction as random factors, while the hybrids were fixed factors. The linear model for the split-plot, with main plots arranged as Randomized Complete Block Design (RCBD), is:

$$Y_{ijk} = \mu + \alpha_i + \gamma_k + \alpha\gamma_{ik} + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$$

Where Y_{ijk} = the observed response variable for the i th level of the main plot factor, j th subplot levels, and k th block; μ = the overall mean of the response variable, i = indexes the main plot levels, j = indexes the subplot levels, k = indexes the blocks, and γ_k represents the effect of the k th block.

Provided that mean square values were significant, a mean comparison was done with Fisher's least significant difference (LSD) test at a 0.05 probability level.²¹ In addition, low N-tolerant hybrids were identified using the iPASTIC created by Pour-Aboughadareh *et al.*⁷ The online toolkit, written in JavaScript, was used to calculate stress indices (Table 1) and the percentage of relative change. The software was also used to calculate the ranking patterns of the hybrids, based on each index. The average sum of ranks (ASR) and standard deviation (SD) were computed to ensure consistency in the indices' ability to identify N stress-tolerant and susceptible maize hybrids.

Maize hybrids exhibiting lower ASR values were identified as the most tolerant. Heat maps constructed using Pearson's²⁹ correlation coefficients were employed to assess the relationships among stress indices and grain yield under both experimental conditions. A three-dimensional plot based on the Stress Tolerance Index (STI) for maize grain yield under optimal and low nitrogen (N) environments facilitated the classification of hybrids into Fernandez's²⁴ four groups (A, B, C, and D). Additionally, principal component analysis (PCA) was performed on nine stress tolerance indices along with grain yield data under both optimal and low N environments for the ten maize hybrids.

Table 1: Indices of tolerance and susceptibility computed using iPASTIC software, with their mathematical formulas

Stress index	Equation / Formula	Selected value	Reference
Tolerance	$TOL = Y_p - Y_s$	Lower value	Rosielle and Hamblin ²²
Stress susceptibility index	$SSI = \frac{1 - Y_s/Y_p}{1 - \bar{Y}_s/\bar{Y}_p}$	Lower value	Fischer and Maurer ²³
Geometric mean productivity	$GMP = \sqrt{Y_s \times Y_p}$	Higher value	Fernandez ²⁴
Stress Tolerance Index	$STI = \frac{Y_s \times Y_p}{(\bar{Y}_p)^2}$	Higher value	Fernandez ²⁴
Harmonic mean	$HM = \frac{2(Y_s \times Y_p)}{Y_s + Y_p}$	Higher value	Bidinger <i>et al.</i> ²⁵
Mean productivity	$MP = \frac{Y_p + Y_s}{2}$	Higher value	Rosielle and Hamblin ²²
Yield index	$YI = \frac{Y_s}{\bar{Y}_s}$	Higher value	Gavuzzi <i>et al.</i> ²⁶
Yield stability index	$YSI = \frac{Y_s}{Y_p}$	Higher value	Bousslama and Schapaugh ²⁷
Relative stress index	$RSI = \frac{Y_s}{Y_p} \frac{\bar{Y}_s}{\bar{Y}_p}$	Higher value	Fischer and Wood ²⁸

Y_p = yield performance under non-stressed conditions, and Y_s = yield performance under stressed conditions for each index.

Source: Pour-Aboughadareh *et al.*⁷

Results

Analysis of Variance for Grain Yield and Agronomic Traits

For each and across N environments, the mean squares for the examined traits were significant ($p < 0.001$, 0.01, or 0.05) for the environment, hybrid, and their interactions (Table 2). Under low N environments, the mean squares of environment for ear aspect, ears per plant, grain yield, and the mean squares of hybrid for plant height and ears per plant showed no significant difference; the mean squares of hybrid \times environment interaction were significant only for the number of days to silking, husk cover, and grain yield. Under the optimal N environments, the mean squares of the environment were significant for most of the traits measured, except for

anthesis-silking interval, ear height, and grain yield. The mean squares of the hybrid showed significant differences for grain yield, husk cover, anthesis-silking interval, and numbers of days to anthesis and silking. The mean squares of hybrid \times environment interaction showed significant differences for the number of days to silking, anthesis-silking interval, plant height, plant and ear aspects. Across the N environments, mean squares of environment, hybrid and their interactions (hybrid \times environment) were significant for the measured traits except mean squares of environment for ear aspect and grain yield, mean squares of hybrid for plant height and ears per plant and mean squares of hybrid \times environment interaction for numbers of days to anthesis, ear height and ears per plant.

Table 2: Mean squares of grain yield and other agronomic traits of extra-early maturing provitamin A-quality protein maize hybrids assessed under low and optimal N environments during the primary growing seasons of 2022 and 2023 at LAUTECH T&R farm, Nigeria.

Source	df	Anthe -sis (days)	Silking (days)	Anthesi-ssilking interval (days)	Plant height (cm)	Ear height (cm)	Plant aspect (1-9)	Ear aspect (1-9)	Husk cover (1-5)	Ears per plant	Grain Yield (kg/ha)	Stay green (1-9)
LOW N ENVIRONMENTS												
ENVIRONMENT (ENV)	1	76.4	336.	92.0	1194.	700.	6.55**	0.01	34.3	0.11	4705	17.2
		1***	18***	5***	90***	60***			8***		1.98	8***
REP(ENV)	6	3.63	6.96*	1.80	180.	102.	1.08	0.94	1.71**	0.01	33572	1.24
					01	54*					6.05**	
HYBRID	10	11.6	27.7	5.88**	188	332.	6.06***	5.5	3.60	0.07	17760	5.5
		3***	4***		.25	40***		3***			58.12***	8***
HYBRID × ENV	10	1.96	7.58*	3.12	80.83	33.50	1.25	1.34	2.05***	0.06	60106	0.93
											9.95***	
Error	60	2.00	3.16	1.94	102.	43.94	0.96	1.14	0.56	0.04	1063	1.01
					58						82.50	
CV (%)		2.36	2.94	32.29	6.53	12.89	18.02	21.25	20.61	23.82	40.85	18.97
R ²		0.66	0.79	0.62	0.45	0.65	0.60	0.52	0.75	0.40	0.80	0.60
OPTIMAL N ENVIRONMENTS												
ENVIRONMENT (ENV)	1	26.3	45.5	2.60	1293.	2.10	38.3	6.06*	71.1	0.17*	1227	-
		8***	7**		52***		9***		5***		956.19	
REP(ENV)	6	5.39	13.1	4.13	319.	126.	1.92	1.77	2.35*	0.02	3604	-
		***	2*		17*	06					05.99	
HYBRID	10	4.98**	26.6	11.0	71.82	148.	1.94	2.48	1.81*	0.04	18998	-
			0***	3***		06					98.56**	
HYBRID × ENV	10	0.84	10.0	6.93**	261.	92.8	2.33*	2.85*	1.01	0.06	9544	-
			9*		07*	8					89.81	
Error	60	1.67	4.58	2.72	110.	85.72	1.05	1.29	0.85	0.04	6983	-
					16						60.50	
CV (%)		2.13	3.49	25.92	6.70	18.54	18.03	23.34	24.10	25.39	46.41	-
R ²		0.54	0.64	0.56	0.50	0.38	0.60	0.47	0.69	0.32	0.43	-
ACROSS ENVIRONMENTS												

ENVIRONMENT (ENV)	3	42.4	140.8	32.2	867.0	263.	15.7	2.39	35.7	0.11*	4317
		7***	4***	2***	6***	92**	4***		8***		92.54
REP(ENV)	12	4.51**	10.0	2.97	249.	114.	1.50	1.35	2.03	0.02	3480
			4**		59**	30			***		66.02
HYBRID	10	11.73	31.9	6.26**	83.28	366.3	5.4	4.67	3.47	0.04	273854
		***	0***			4***	4***	***	***		8.04***
HYBRID × ENV	30	2.56	13.3	6.90	172.	80.16	2.04**	2.51**	1.67	0.06	83081
			7***	***	83*				***		4.81***
Error	120	1.83	3.87	2.33	106	64.83	1.00	1.22	0.71	0.04	4023
					.37						71.5
CV (%)		2.24	3.23	28.59	6.61	15.89	18.03	22.29	22.53	24.66	48.58
		0.63	0.73	0.59	0.48	0.52	0.60	0.50	0.72	0.36	577
R ²											0.545
											758

*, **, ***, Significant at 0.05, 0.01, and 0.001 probability levels, respectively
 CV = coefficient of variation; R² = coefficient of determination

Selection of Maize Hybrids Based on Stress Indices and Grain Yield Response

At the optimal N environment, maize grain yield (Y_p) ranged from 594.9 to 2,022.0 kg ha⁻¹. TZEEIORQ 63 × TZEEIORQ 53, followed by TZEEIORQ 63 × TZEEIORQ 52, had the highest mean grain yield performance. Under low N environment, grain yield (Y_s) varied from 360.0 to 901.5 kg ha⁻¹, and TZEEIORQ 63 × TZEEIORQ 53 followed by TZEEIORQ 53 × TZEEIORQ 52 showed the highest values under low N environment (Table 3). Comparison of the hybrids' grain yield performance under low and optimal N conditions in the two years of evaluation revealed yield reductions ranging from 15% for TZEEIORQ 53 × TZEEIORQ 52 to 59% for TZEEIORQ 63 × TZEEIORQ 57, with a mean of 36%. TZEEIORQ 63 × TZEEIORQ 53, TZEEIORQ 63 × TZEEIORQ 52, and TZEEIORQ 53 × TZEEIORQ 52 had high values for STI, MP, GMP HM, and YI indices, which are indicators of good performance under low and optimum N environments. Our findings showed the selection of superior maize hybrids based on more than one indicator. The stability of the hybrids under both N conditions was further assessed with the YSI and RSI indices, TZEEIORQ 53 × TZEEIORQ 52, TZEEIORQ 57 × TZEEIORQ 53, and TZEEIORQ 55 × TZEEIORQ 53 had high values with a similar ranking pattern for these indices in the identification of N-tolerant and stable hybrids.

The maize hybrids with the lowest reduction percentage (<32%) in grain yield under low N environments were TZEEIORQ 53 × TZEEIORQ 52 (15%), TZEEIORQ 57 × TZEEIORQ 52 (30%), TZEEIORQ 55 × TZEEIORQ 53 (21%), and TZEEIORQ 57 × TZEEIORQ 53 (19%). The identified hybrids with lower percent relative change also had the low values for TOL with SSI < 1, emphasizing their tolerance to low N, whereas TZEEIORQ 63 × TZEEIORQ 57, with the highest percent yield reduction, was sensitive to low N conditions.

The results based on a single index may not be reliable, and hence, an average sum of rank (ASR) based on the combination of all nine indices was used to depict the hybrid's N stress tolerance nature. The SR values of the hybrids were between 26 and 92, and the hybrid with the lowest ASR value indicates N stress tolerance. Thus, the top three N stress-tolerant

hybrids identified were TZEEIORQ 53 × TZEEIORQ 52 (ASR = 2.4; SD = 1.2), TZEEIORQ 63 × TZEEIORQ 52 (ASR = 3.5; SD = 1.8), and TZEEIORQ 63 × TZEEIORQ 53 (ASR = 4.0; SD = 4.2).

Table 3: Ranking of the 10 extra-early maize hybrids along with the relative change and tolerance indices

Hybrid	Yp	Ys(%)	RC	TOL	MP	GMP	HM	SSI	STI	YI	YSI	RSI	SR	ASR	SD
TZEEIORQ 53 × TZEEIORQ 52	801.4	682.2	14.9	119.2	741.8	739.4	737.0	0.4	0.7	1.3	0.9	1.4	26	2.4	1.2
TZEEIORQ 55 × TZEEIORQ 52	647.0	369.1	42.9	277.8	508.1	488.7	470.1	1.1	0.3	0.7	0.6	0.9	92	8.4	1.7
TZEEIORQ 57 × TZEEIORQ 52	621.6	432.7	30.4	188.9	527.2	518.7	510.3	0.8	0.3	0.8	0.7	1.1	76	6.9	2.3
TZEEIORQ 63 × TZEEIORQ 52	943.8	639.7	32.2	304.0	791.7	777.0	762.6	0.8	0.8	1.2	0.7	1.1	38	3.5	1.8
TZEEIORQ 55 × TZEEIORQ 53	724.8	571.6	21.1	153.2	648.2	643.6	639.1	0.5	0.5	1.1	0.8	1.3	47	4.3	1.3
TZEEIORQ 57 × TZEEIORQ 53	594.9	484.3	18.6	110.6	539.6	536.7	533.9	0.5	0.4	0.9	0.8	1.3	60	5.5	3.1
TZEEIORQ 63 × TZEEIORQ 53	2022.0	901.5	55.4	1120.5	1461.7	1350.1	1247.0	1.4	2.3	1.7	0.4	0.7	44	4.0	4.2
TZEEIORQ 57 × TZEEIORQ 55	728.6	435.2	40.3	293.4	581.9	563.1	544.9	1.0	0.4	0.8	0.6	1.0	69	6.3	0.5
TZEEIORQ 63 × TZEEIORQ 55	922.3	513.9	44.3	408.4	718.1	688.5	660.0	1.1	0.6	1.0	0.6	0.9	61	5.5	2.0
TZEEIORQ 63 × TZEEIORQ 57	869.5	360.0	58.6	509.5	614.8	559.5	509.2	1.5	0.4	0.7	0.4	0.7	92	8.4	2.1

Yp = grain yield under optimum N; Ys = grain yield under low N; RC = % relative change; TOL = tolerance index; RSI = relative stress index; MP = mean productivity; HM = harmonic mean; YSI = yield stability index; GMP = geometric mean productivity; SSI = stress susceptibility index; STI = stress tolerance index; YI = yield index; SR = sum ranks; ASR = average sum of ranks; SD = standard deviation

Response of Maize Hybrids for Agronomic Traits Under The Low and Optimal N Environments

The mean days to anthesis and silking were 60 days under low N environments compared to 61 days in

optimal N environments (Table S1). Under low N environments, TZEEIORQ 63 × TZEEIORQ 52 and TZEEIORQ 55 × TZEEIORQ 53 had the shortest anthesis-silking interval (0.1). The tallest hybrid

Multivariate Analysis

The first two principal components (PC) with eigenvalues >1 explained 99.6% (PC1 = 71.2 and PC2 = 28.4) of the overall variation in yield performance, according to the PCA results based on the correlation matrix (Figure 2). Grain yield favourably influenced PC1 under both environments and all stress indices except RSI and YSI, as observed also on the biplot. Only TOL and STI had a negative association with PC2. Hence, selection based on high values of indices in these PCs could help to identify N-tolerant hybrids.

The biplot of PCA1 and PCA2 classified the 10 single-cross hybrids into four different quadrants based on their yield response under both low and optimal N environments, and only one hybrid (TZEEIORQ 63 × TZEEIORQ 53) was located near the tolerance indices, such as STI, MP, GMP, HM, and YI. On the other hand, the majority of hybrids

were categorized as N stress-susceptible hybrids. The hybrid (TZEEIORQ 63 × TZEEIORQ 53) identified as superior by the PCA biplot was further backed up by the 3D plot's findings. This hybrid has STI >1, and according to Fernandez's 24 theory, it falls into the A group, which implies a comparatively consistent performance in both optimal and low N environments (Figure S1). Also, TZEEIORQ 53 × TZEEIORQ 52 was seen on the borderline of group C, which indicates high performance under low N conditions. Other hybrids were in group D, suggesting low performance under both N environments and the lowest STI.

In general, the biplot and 3D plot results showed that TZEEIORQ 63 × TZEEIORQ 53 performed best across both N environments. Thus, based on STI, plant breeders can select hybrids with yield potential under low N stress conditions.

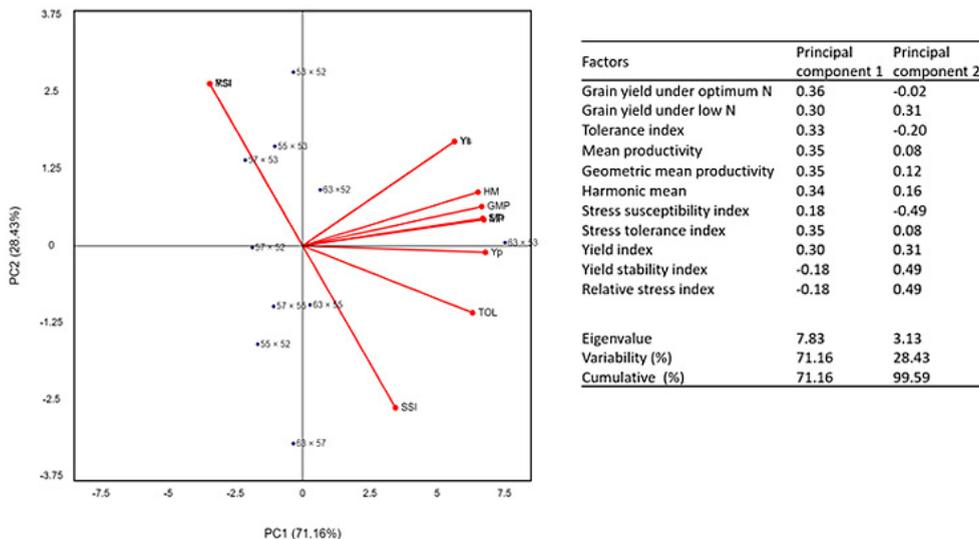


Fig. 2: Principal components analysis (PCA) based on the correlation matrix of grain yield under optimum N (Yp), grain yield under low N (Ys) and stress tolerance indices.

Discussion

The highly significant mean squares of hybrids observed for most traits, including grain yield under both research conditions and stay green characteristics under low N conditions, indicate substantial genetic variability among the extra-early maize hybrids. This variability suggests the potential for selecting hybrids that are tolerant to N stress.³⁰

In line with the present results, Obeng-Bio *et al*⁹ found significant variation among early PVA-QPM maize hybrids for grain yield under low and optimal N conditions. Furthermore, Li *et al*³¹ reported significant differences in grain yields among maize cultivars grown under varying N research conditions. The distinct N environments effectively revealed genetic differences among the hybrids. Additionally, the

significant mean squares of hybrid \times environment interaction observed for grain yield and most traits under each and across N environments show that environmental variation influences trait expression. This highlights the importance of evaluating hybrids across multiple N environments to assess grain yield performance accurately.

It is important to mention that the inbred lines used to develop the hybrids evaluated in this study had grain yields ranging from 250 to 2372 kg/ha under diverse environments.¹⁷⁻¹⁸ However, the hybrids developed exhibited abysmally low yields despite their responsiveness to nutrients and heavy feeding on N. Plausible reasons for this could include deleterious alleles in the genetic background of the inbreds negatively affecting the hybrids' performance, poor genetic compatibility, or poor general and specific combining abilities, resulting in hybrids with low yields. Ideally, extra-early maize varieties fit well into multiple cropping cycles due to their relatively short growing periods. Nevertheless, they often incur an undesirable yield penalty because the shorter time to accumulate and translocate sufficient photo-assimilate reserves results in low grain yields, as evidenced in this study and reported by Semahegn *et al.*¹¹

Grain yield variation between hybrids grown under low and optimal N environments underscores the differential response of the hybrids to N application, in agreement with the report of Aga *et al.*³⁴ This further emphasizes grain yield as a complex quantitative trait controlled by multiple genes and impacted by environmental factors.³⁵ The result of maize hybrids with the lowest reduction percentage in this study corroborates the reports of Aga *et al.*,²⁸ who reported yield reduction of 22-32% in durum wheat genotype under low N inputs.

Among the 10 single-cross hybrids evaluated, TZEEIORQ 63 \times TZEEIORQ 53 showed the highest values for MP, GMP, HM, STI, YI, and TOL. Anand *et al.*³² observed a similar trend for these indices in the selection of high grain yield in rice under both water-stressed and non-stressed conditions, while Shahrokhi *et al.*³³ and Sánchez-Reinoso *et al.*¹³ reported the usefulness of these indices in screening maize and bush bean for drought tolerance. The consistent identification

of TZEEIORQ 63 \times TZEEIORQ 53 and TZEEIORQ 63 \times TZEEIORQ 52 as high-yielding and tolerant to N stress demonstrates the efficiency of ASR. This provides greater insight into the response of the evaluated hybrids, and this approach has been widely utilized to select superior genotypes in various breeding programmes.^{12,36}

According to Horváth *et al.*,³⁷ indices that correlate with yield performance in both stress and non-stress research conditions are known as the best tolerance indicators because they can be used interchangeably to select low N-tolerant hybrids. Therefore, TOL, MP, GMP, HM, STI, and YI, which had a strong and significant relationship with grain yield under optimal (Y_p) and low (Y_s) N environments, can identify maize hybrids with high yield potential and relatively uniform performance under both N conditions, corroborating the report of Bonea¹⁴ and Singamsetti *et al.*³⁸ who found similar strong positive correlation patterns between stress indices and grain yield under stress and optimal conditions in maize. Likewise, previous studies on other crops like canola³⁹ and wheat⁴⁰ confirm the same trend of association. Significant positive correlations between Y_p and Y_s have been reported by Grzesiak *et al.*⁴¹, Sedri *et al.*⁴² and Shojaei *et al.*⁴⁰ The perfect positive or negative correlation coefficient ($r = 1$ or -1 , $p < 0.05$) between Y_s and YI, YSI and RSI, SSI with YSI and RSI, observed in this study, were similar to the reports of Mickky *et al.*⁴³ and Nazari *et al.*⁴⁴

Furthermore, the biplot categorized the indices into two groups. The first group was those with high and positive values in PC1 (MP, GMP, HM, STI, and YI) and negative values in PC2 (TOL and SSI). The second group had an index with a negative value in PC1 (RSI and YSI), while the index in PC2 had positive values. According to Nazari *et al.*,³⁸ indices whose vectors have been placed between the vectors of Y_p and Y_s are appropriate for the selection of stress-tolerant hybrids. Similar results have been reported in other crops.⁴⁵⁻⁴⁶ The hybrid TZEEIORQ 63 \times TZEEIORQ 53 was found to be superior for grain yield and tolerant to low N. Its performance under both low and optimal N conditions surpasses the average performance of all other hybrids under optimal conditions. Similar performance has been reported by Ivić *et al.*⁴⁰ and Choudhary *et al.*¹⁶ in wheat and sorghum, respectively.

Conclusion

This study highlights the existence of considerable variability among the maize hybrids for grain yield and other agronomic traits, which can further be exploited for improvement. Superior low N-tolerant hybrids based on each index were identified. Among the nine indices, only four (MP, GMP, HM, STI, and YI) were similar in classifying TZEEIORQ 63 × TZEEIORQ 53, TZEEIORQ 63 × TZEEIORQ 52, and TZEEIORQ 53 × TZEEIORQ 52 as low N-tolerant hybrids. According to the YSI and RS1 indices, TZEEIORQ 53 × TZEEIORQ 52 and TZEEIORQ 57 × TZEEIORQ 53 were the most stable low N-tolerant hybrids. All the other indices, except TOL, SSI, YSI, and RSI, were consistent in identifying TZEEIORQ 63 × TZEEIORQ 53 as the superior low N-tolerant hybrid. In addition to the consistency of the indices for identifying low N-tolerant hybrids, the rank sum also emphasized TZEEIORQ 53 × TZEEIORQ 52, TZEEIORQ 63 × TZEEIORQ 52, and TZEEIORQ 63 × TZEEIORQ 53 as outstanding for grain yield under low N environments. Therefore, based on grain yield, the stress indices computed with the iPASTIC software were able to identify superior and stable hybrids under low and optimal N environments. The identified low N-tolerant hybrids may be useful for cultivation in areas where soil N stress is a challenge. They may also serve as potential donors in the development of N-efficient maize varieties.

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Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

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Permission to Reproduce Material from other Sources

Not Applicable

Author Contributions

- **Adesike Oladoyin Olayinka:** Conceptualization, Methodology, Analysis, Writing – Original Draft.
- **Adesike Oladoyin Olayinka, Moses Adeolu Adebayo, Solomon Adeyemi Oyekale, Ibrahim Akintunde Raji:** Data Collection, Writing – Review & Editing.
- **Adesike Oladoyin Olayinka, Moses Adeolu Adebayo:** Visualization, Supervision, Project Administration.
- **Adesike Oladoyin Olayinka and Solomon Adeyemi Oyekale:** Resources, Supervision.

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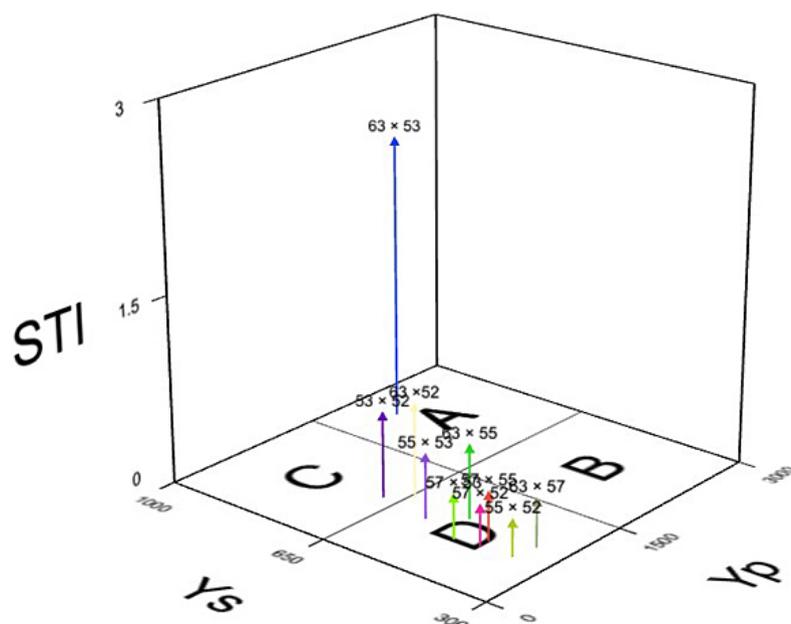


Fig. S1. Interactive three-dimensional (3D) plot based on stress tolerance index (STI) and yield (Yp: yield performance under optimum N environment, and Ys: yield performance under low N environment).

Table S1: Grain yield and other agronomic traits of 10 extra-early provitamin A - quality protein maize hybrids and one checks evaluated under low and optimal N environments in 2022 and 2023 growing seasons at LAUTECH T&R farm, Nigeria

HYBRID	Anthesis (days)	Silking (days)	Anthesis-silking interval (days)	Plant height (cm)	Ear height (cm)	Plant aspect (1-9)	Ear aspect (1-9)	Husk cover (1-5)	Ears per plant	Grain Yield (kg/ha)	Stay green (1-9)
LOW N ENVIRONMENTS											
TZEEIORQ 53 x TZEIIORQ 52	59.75	60.13	0.38	160.50	55.15	4.75	5.13	3.75	0.78	682.22	4.88
TZEEIORQ 55 x TZEIIORQ 52	59.88	60.75	0.88	151.41	47.98	5.50	5.50	4.88	0.79	369.14	5.25
TZEEIORQ 57 x TZEIIORQ 52	60.00	60.00	0.50	153.04	49.47	6.50	5.63	3.63	0.75	432.73	5.63
TZEEIORQ 63 x TZEIIORQ 52	59.13	59.25	0.13	155.89	51.74	5.00	4.75	3.63	0.83	639.73	4.88
TZEEIORQ 55 x TZEIIORQ 53	59.25	59.38	0.13	152.53	47.21	6.13	5.75	4.25	0.82	571.57	6.25
TZEEIORQ 57 x TZEIIORQ 53	60.25	60.50	0.25	148.06	49.75	6.63	5.38	4.13	0.83	484.26	6.38
TZEEIORQ 63 x TZEIIORQ 53	59.38	60.00	0.63	163.30	54.33	4.75	5.13	3.25	0.94	901.49	4.75
TZEEIORQ 57 x TZEIIORQ 55	59.88	61.50	1.63	149.00	44.00	6.13	6.13	3.88	0.63	435.16	6.00
TZEEIORQ 63 x TZEIIORQ 55	58.75	59.25	0.50	159.03	48.66	5.13	4.25	3.13	0.73	513.9	5.00
TZEEIORQ 63 x TZEIIORQ 57	58.88	59.13	0.25	156.04	48.86	5.50	4.63	2.88	0.72	360.02	5.88
Mean	59.51	59.98	0.53	154.88	49.72	5.60	5.23	3.74	0.78	539.02	5.49
Check	63.25	65.50	2.25	158.39	68.38	3.75	3.13	2.50	0.94	1481.14	3.50
OPTIMAL N ENVIRONMENTS											
TZEEIORQ 53 x TZEIIORQ 52	60.50	61.00	0.50	157.06	49.34	5.63	4.25	3.88	0.74	801.41	-
TZEEIORQ 55 x TZEIIORQ 52	59.25	59.75	0.50	162.13	44.23	5.13	5.13	4.00	0.84	646.98	-
TZEEIORQ 57 x TZEIIORQ 52	60.50	61.63	1.13	157.86	48.95	5.50	5.13	4.25	0.80	621.63	-
TZEEIORQ 63 x TZEIIORQ 52	59.88	60.50	0.63	159.52	52.38	5.63	5.00	3.50	0.85	943.75	-
TZEEIORQ 55 x TZEIIORQ 53	60.13	60.38	0.25	154.14	46.78	6.13	5.75	3.75	0.95	724.77	-
TZEEIORQ 57 x TZEIIORQ 53	59.97	60.95	0.98	156.55	47.47	6.02	4.89	4.22	0.91	594.9	-
TZEEIORQ 63 x TZEIIORQ 53	62.13	64.88	2.75	156.06	58.55	4.88	4.50	3.50	0.80	2022	-
TZEEIORQ 57 x TZEIIORQ 55	59.13	60.00	0.88	160.01	52.33	5.63	4.13	3.50	0.91	728.55	-
TZEEIORQ 63 x TZEIIORQ 55	60.75	62.25	1.50	152.58	47.10	6.38	5.25	4.63	0.80	922.33	-
TZEEIORQ 63 x TZEIIORQ 57	61.00	62.25	1.25	152.74	47.07	6.25	5.50	4.00	0.77	869.49	-
Mean	60.32	61.34	1.04	156.86	49.42	5.71	4.95	3.92	0.84	887.58	-

Check	62.00	63.75	1.75	156.08	55.69	5.13	4.13	2.88	0.78	2050.53	-
Statistics											
Minimum	58.75	58.88	0.50	148.06	44.00	3.75	3.13	2.50	0.63	360.02	-
Maximum	63.25	65.50	2.75	163.30	68.38	6.63	6.13	4.88	0.95	2050.53	-
Grand mean	60.33	60.87	0.87	156.00	50.70	5.55	4.96	3.73	0.81	808.99	-
LSD (0.05)	0.95	1.38	1.07	7.22	5.64	0.70	0.77	0.59	0.14	444.04	-
Standard Error (\pm)	0.13	0.22	0.11	0.75	0.99	0.15	0.10	0.10	0.02	88.53	-